SYNTHESIS AND REACTIONS OF FLAVAZOLES

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY

By

P. RAMABHADRAN

DEPARTMENT OF APPLIED CHEMISTRY
UNIVERSITY OF COCHIN, COCHIN 682 022
JULY 1984

CERTIFICATE

Certified that this thesis is based on the work done by Mr.P. Ramabhadran under my guidance in the Department of Applied Chemistry, University of Cochin and no part of this has been presented by him for any other degree.

P. Madhavan Pillai

Dr.P. Madhavan Pillai
(Supervising Teacher)
Reader
Dept.of Applied Chemistry
University of Cochin

Cochin 682022,
July 18, 1984.

DECLARATION

Certified that the work presented in this thesis is based on the original work done by me under the guidance of Dr.P. Madhavan Pillai, Reader, Department of Applied Chemistry, University of Cochin and has not been included in any other thesis submitted for the award of any degree.

P. Ramabhadran

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CHAPTER I

INTRODUCTION

The flavazole (1H-pyrazolo[3,4-b]quinoxaline) ring system was first prepared by H.Ohle and co-workers in 1941 by the reaction of glucose with o-phenylene diamine and phenylhydrazine in the presence of acetic acid. Later G.Neumüller extended the same procedure to disaccharides and a trisaccharide obtained from the action of malt on starch. The reaction has been established as a general one for reducing sugars which are not substituted at positions 2 and 3. Flavazoles are readily crystallisable and they may be identified by means of their melting points or powder X-ray diffraction patterns.

Although many sugars—monosaccharides, disaccharides and trisaccharides—have been converted into their 1-phenylflavazoles mainly as a means of characterisation of the carbohydrates, the basic chemistry of the ring system has not been thoroughly understood. Also, recent reports of the biological activity of some of these compounds as potential diuretic, anti-inflammatory, analgesic, anti-leukaemic, tuberculostatic and immunochemical agents have created added interest in synthesising new types of flavazole

derivatives with a view to testing their pharmacological activity.

The present work has produced for the first time 1-phenylflavazoles with chloro, amino, hydroxy, chloromethyl, carboxamido, trichloromethyl, N-pyrrolidyl and N-pyrrolidylmethyl groups substituted at position 3. The interconversions of 3-amino, 3-hydroxy and 3-chloro-1-phenylflavazoles were also investigated. Further, an unusual phenylation reaction was found to take place if stored or air-oxidised phenylhydrazine was used as the condensing agent for the formation of flavazoles from quinoxaline-2-carboxaldehyde phenylhydrazones. By this phenylation reaction 1,3-diphenyl, 1-p-tolyl-3-phenyl, 1-p-chlorophenyl-3-phenyl, 1-p-bromophenyl-3-phenyl and 1-phenyl-3-p-tolylflavazoles were prepared. In addition to establishing the structure of the phenylation products, the reaction was shown to take place by a free radical mechanism involving phenyl radicals formed from oxidised phenylhydrazine. Also the oxidation, reduction and bromination reactions of 1-phenylflavazole were investigated. The product obtained when a mixture of 1-phenylflavazole and sodium-

borohydride in isopropanol was heated under reflux was shown to be 2-anilinoquinoxaline-3-carboxamide which when refluxed with concentrated hydrochloric acid gave the known 2-anilinoquinoxaline.

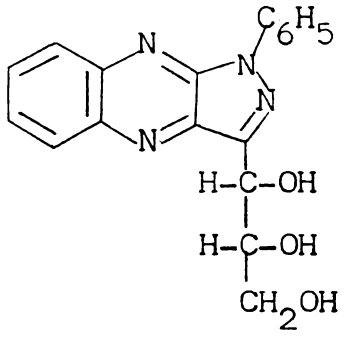
New procedures were worked out for the oxidative cyclisation reactions of quinoxaline-2-carboxaldehyde phenylhydrazones to 1-phenylflavazoles in excellent yields using azobenzene as a dehydrogenating agent. These cyclisations were also shown to take place, though in low yield, if the quinoxaline-2-carboxaldehyde phenylhydrazones were heated above their melting points in an atmosphere containing oxygen.

CHAPTER II

HISTORICAL REVIEW

2.1 Introduction

The application of carbohydrates as starting materials for the synthesis of heterocyclic compounds is well-known[1,2]. The reaction of glucose with o-phenylene diamine and phenylhydrazine in the presence of an acid gave rise to a derivative, 1 of a new heterocyclic ring system, 1H-pyrazolo[3,4-b]quinoxaline (2) which was called a 'flavazole' because of its yellow colour[3,4]. The reaction was found to be a general one



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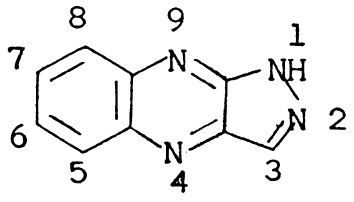


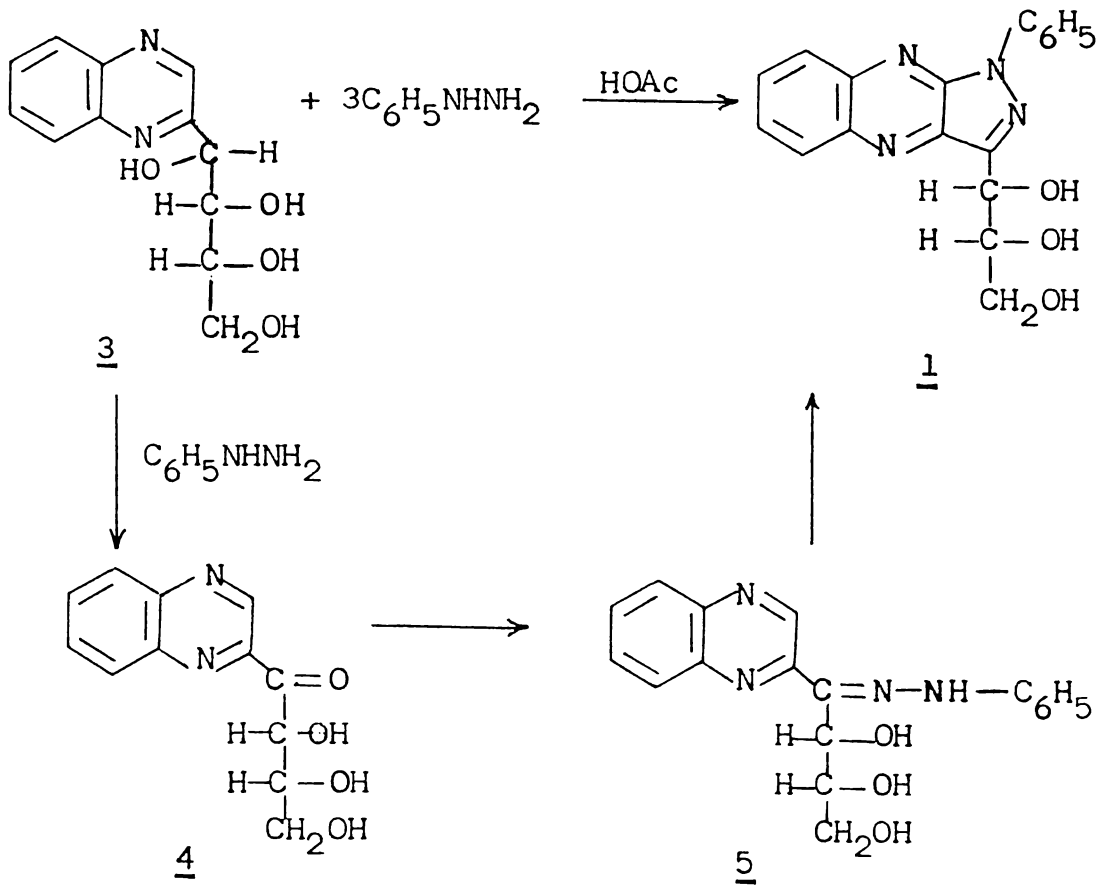
2

for reducing sugars which are not substituted at positions 2 and 3. As flavazoles generally crystallised readily and could be identified by means of their melting points or powder X-ray diffraction patterns, their formation was used for the characterisation of the reacting sugars[5,6].

2.2 Preparation of flavazoles

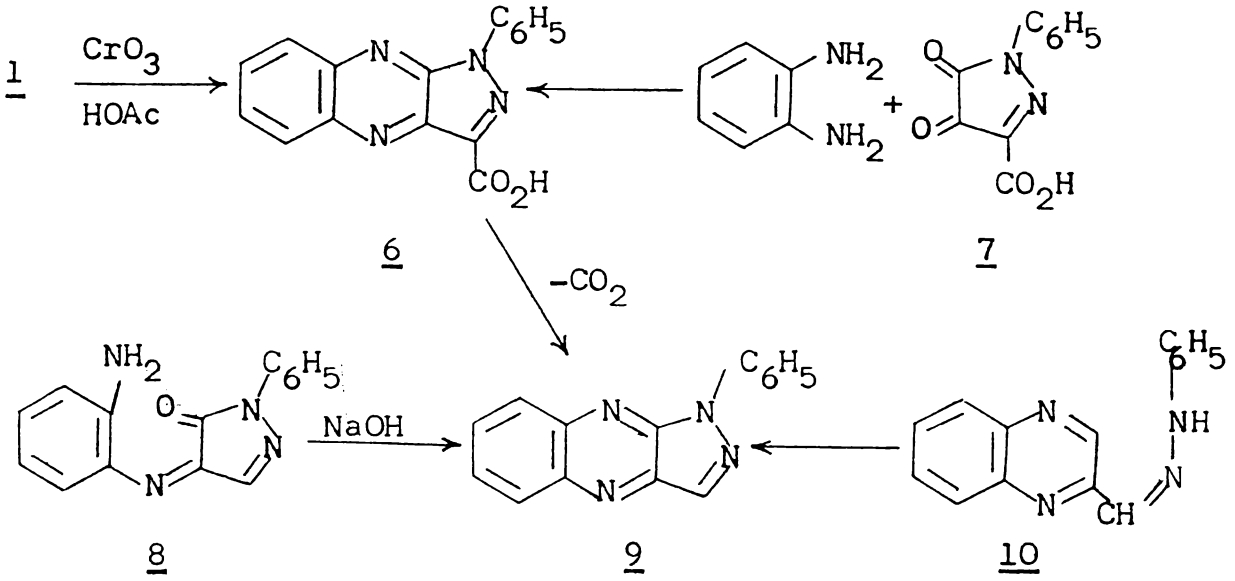
2.2.1 1-Phenylflavazoles

The flavazole ring system[7] was first prepared by Ohle and co-workers[3,4] in 1941 by the treatment of 3-(D-*arabino*-tetrahydroxybutyl)quinoxaline (3) with phenylhydrazine in acetic acid when 1-phenyl-3-(D-*erythro*-1,2,3-trihydroxypropyl) flavazole (1) was obtained in 97% yield. It was suggested that phenylhydrazine first dehydrogenated 3 to the ketoderivative, 4 which then condensed with another molecule of phenylhydrazine to form a phenylhydrazone, 5. A third molecule of phenylhydrazine was used up for the oxidative cyclisation of 5 to the flavazole derivative, 1. In an



experiment involving 3 with 5 mole equivalents of phenylhydrazine in water in the absence of acetic acid, it was shown that ammonia (18%) and aniline (11%) were produced and 9% of 5 was also isolated[3].

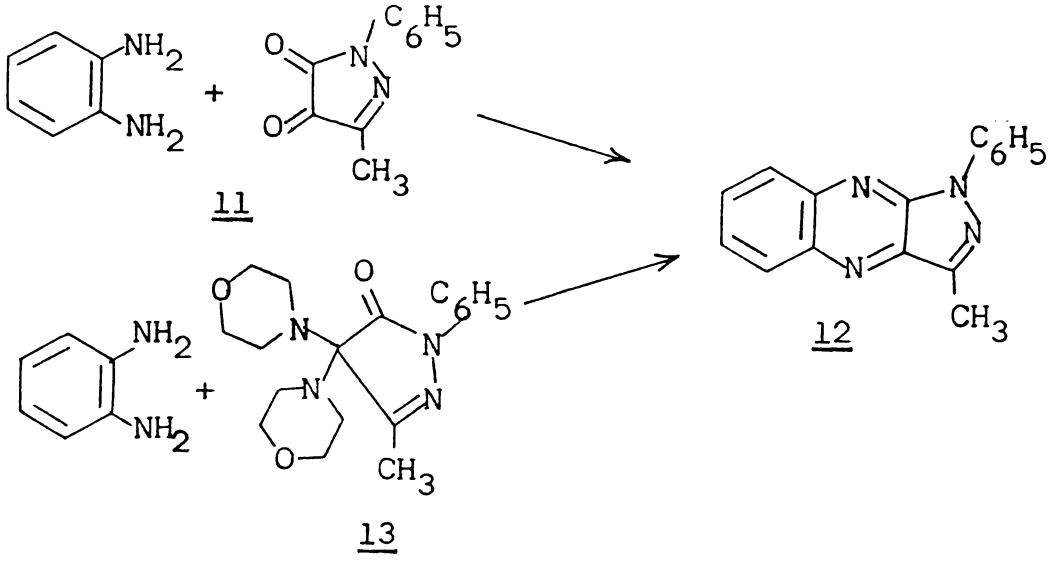
The structure of 1 including the position of the sugar residue was established[8] by oxidation of 1 with chromic anhydride in acetic acid to 1-phenyl-flavazole-3-caroxylic acid (6) and the formation of the same compound by the condensation of o-phenylene diamine with 1-phenyl-4,5-dioxo-2-pyrazoline-3-carboxylic acid (7). When 6 was heated above its melting point, it readily lost carbon dioxide and



provided 1-phenylflavazole (9) which was also obtained by the cyclisation of 8 in the presence of sodium hydroxide

solution[8]. Henseke and co-workers later obtained 1-phenylflavazole (9) when quinoxaline-2-carboxaldehyde phenylhydrazone (10) was dehydrogenated using phenylhydrazine in an acetic acid solution[9].

3-Methyl-1-phenylflavazole, 12 was prepared by the reaction of 3-methyl-1-phenyl-4,5-dioxo-2-pyrazoline (11) with o-phenylene diamine[8]. The treatment of the aminal, 13 with o-phenylene diamine also



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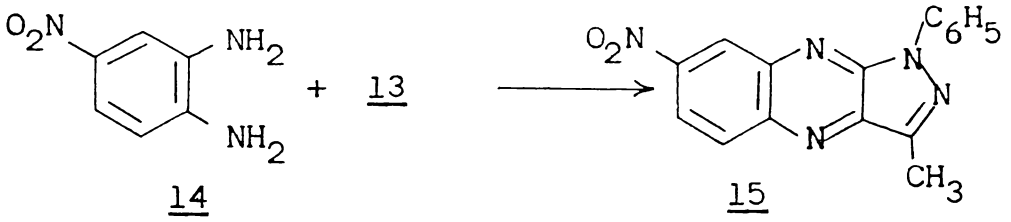
12

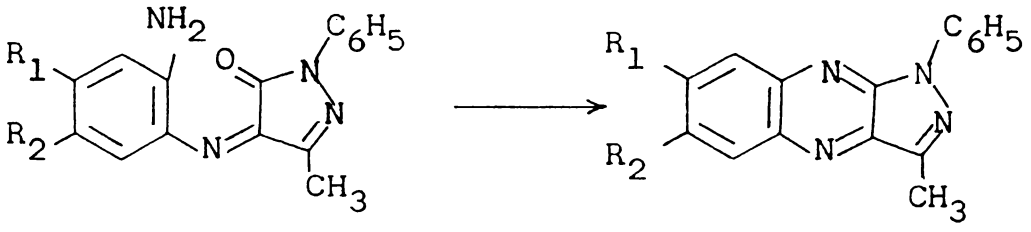
provided[10] 12. The methyl group in 12 was found to be highly unreactive towards oxidising agents and benzaldehyde[8]. Klicnar reported[11] the preparation of 3-methyl-7-nitro-1-phenylflavazole (15) by the condensation of 13 with 4-nitro-o-phenylene diamine (14). Reduction of 15 with hydrazine in the presence of Raney Nickel gave

the amino derivative, 17. 7-Amino-3-methyl-1-phenyl-flavazole (17) and two of its analogs 19 and 21 had



14 15

already been prepared by Vanicek by the cyclisation of the indoaniline dyes 16,18 and 20 by heating them in acetic acid solutions[12].



16, $R_1 = NH_2$, $R_2 = H$ 17, $R_1 = NH_2$, $R_2 = H$

18, $R_1 = NH_2$, $R_2 = CH_3$ 19, $R_1 = NH_2$, $R_2 = CH_3$

20, $R_1 = N(CH_3)_2$, $R_2 = H$ 21, $R_1 = N(CH_3)_2$, $R_2 = H$

Ohle and Liebig proposed two methods for the preparation of flavazoles from carbohydrates[13]. In the

first method, an aqueous solution of the sugar was first heated with o-phenylene diamine in the presence of hydrazine hydrate, boric acid and acetic acid under a stream of carbon dioxide and the resulting dark brown solution of quinoxaline derivative was mixed with phenylhydrazine, acetic acid and hydrochloric acid and the mixture heated under a carbon dioxide atmosphere when the flavazole derivative separated out as a solid. In the second method, a mixture of the sugar solution in water, o-phenylene diamine and phenylhydrazine was heated in the presence of acetic acid and hydrochloric acid for 20 to 24 hours under a stream of carbon dioxide. A comparison of the two methods in the yields of the products formed from a few sugars is given in Table I.

Whereas the osazone reaction of the aldoses involve only one asymmetric carbon atom, namely C-2, the flavazole reaction involves C-2 and C-3. Ohle and Liebig therefore suggested that if two sugars give different osazones but the same flavazole, they have opposite configuration at C-3 but have the same stereochemistry for the remaining carbon atoms[13].

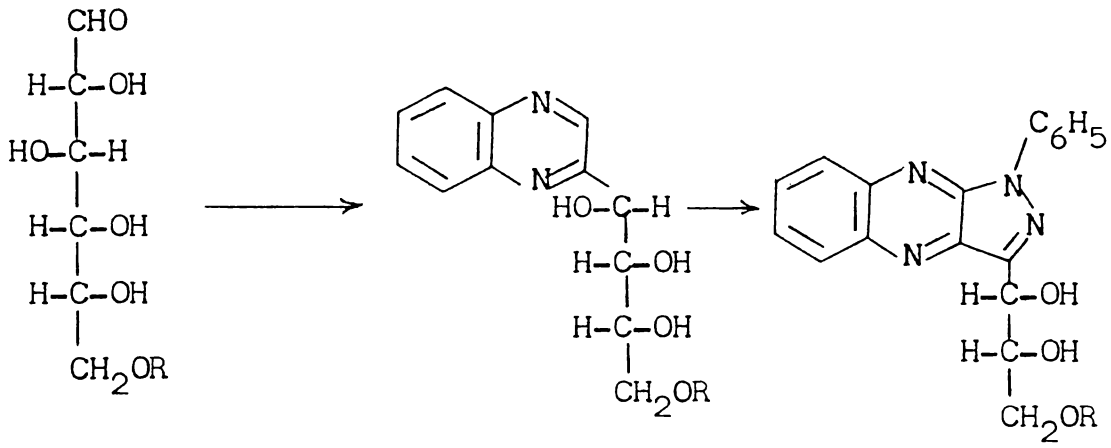
A general procedure for the characterisation of sugars and sugar derivatives as their 1-phenyl-

Table I

Percentage yields of 1-phenylflavazole formation by two different methods[13]

Name of sugar	Name of product	Percentage yield	
		Method I	Method II
D-Galactose	1-Phenyl-3-(D-threo-trihydroxypropyl) flavazole	10	33
L-Sorbose	1-Phenyl-3-(L-threo-trihydroxypropyl) flavazole	3.3	12
D-Xylose	1-Phenyl-3-(D-dihydroxy-ethyl) flavazole	12	11.4
L-Arabinose	1-Phenyl-3-(L-dihydroxy-ethyl) flavazole	10	10
L-Rhamnose	1-Phenyl-3-(L-erythro-dihydroxypropyl) flavazole	25	14

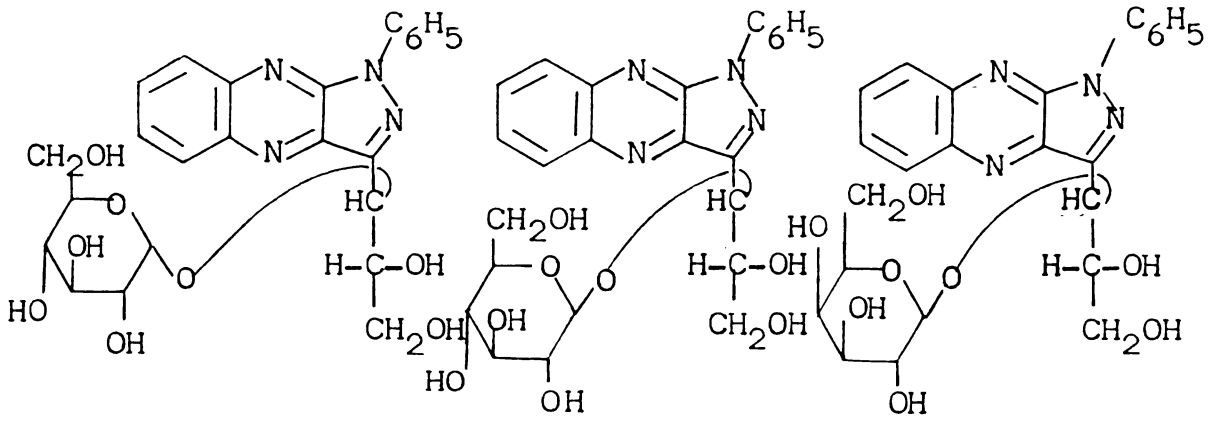
flavazoles was also worked out by Ohle and Kruyff[14]. This method involved the conversion of the carbohydrate into the quinoxaline derivative by treatment with o-phenylene diamine, hydrazine hydrate and acetic acid in pyridine, separation of the byproduct azine by decantation and subsequent reaction of the quinoxaline derivative with phenylhydrazine. Using this procedure the 6-substituted glucoses, 6-O-methyl-D-glucose (22) and 6-O-phenyl-D-glucose (23) were converted into the 1-phenylflavazole derivatives, 1-phenyl-3-(3-O-methyl-D-*erythro*-trihydroxypropyl) flavazole (26) and 1-phenyl-3-(3-O-phenyl-D-*erythro*-trihydroxypropyl)flavazole (27) through the corresponding quinoxaline derivatives, 24 and 25.



22, R = CH_3 24, R = CH_3 26, R = CH_3

23, R = C_6H_5 25, R = C_6H_5 27, R = C_6H_5

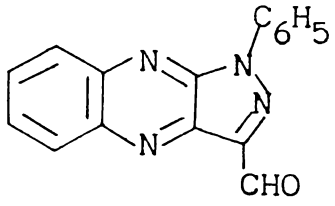
1-Phenylflavazole derivatives 28, 29 and 30 of the disaccharides, maltose, cellobiose and lactose respectively and that of a trisaccharide, dextrin obtained by the action of malt on starch were prepared and characterised by Neumüller for possible use in the determination of the structure of oligosaccharides obtained by enzymic action.[15]



28 29 30

Flavazoles 28, 29 and 30 consumed 4 molecules each of periodic acid in the presence of acetic acid but the reaction did not stop at this point because of further slow oxidation of the products obtained. The flavazole of the starch dextrin consumed 5 molecules of periodic acid under the same condition, but its structure was not proved conclusively as the product of periodic acid oxidation could not be isolated[15]. However, the

structure of the fermentation product obtained from barley with malt amylase was shown to be an isomaltose containing trisaccharide from the fact that it consumed 5 molecules of periodic acid and that the yield of the product did not correspond to 1-phenyl flavazole-3-carboxaldehyde (31) but to a material having a 1,6-glycoside linkage[16].



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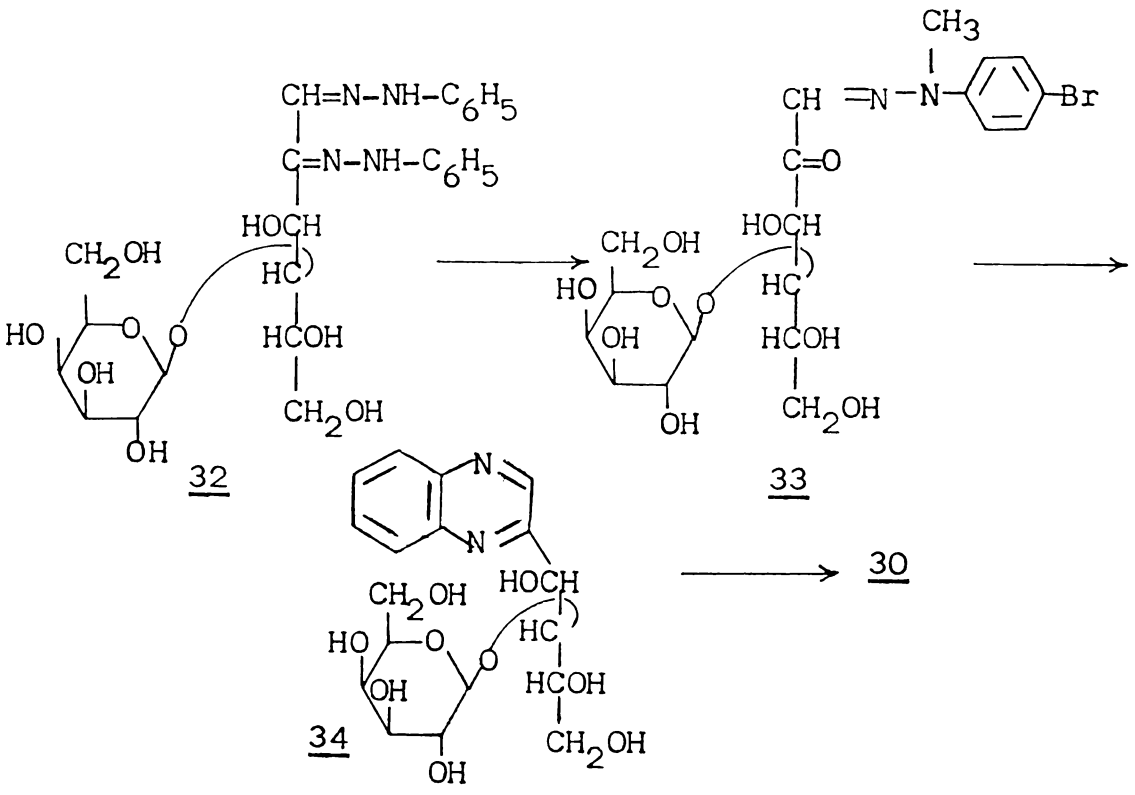
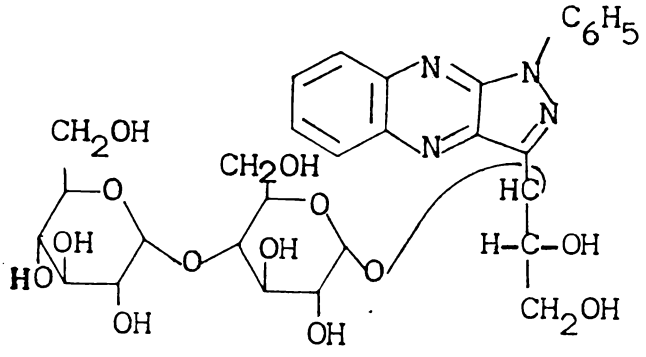
3-(1-β-D-Galactosido -D-erythro-trihydroxy-propyl)-1-phenylflavazole (30) was also obtained from lactose phenylosazone[17] (32). Conversion of 32 into lactosone-1-α-methyl-p-bromophenylhydrazone (33) and subsequent reaction with o-phenylene diamine gave 2-(1-β-D-galactosido-D-arabino-trihydroxybutyl)quinoxaline (34) which when treated with pehnylhydrazine provided 30.

The trisaccharide, maltotriose was converted into 3-[1-(O-α-D-glucopyranosyl(1→4)-O-α-D-glucopyranosyl)-D-erythro-trihydroxypropyl]-1-phenylflavazole (35) in



12% yield by a one step reaction involving o-phenylene diamine and phenylhydrazine[18].



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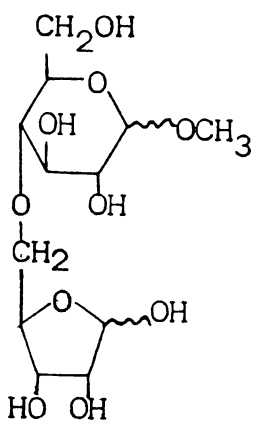
Courtois and Ariyoshi prepared and characterised the 1-phenylflavazole derivatives of a number

of sugars[19] (See Table II) as a means of their identification according to the method of French and co-workers who had prepared the flavazole derivative of mannitotriose[20].

The singly branched dextrins containing 4 to 7 glucose units obtained by extended action of salivary amylase on waxy corn starch was converted into the flavazole derivatives by Nordin and French[21]. The individual dextrin flavazoles reacted with amyloglucosidase to yield a singly branched flavazole derivative containing 4 glucose units[21]. An anomeric mixture of the methylglycoside, 36 of the disaccharide obtained by the hydrolysis of exotoxin[22] was converted into a mixture of the 1-phenylflavazoles, 37. An nmr spectral analysis of the tetraacetate, 38 showed that the mixture contained 80% of the β-anomer.



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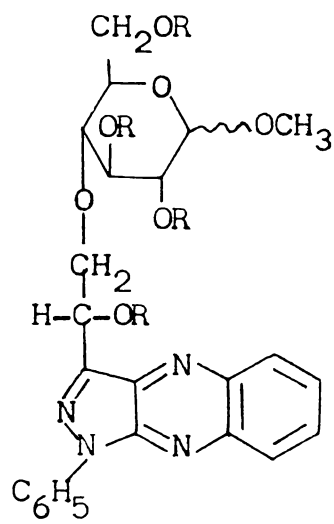


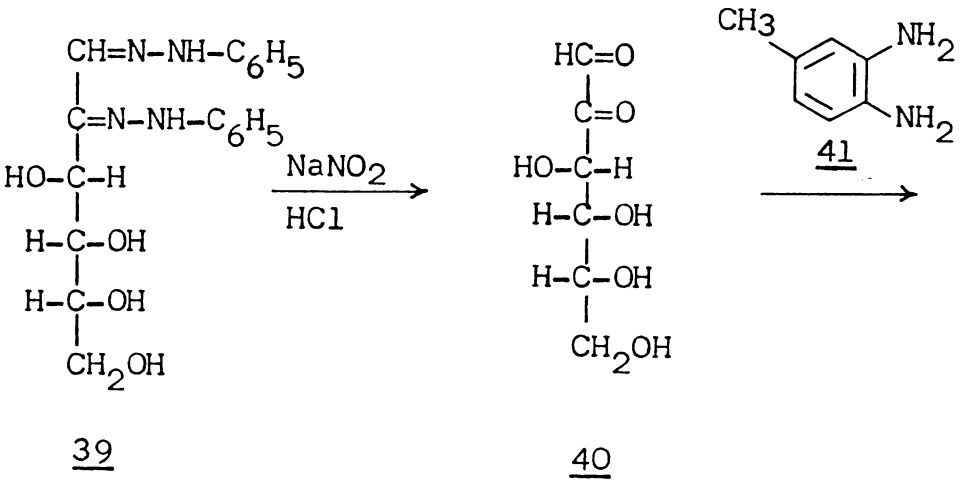
37, R = H
38, R = Ac

Table II

Physical constants of the 1-phenylflavazole derivatives of some sugars

Name of sugar	Yield (%) of 1-phenylflavazole derivative	Solvent of crystallisation	Melting point	$[\alpha]_D^{20}$ c=1 (pyridine)
L-Arabinose	21.5	25% EtOH	215°	-6.9°
L-Rhamnose	25.6	MeOH	214°	+43.8°
D-Glucose	25.5	95% EtOH	218°	-20°
D-Galactose	23.5	95% EtOH	194-5°	-49.3°
Maltose	14.4	1:9-C_5H_5N-EtOH	265°	+53.50°
Gentiobiose	32.3	95% EtOH	245-7°	-43°
Melibiose	34.5	1:9-C_5H_5N-EtOH	218-21°	+45°
Lactose	24.1	1:9-C_5H_5N-EtOH	272°	-88°
Vicianose	23.3	MeOH	216-20°	--
Manninotriose	--	EtOH	236-8°	+60°
Trigalactosidoglucose	34.0	MeOH and MeCOEt	257-62°	+80°

1-Phenylflavazoles substituted with a methyl group at position 6(7) was prepared by Henseke and Bahner[23] starting from D-fructose phenylosazone (39). Conversion of 39 to the osone, 40 followed by treatment with 3,4-diaminotoluene (41) gave 6(7)-methyl-2-(D-arabino-tetrahydroxybutyl)quinoxaline (42). Condensation of 42 with phenylhydrazine in the presence of acid gave 6(7)-methyl-1-phenyl-3-(D-erythro-trihydroxypropyl)flavazole[23] (43). The exact position of the methyl group was not established.



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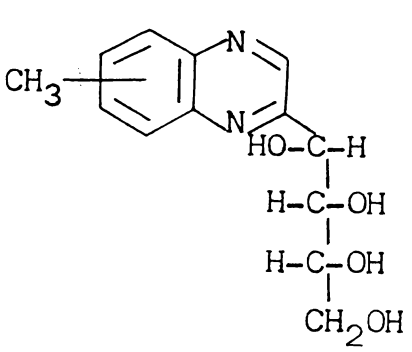

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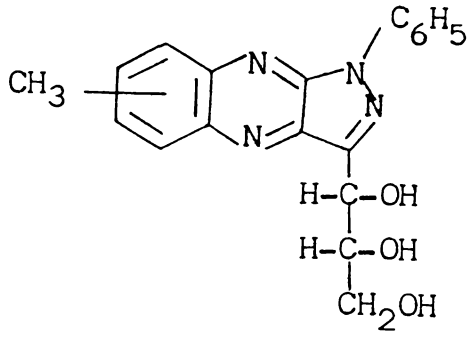
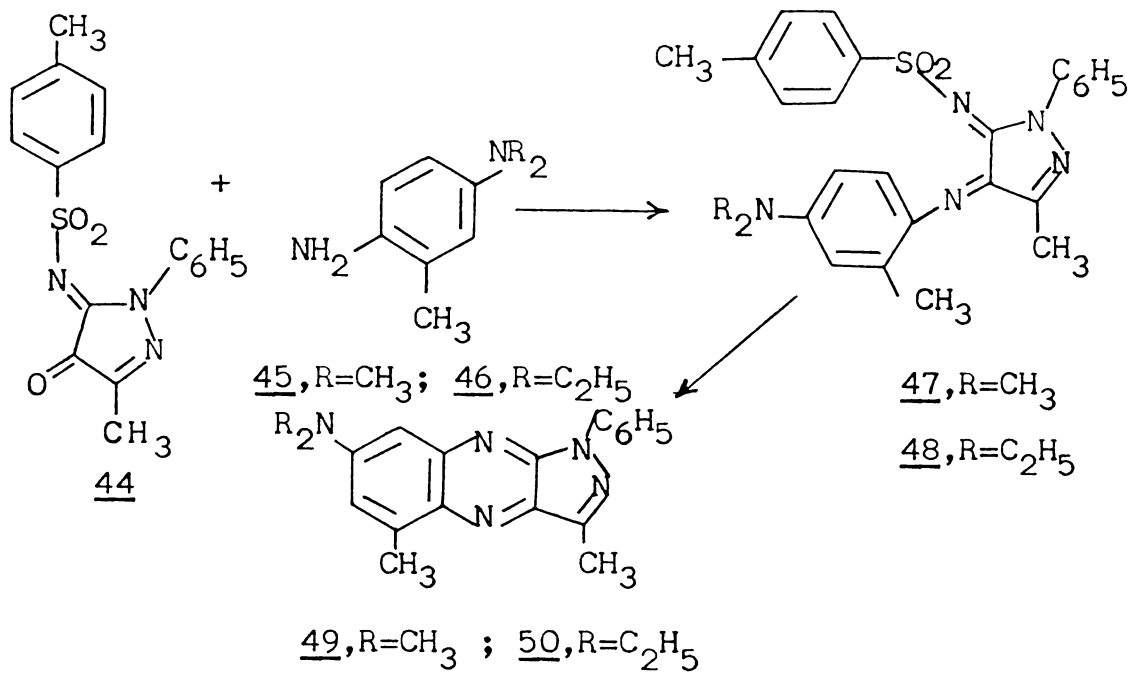
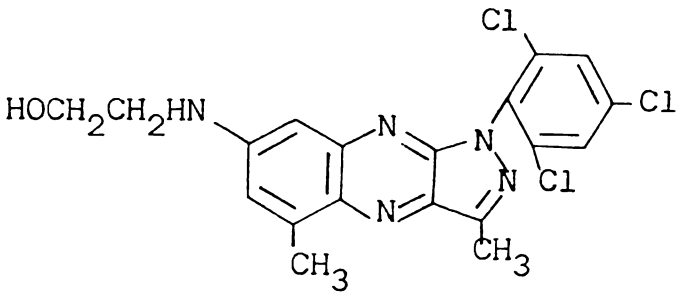


43

Coupling of azomethine dyes to form flavazoles which may be used as fluorescent dyes was reported by Credner[24,25]. Thus the coupling of 5-(p-toluenesulphonimido)-3-methyl-1-phenyl-4-pyrazolone (44) with 2-amino-5-dialkylaminotoluenes, 45 and 46 in the presence of potassium persulphate or silver bromide gave the

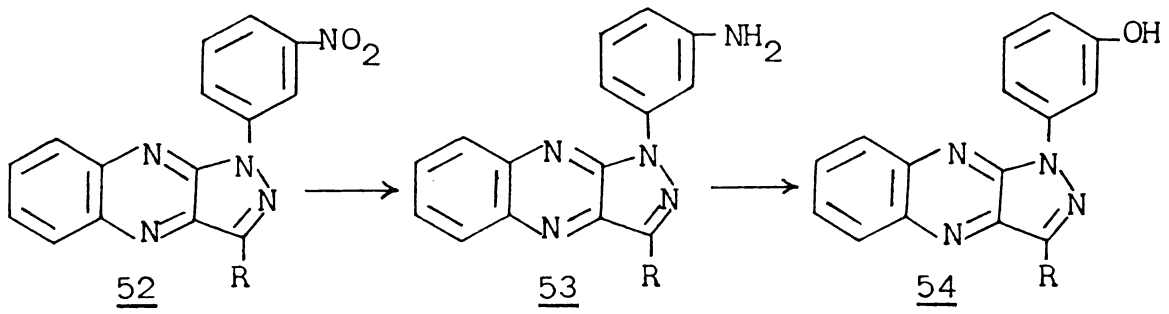


corresponding unstable azomethine dyes, 47 and 48 which on heating itself or by treatment with hydrochloric acid gave the fluorescent 7-alkylamino-3,5-dimethyl-1-phenyl-flavazoles 49 and 50. The preparation of a similar compound, 51 with a trichlorophenyl group at position 1 was also reported by Credner and Pueschel[25].



51

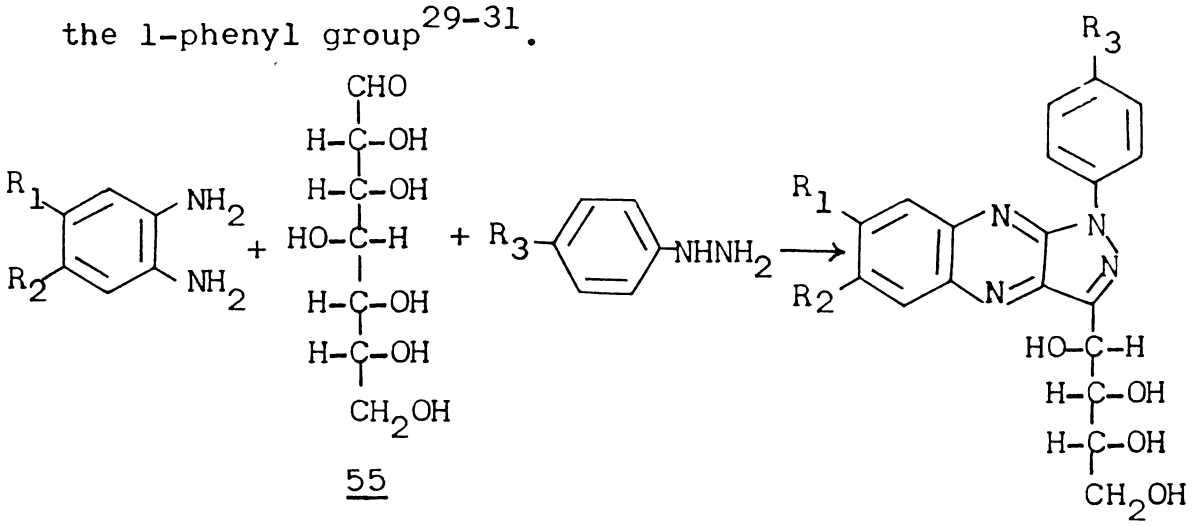
1-(m-Nitrophenyl) flavazole (52) of the oligosaccharides of isomaltose, maltose and cellobiose series were prepared by treatment of the oligosaccharides with o-phenylene diamine and m-nitrophenylhydrazine[26]. The nitroderivatives of 21 such oligosaccharides were catalytically hydrogenated to the corresponding 1-(m-aminophenyl) flavazoles[27] (53). The amino group was converted into hydroxyl group by diazotisation to give 1-(m-hydroxyphenyl) flavazoles[28] (54).



R = sugar residue

Condensation of o-phenylene diamines with a heptose, D-glycero-D-guloheptose (55) and phenylhydrazine or

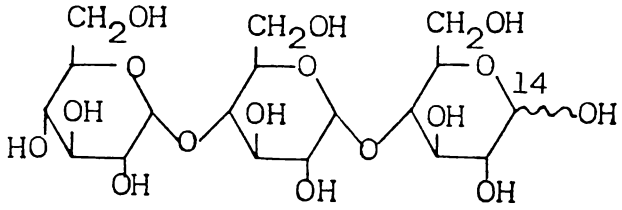
p-fluorophenylhydrazine gave flavazoles 56 to 59 with substituents at the quinoxaline ring and on the 1-phenyl group[29-31].



55

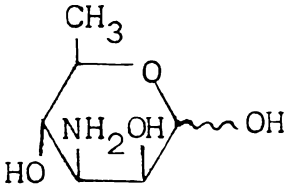
56, $R_1=R_2=R_3=H$
57, $R_1=R_3=H$, $R_2=Cl$
58, $R_1=R_2=CH_3$, $R_3=H$
59, $R_1=R_2=H$, $R_3=F$

The 1-phenylflavazole of a ^{14}C labelled trisaccharide, 1-^{14}C-amylotriose was prepared[32]. The X-ray diffraction data of this crystalline derivative confirmed the structure of amylotriose as O-α-D-glucopyranosyl (1→4)-O-α-D-glucopyranosyl (1→4)-D-1-^{14}C-glucose (60).



60

The 3-amino-3,6-dideoxy-D-aldohexose, mycosamine (61) was converted into its 1-phenylflavazole (62). The configuration at C-4 of 61 was established from the fact that 62 was the enantiomorph of the 1-phenylflavazole (63) formed from L-rhamnose[33].



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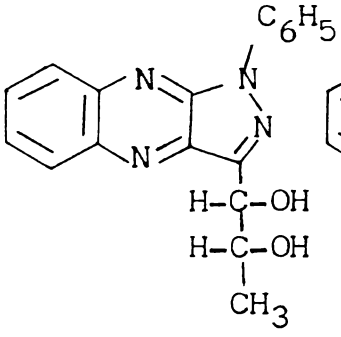

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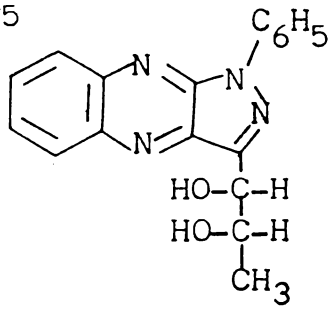
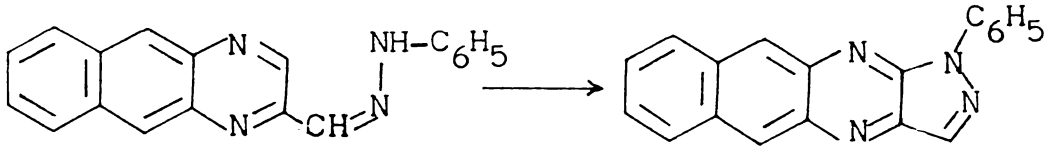


63

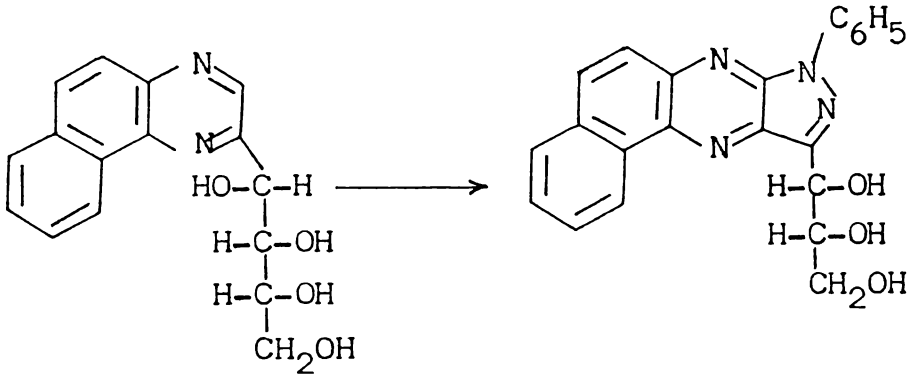
1-Phenylflavazole derivative of amylose was prepared by its reaction with o-phenylene diamine and phenylhydrazine[34].

Henseke and co-workers[9] obtained a higher condensed flavazole, 1-phenyl-6,7-benzoflavazole (65) by the dehydrogenation of 6,7-benzoquinoxaline-2-carboxaldehyde phenylhydrazone (64) with phenylhydrazine in acetic acid medium.



64 65

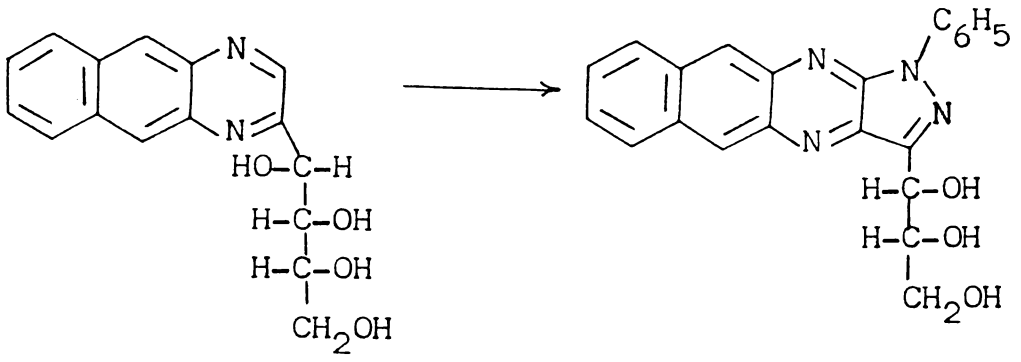
Also 3-(D-arabino-tetrahydroxybutyl)-5,6-benzoquinoxaline (66) obtained by the condensation of 1,2-diaminonaphthalene with D-fructosone-1-methylphenylhydrazone[35] gave 3-(D-erythro-trihydroxypropyl)-1-phenyl-5,6-benzoflavazole (67) when treated



66 67

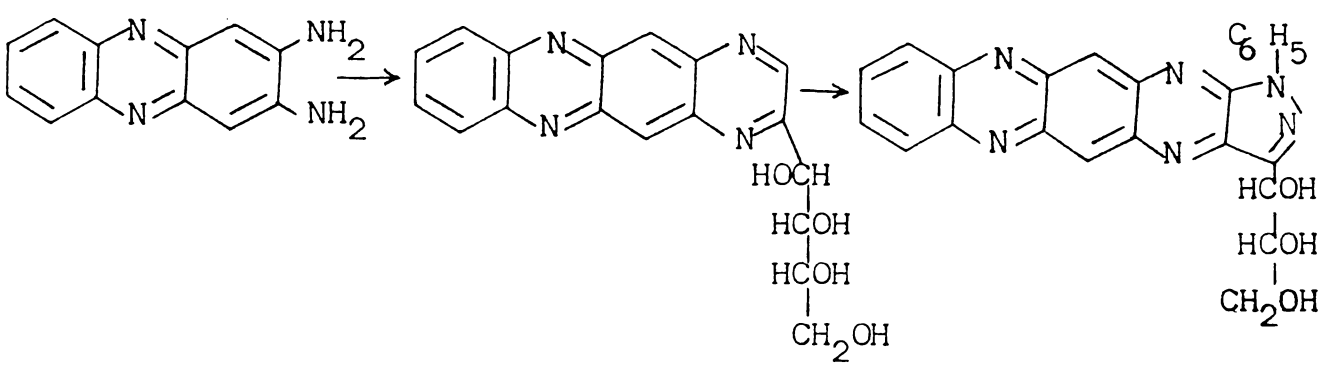
with phenylhydrazine under acidic conditions[36]. Similarly, D-fructosone-1-methylphenylhydrazone condensed with 2,3-diaminonaphthalene to give

3-(D-arabino-tetrahydroxybutyl)-6,7-benzoquinoxaline (68) which was converted into the flavazole, 69 by treatment with phenylhydrazine.



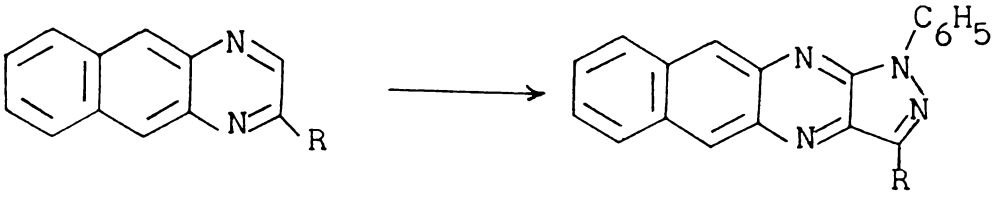
68 69

A still higher condensed flavazole, 3-(D-erythro-trihydroxypropyl)-1-phenylflavazolo[6,7-b] quinoxaline (72) was prepared by the condensation of phenylhydrazine with 2-(D-arabino-tetrahydroxybutyl) quinoxalino[6,7-b] quinoxaline (71) which in turn was obtained by the reaction of 2,3-diaminophenazine (70) with D-fructose-1-methylphenylhydrazone[36].



70 71 72

Henseke and Bauer reported the preparation of benzoflavazoles 77-80 by reaction of the corresponding benzoquinoxaline derivatives 73-76 with phenylhydrazine in the presence of acid[37].



73-76 77-80

73, R = D-lyxo-tetrahydroxybutyl

74, R = L-xylo-tetrahydroxybutyl

75, R = D-threo-trihydroxypropyl

76, R = L-erythro-trihydroxypropyl

77, R = D-threo-trihydroxypropyl

78, R = L-threo-trihydroxypropyl

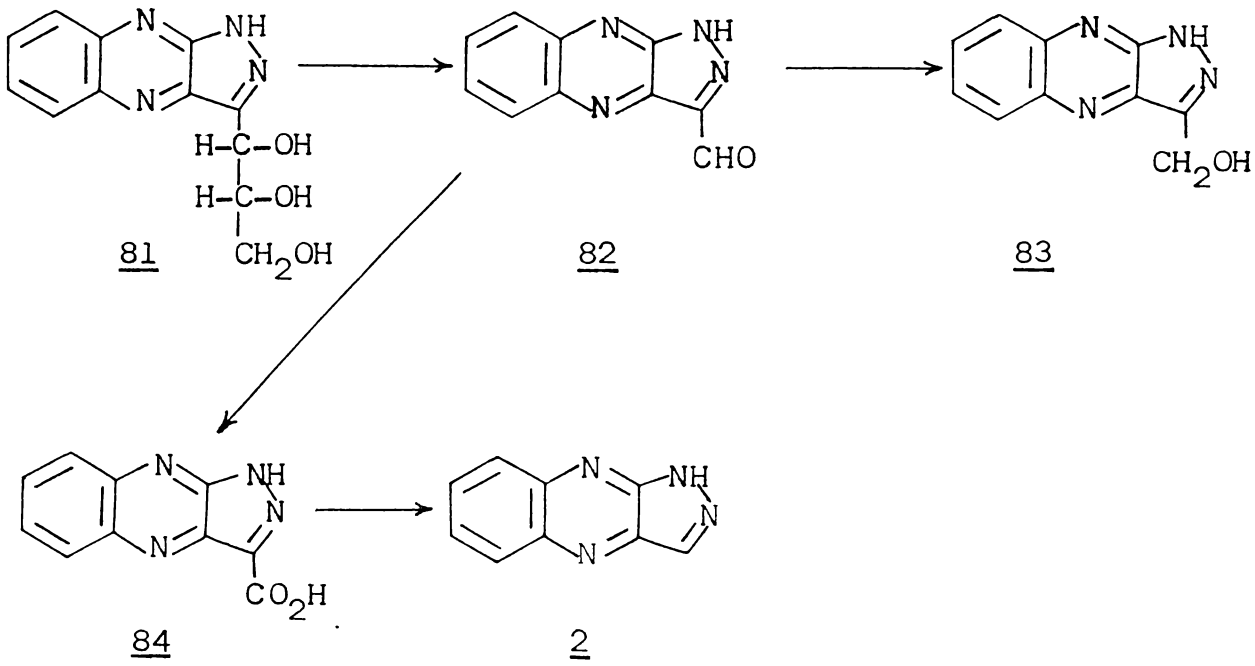
79, R = D-dihydroxyethyl

80, R = L-dihydroxyethyl

2.2.2 Flavazoles unsubstituted at position 1

Ohle and Iltgen prepared the parent, unsubstituted flavazole, 2, as follows:[38] Condensation of 2-(D-arabino-tetrahydroxybutyl)quinoxaline[4](3) with

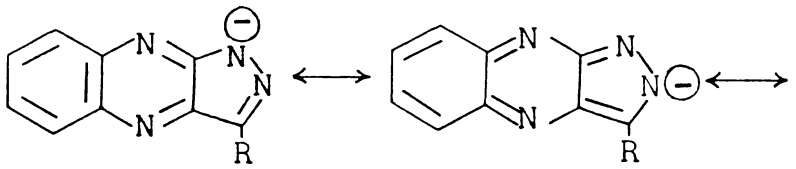
hydrazine yielded 40% of 3-(D-*erythro*-trihydroxypropyl) flavazole (81) under optimum conditions. The oxidation of 81 with lead tetraacetate gave the carboxaldehyde, 82 in poor yield, but the yield was increased if periodic acid was used as the oxidising agent. Treatment of 82 with hot concentrated alkali under the Cannizzaro's reaction conditions gave a mixture of the primary alcohol, 83 and carboxylic acid, 84. Oxidation of 82 with chromic anhydride in 50% sulphuric acid also provided 84, which readily lost carbon dioxide when sublimed under atmospheric pressure and provided the parent flavazole, 2. It was observed that all flavazoles with a free 1-position form orange to yellow



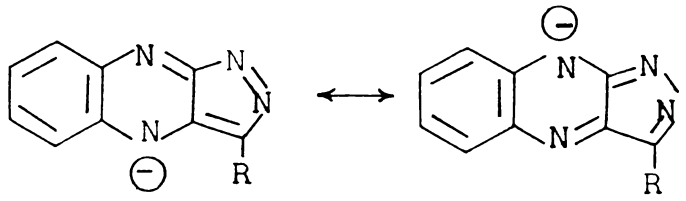
81 82 83

84 2

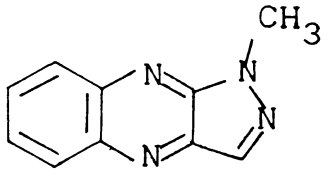
metal salts, the colour being ascribed to the flavazole anion, 85 which may have a number of resonance forms as shown below.



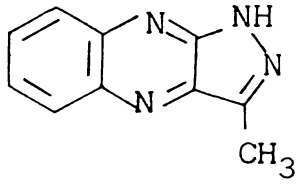
85



The preparation of 1-methylflavazole (86) starting from methylhydrazine instead of hydrazine in the reaction with 3 and that of 3-methylflavazole (87) by the reduction of 82 with hydrazine have also been described by the same authors[38]. Flavazole, 2 was also prepared starting from pyruvic acid[39]. Treatment

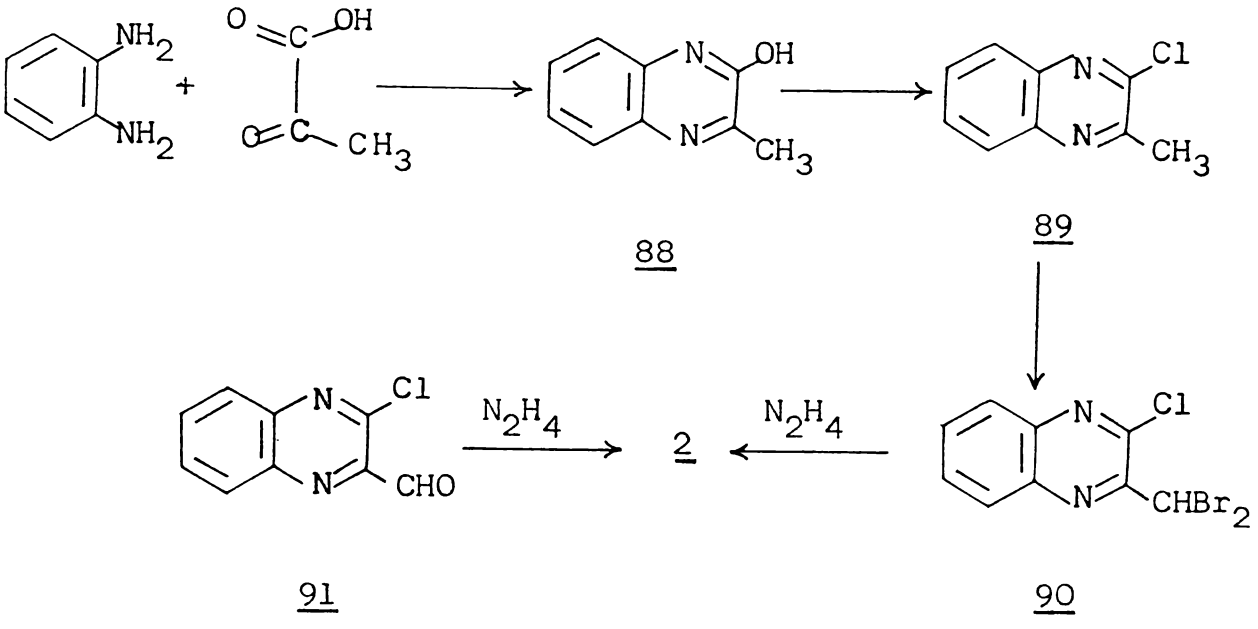


86



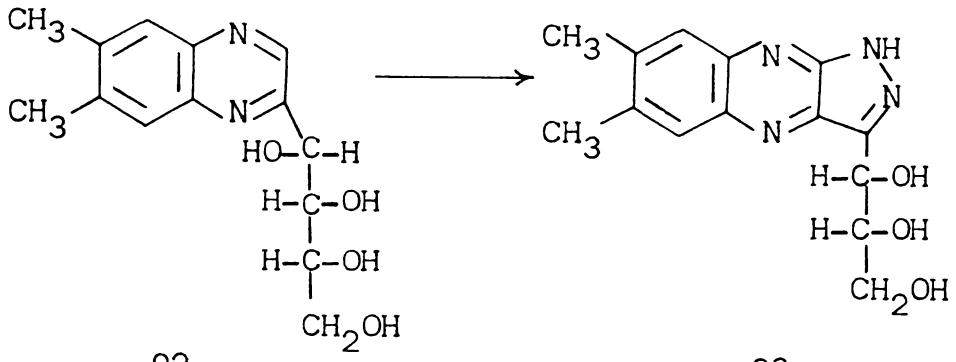
87

of o-phenylene diamine with pyruvic acid gave 2-hydroxy-3-methylquinoxaline (88). Conversion of 88 into the chloroderivative 89 by reaction with $POCl_3$ followed by bromination of the methyl group to get 90 and subsequent treatment with hydrazine hydrate provided the unsubstituted flavazole, 2. Romenenko and Burmistrov also prepared 2 in 71% yield by the reaction of 2-chloroquinoxaline-3-carboxaldehyde (91) with hydrazine[40].



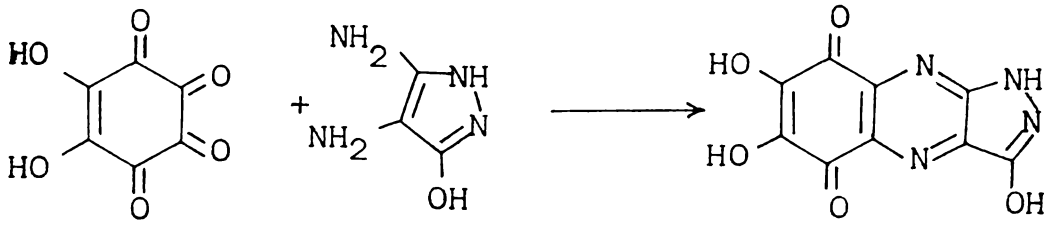
7,8-Dimethylflavazoles substituted at position 3 were synthesised in order to investigate their pharmacological activity[41]. 2-(D-Arabino-tetrahydroxybutyl)-6,7-dimethylquinoxaline (92) was treated with hydrazine-

hydrate in acetic acid and copper powder to give 3-(D-erythro-trihydroxypropyl)-6,7-dimethylflavazole (93).



92 93

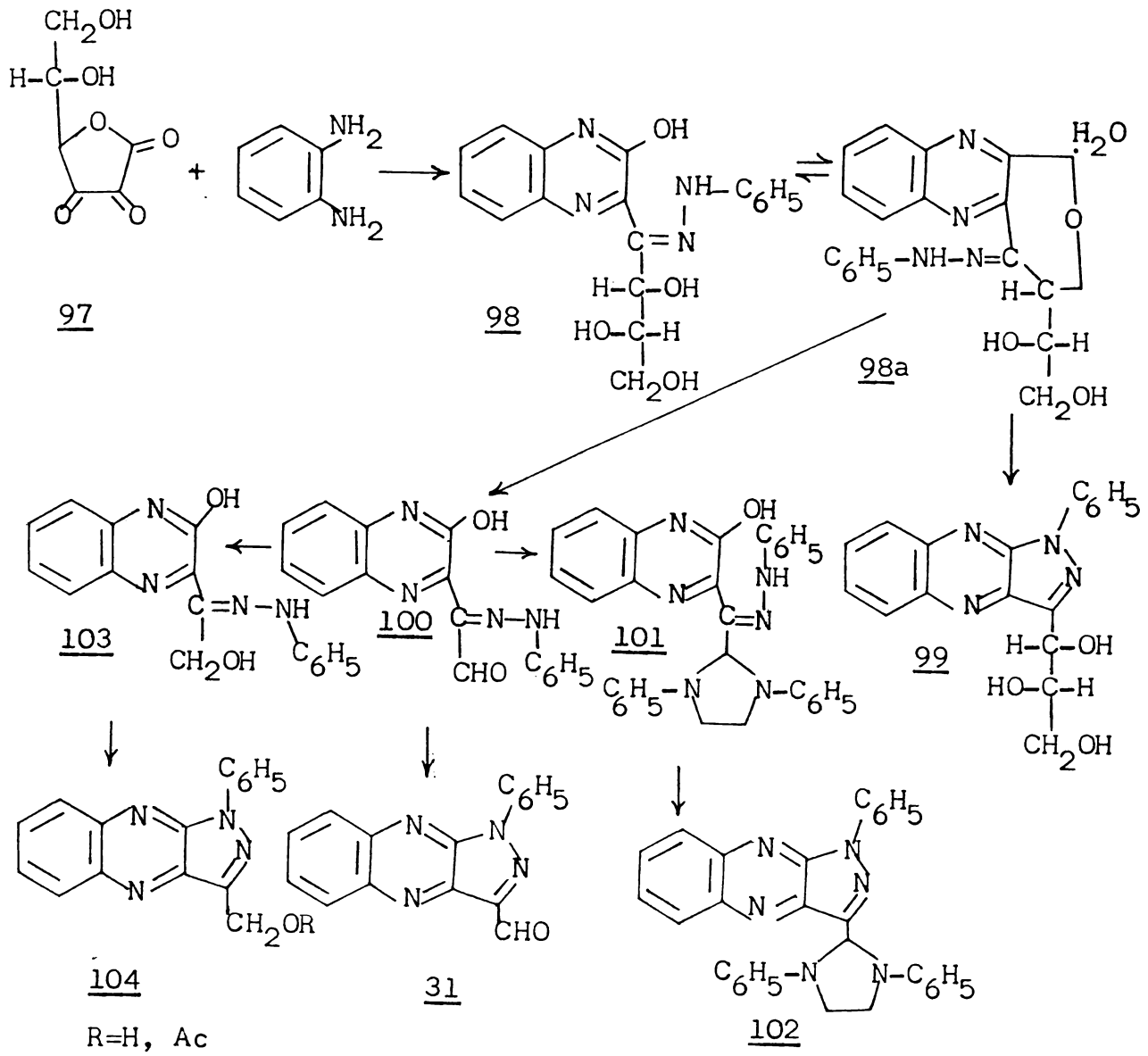
A highly oxidised flavazole derivative, 3,6,7-trihydroxyflavazole-5,8-dione (96) was prepared by the treatment of a solution of dipotassium rhodizonate 94 with 3,4-diamino-5-hydroxypyrazole, 95 in the presence of sulphuric acid[42].



94 95 96

2.2.3 Flavazoles formed from dehydro-L-ascorbic acid and related compounds

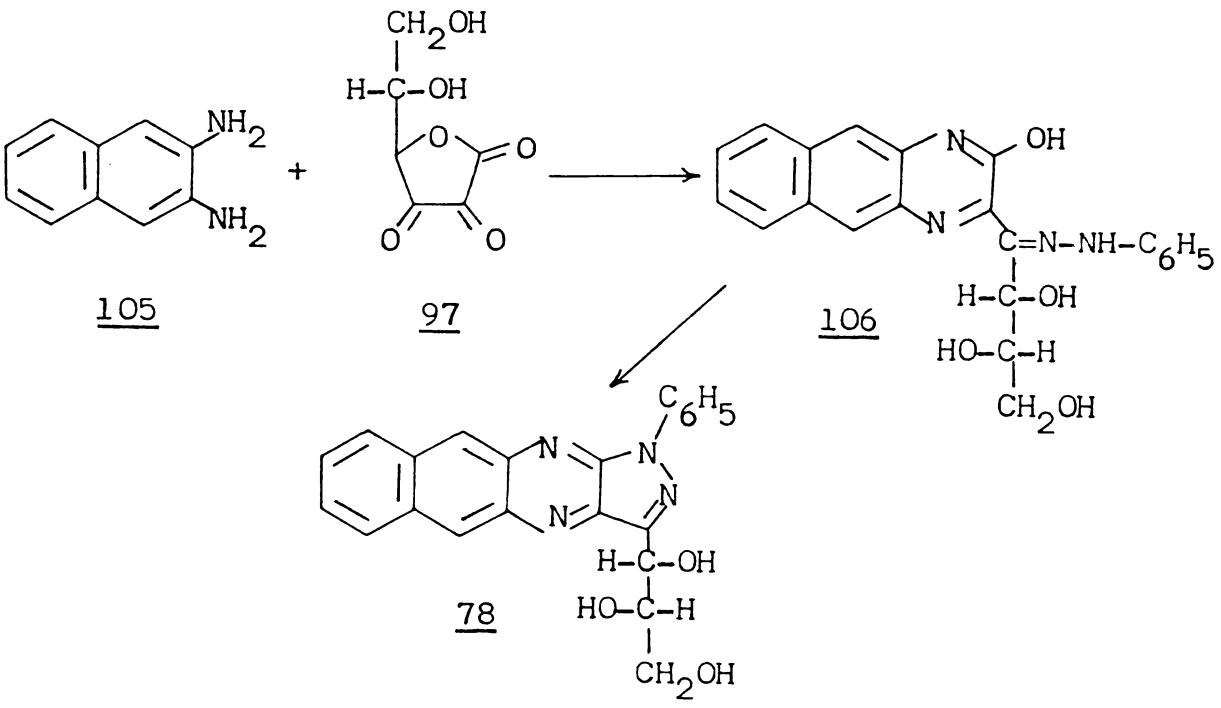
The simultaneous action of o-phenylene diamine and phenylhydrazine on dehydro-L-ascorbic acid (97) yielded 2-hydroxy-3-(1-phenylhydrazono-L-threo-2,3,4-trihydroxybutyl)quinoxaline[43] (98) which was first believed



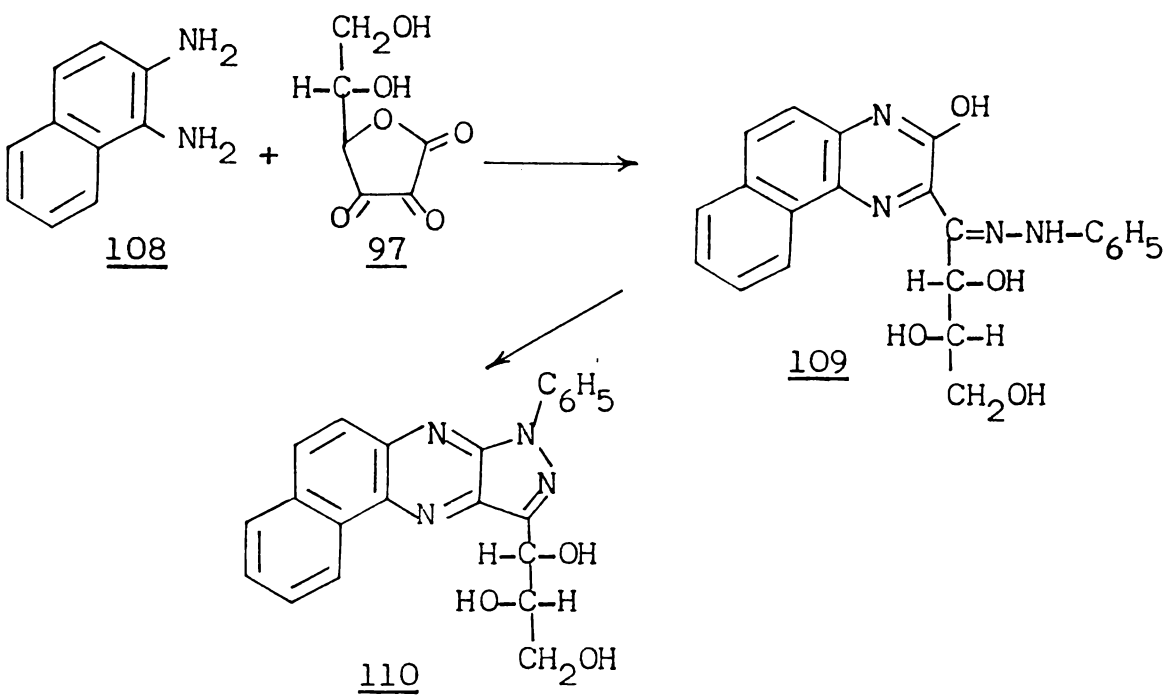
to have the cyclic structure, 98a. When 98 was heated with sodium hydroxide solution, 1-phenyl-3-(L-threo-trihydroxypropyl)flavazole (99) was obtained in 95% yield[43].

Also oxidation of 98 with aqueous periodic acid gave the carboxaldehyde, 100 which when treated with 1,2-dianilinoethane gave 101 and subsequent cyclisation in the presence of methanolic sodium hydroxide provided 1-phenyl-3-(1,3-diphenyl-2-imidazolinyl) flavazole[44](102). 1-[2-Hydroxyquinoxalyl-(3)-]glyoxal-1-phenylhydrazone (100) itself cyclised to give 1-phenylflavazole-3-carboxaldehyde (31) in the presence of alkali. Compound 31 was also converted into 102 by treatment with 1,2-dianilinoethane[44]. Reduction of 100 with sodium borohydride to 103 followed by treatment with base or acetic anhydride also provided the flavazole, 104 either with the free or the acetylated hydroxyl group[45].

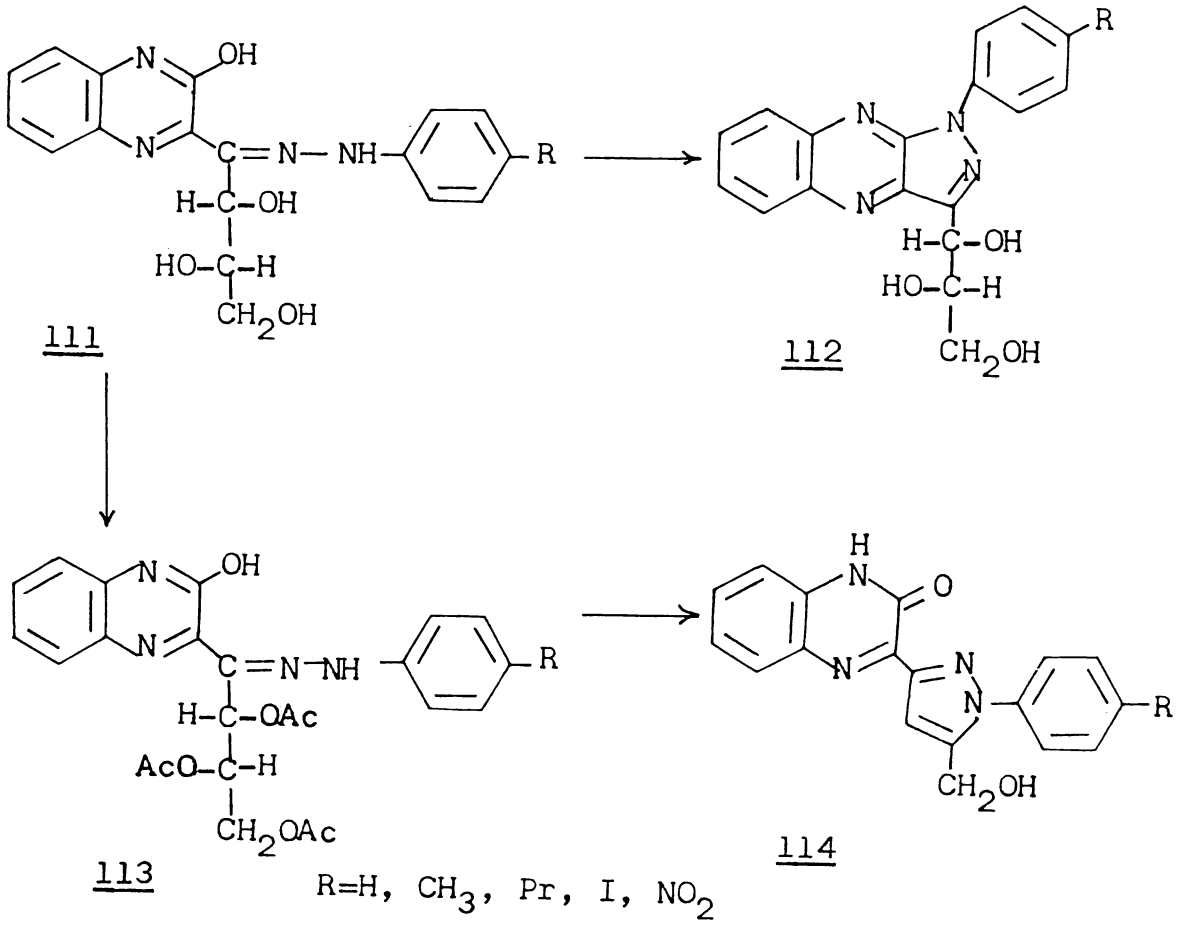
L-Dehydroascorbic acid obtained by the oxidation of L-ascorbic acid with p-benzoquinone gave 2-hydroxy-3-(1-phenylhydrazono-L-threo-trihydroxybutyl)-6,7-benzoquinoxaline (106) on treatment with 2,3-diaminonaphthalene (105). Cyclisation of 106 with sodium hydroxide solution in the presence of n-butanol provided 1-phenyl-3-(L-threo-trihydroxypropyl)-6,7-benzoflavazole[44] (78). Similarly L-dehydroascorbic



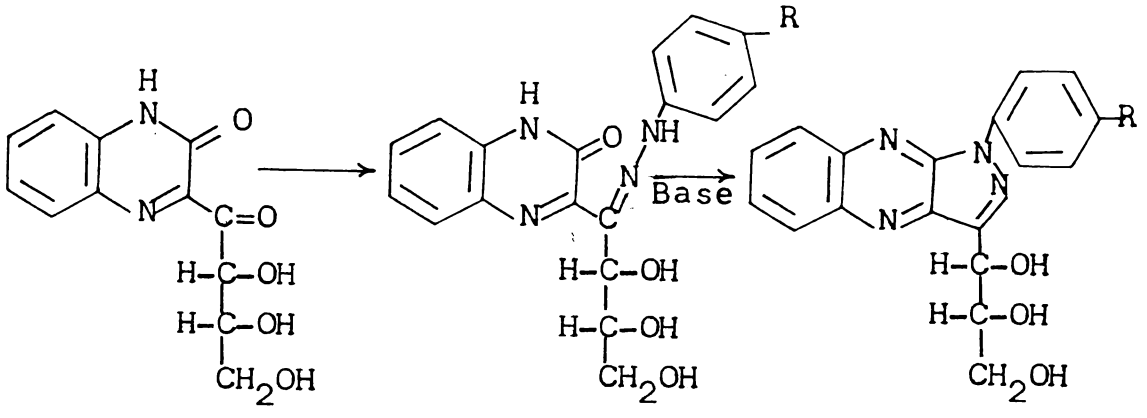
acid on treatment with 1,2-diaminonaphthalene (108) and phenylhydrazine yielded 2-hydroxy-3-(1-phenylhydrazone-L-threo-trihydroxybutyl)-5,6-benzoquinoxaline (109) which was cyclised to the flavazole, 110 by treatment with aqueous sodium hydroxide in the presence of n-butanol[44].



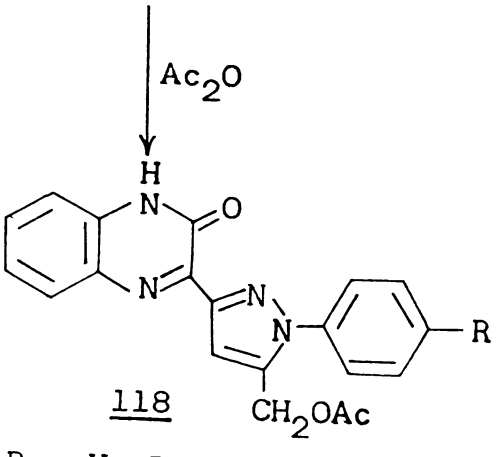
El Ashry and co-workers prepared a number of *para*-substituted 1-phenyl-3-(L-*threo*-trihydroxypropyl)flavazole (*112*) by the simultaneous treatment of dehydro-L-ascorbic acid with o-phenylene diamine and *para*-substituted phenylhydrazine and subsequent cyclisation of the quinoxaline derivatives (*111*) in the presence of alkali[46]. However the corresponding acetylated quinoxalines (*113*) on treatment with alkali underwent deacetylation and dehydration to 3-(1-aryl-5-hydroxy-methylpyrazol-3-yl)-2-quinoxalinones[46] (*114*).



The D-isomer of 97 also reacted with o-phenylene diamine and its 4-chloro analogue in the same manner producing 3-(D-erythro-2,3,4-trihydroxy-1-oxobutyl)-2-quinoxalinone (115) and its 6-chloroderivative[47]. These quinoxaline derivatives reacted with a number of para-substituted phenylhydrazines producing the corresponding phenylhydrazones (116). While the reaction of 116 with alkali produced the flavazoles, 117, cyclisation in the presence of acetic anhydride gave only 118 which was identical to the O-acetyl derivative of 114[48].



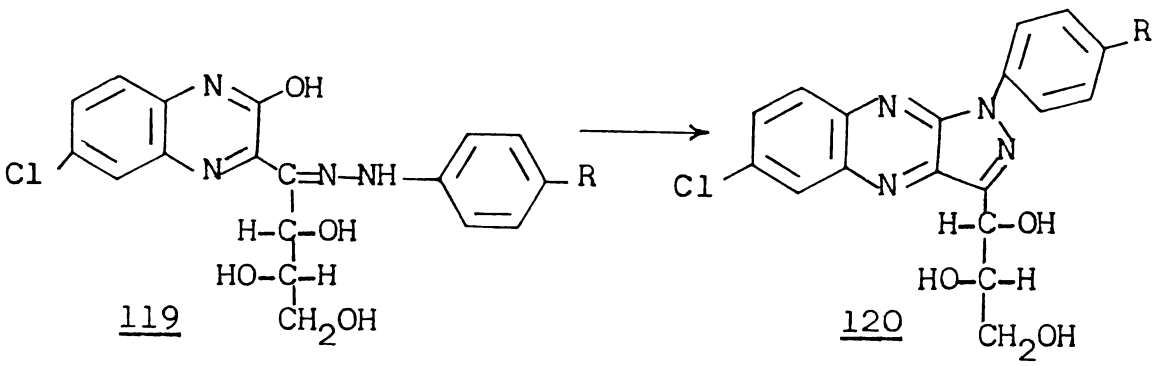
115 116 117



118

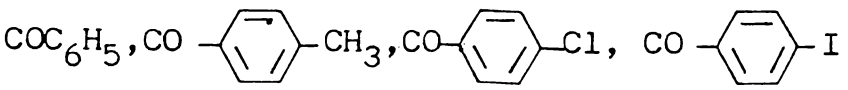
R = H, Br, I, NO_2

Also, the reaction of dehydro-L-ascorbic acid with 4-chloro-o-phenylene diamine followed by treatment with para-substituted pehnylhydrazines gave 6-chloro-3-(1-arylhydrazono-L-threo-2,3,4-trihydroxybutyl)-2-quinoxalinones[48](119). The formation of only one isomer from the 4-chloro-o-phenylene diamine has been explained as due to the difference in the reactivity of the two amino groups because of the effect of the chlorine substituent. Elimination of a molecule of water by treatment with alkali gave the corresponding flavazoles (120). 3-(L-Threo-trihydroxypropyl)-1-(p-sulphamylphenyl) flavazole (122) was similarly

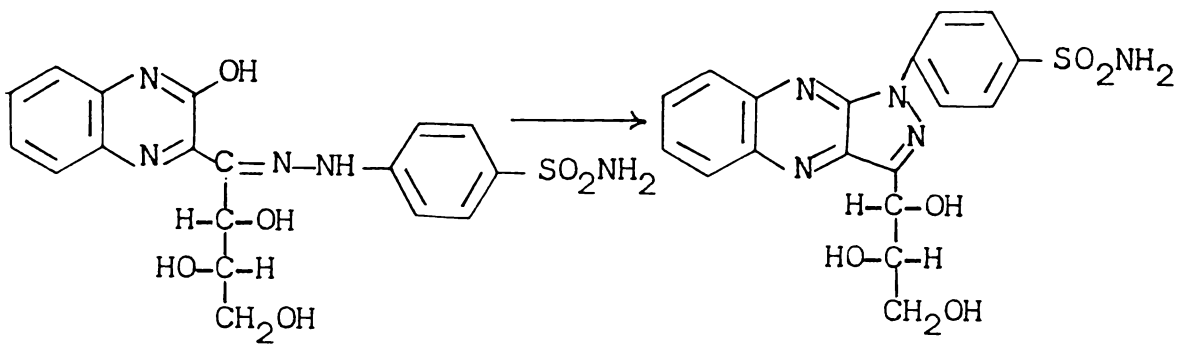


119 120

R = CH_3, OCH_3, Br, I, NO_2, SO_2NH_2,

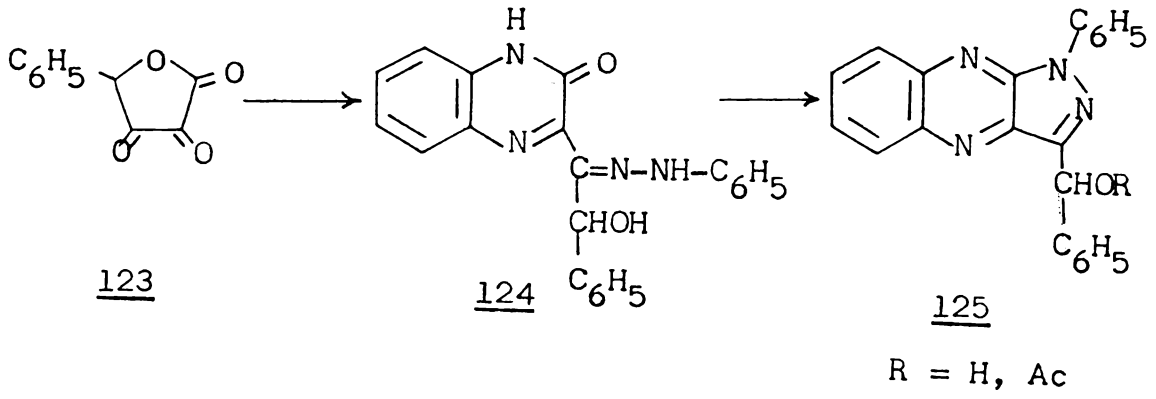


prepared[49] by the cyclisation of the corresponding quinoxaline derivative, 121. 4-Phenyl-2,3-dibutano-1,4-lactone (123) also reacted with o-phenylene diamine



121 122

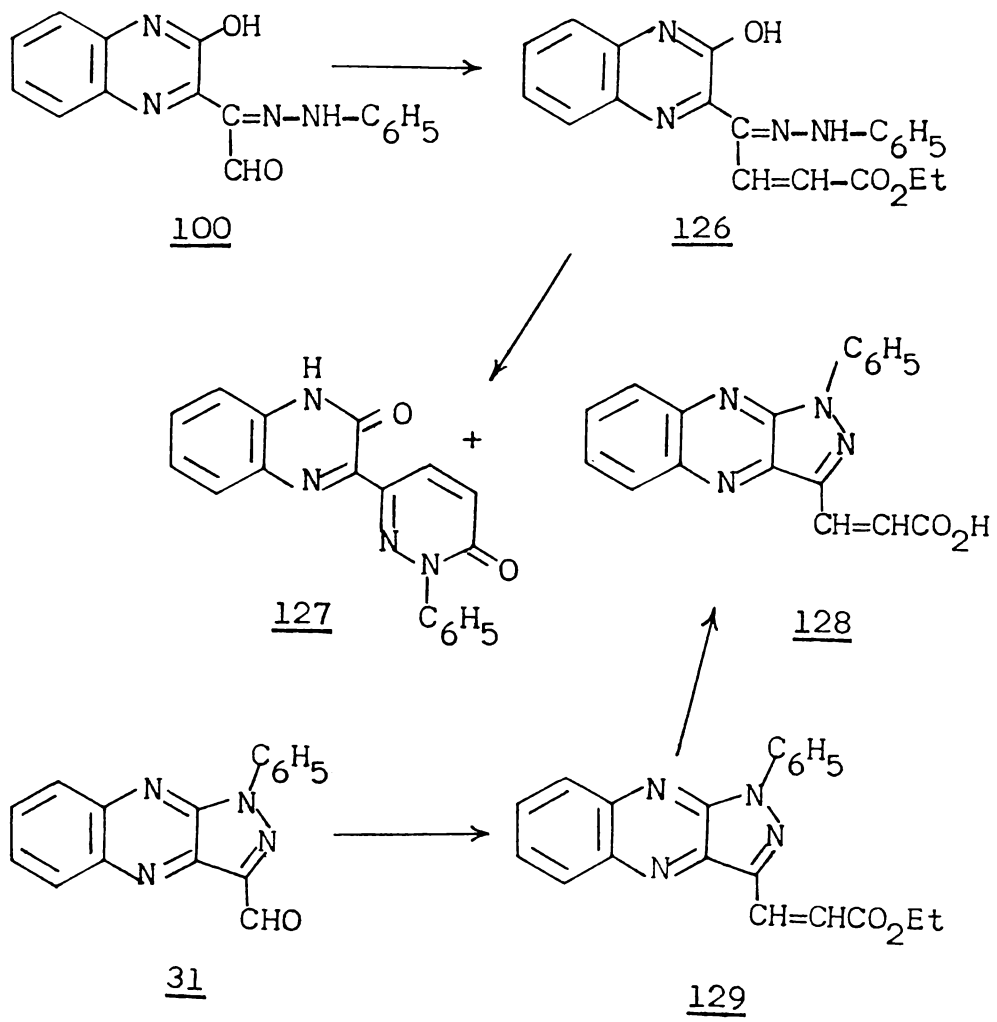
and phenylhydrazine in an analogous manner producing 3-(2-aryl-1-phenylhydrazono-2-hydroxyethyl)-2-quinoxalinone[47](124). The flavazole, 3-(α-hydroxybenzyl)-1-phenylflavazole (125) was obtained by the cyclisation of 124[47].



123 124 125

R = H, Ac

A Wittig reaction of the carboxaldehyde,[44] 100 with carboethoxymethylidenetriphenylphosphorane gave the condensation product,[50] 126. Cyclisation of 126 with alkali gave two products, the pyridazinone, 127 and the flavazole, 128. The formation of the free carboxylic acid, 128 may be explained as the hydrolysis product of the ester 129 which was also obtained by a Wittig reaction of the 1-phenylflavazole-3-carboxaldehyde, 31.

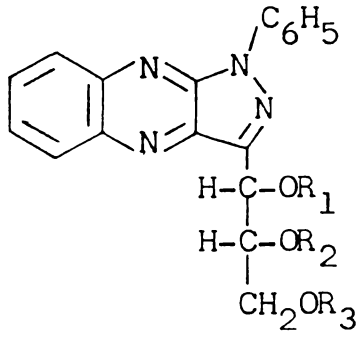


2.3 Reactions of flavazoles

A large number of the chemical reactions of flavazoles have been studied as summarised below.

2.3.1 Reactions involving the sugar residue at position 3

As most of the earlier work on flavazoles was carried out with flavazoles prepared from carbohydrates, they all had a sugar residue at position 3. The reactions of these sugar residues have been studied by a number of authors. Ohle and co-workers, who first synthesised this ring system, prepared a number of derivatives of the hydroxyl groups in order to prove the number and nature of the hydroxyl groups[3,4]. Thus the tri-O-acetyl derivative, 130 was obtained by acetylation of 1 with acetic anhydride and pyridine[3]. With triphenylmethyl-chloride in pyridine at 20°, 1 readily formed the mono-trityl ether, 131 at the primary carbon. Acetylation of 131 yielded the di-O-acetate, 132. Reaction of 1 with acetone gave a mixture of isopropylidene derivatives 133 and 134, 133 being the major product. Benzoylation of the mixture of isopropylidenes gave a mixture of 135 and 136 respectively. Alkali hydrolysis of 135 gave



1 , $R_1 = R_2 = R_3 = H$

130, $R_1 = R_2 = R_3 = Ac$

131, $R_1 = R_2 = H$, $R_3 = -C(C_6H_5)_3$

132, $R_1 = R_2 = Ac$, $R_3 = -C(C_6H_5)_3$

133, $R_1 = H$, $R_2, R_3 = \succ C(CH_3)_2$

134, $R_1, R_2 = \succ C(CH_3)_2$, $R_3 = H$

135, $R_1 = COC_6H_5$, $R_2, R_3 = \succ C(CH_3)_2$

136, $R_1, R_2 = \succ C(CH_3)_2$, $R_3 = COC_6H_5$

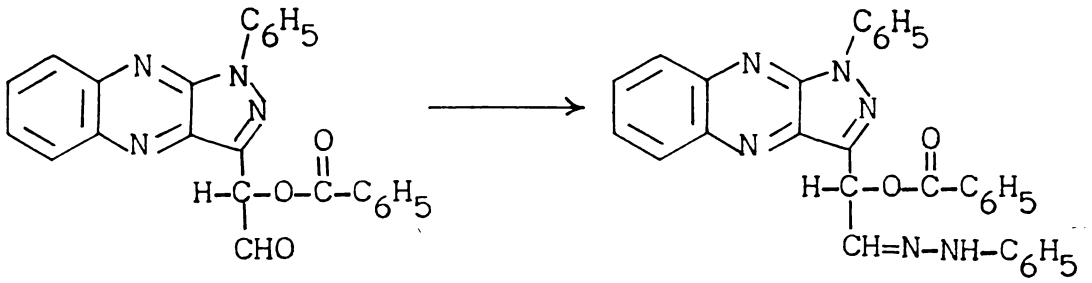
137, $R_1 = COC_6H_5$, $R_2 = R_3 = H$

138, $R_1 = R_2 = R_3 = COC_6H_5$

139, $R_1 = R_2 = H$, $R_3 = COC_6H_5$

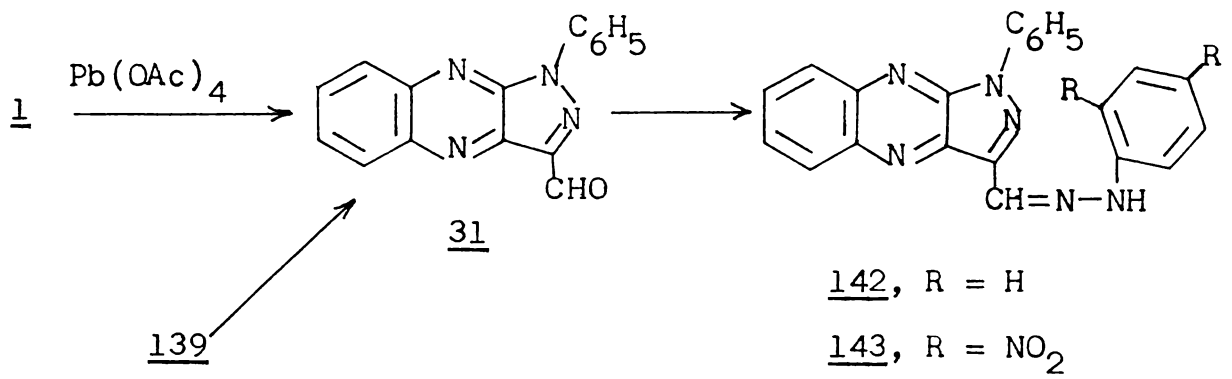
back 133, while a mild acid hydrolysis yielded the monobenzoate, 137. The tribenzoate, 138 was prepared by benzoylation of 1 with excess of benzoyl chloride in pyridine at 100°, while with one mole of benzoyl chloride in pyridine at 20° for 15 hours provided

the monobenzoate, 139. With acetone and a catalytic amount of sulphuric acid, 139 yielded the isopropylidene derivative, 136. Cleavage of 137 with lead tetraacetate in benzene yielded (1-phenyl-3-flavazolyl)-O-benzoyl-glyoxal, 140 which formed a phenylhydrazone, 141. Oxidation of 139 with lead tetraacetate in benzene gave



140 141

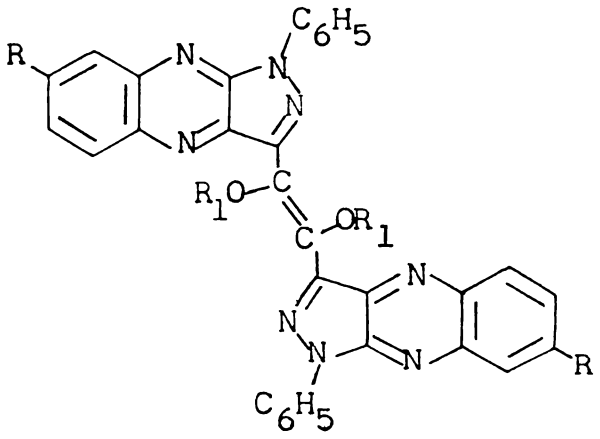
the 1-phenylflavazole-3-carboxaldehyde, 31 which was also obtained by the direct oxidation of 1 with lead tetraacetate. The phenylhydrazone, 142 and 2,4-dinitro-phenylhydrazone, 143 of 31 were also reported[4].

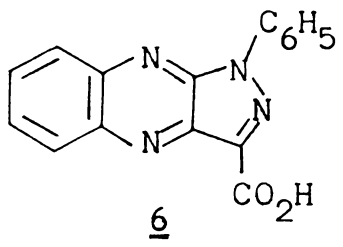


142, R = H

143, R = NO_2

The carboxaldehyde, 31 underwent an acyloin reaction when treated with aqueous potassium cyanide and the resulting solution boiled with methanol[44]. The product was identified to be 1,1'-diphenylflavazoin (144) and the mother liquor on evaporation gave 1-phenylflavazole-3-carboxylic acid (6) which was also obtained by the oxidation of 31 with chromium trioxide in acetic acid[51].





6

144, R = R_1 = H

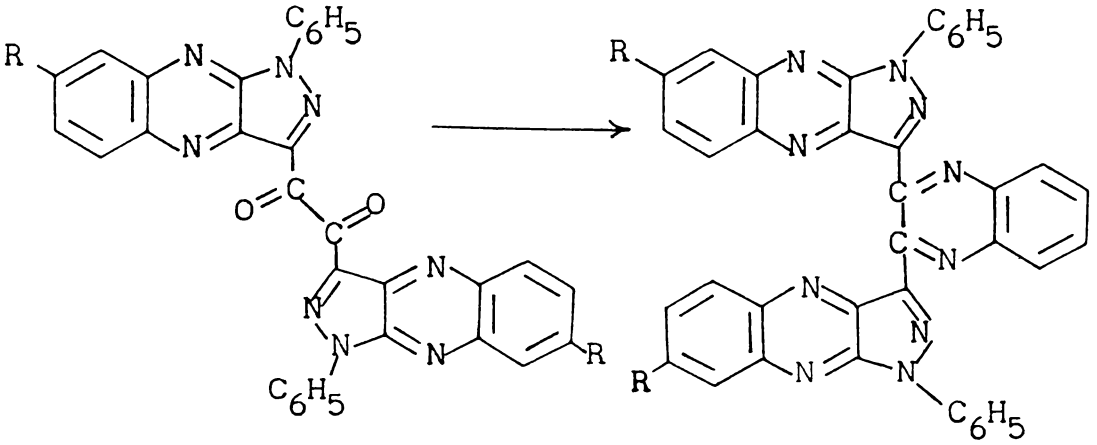
145, R = Cl, R_1 = H

146, R = Cl, R_1 = Ac

147, R = Cl, R_1 = C_6H_5CO

7-Chloro-1-phenylflavazole-3-carboxaldehyde was converted into the flavazoin 145, which was also characterised as its

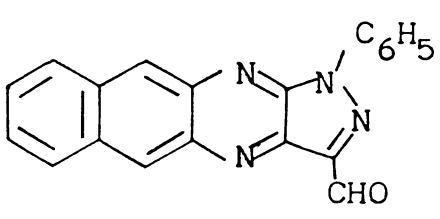
diacetate, 146 and dibenzoate[52], 147. The flavazoins were easily oxidised by air to the corresponding flavazils, 149 and 150. Treatment of the flavazil 149 with o-phenylene diamine provided 2,3-bis(1-phenyl-flavazol-3-yl)quinoxaline[52] (151).



149, R = H
150, R = Cl

151, R = H

Oxidation of the sugar residue in higher condensed flavazoles by lead tetraacetate or periodic acid provided the corresponding 3-formylflavazoles. Thus compounds 152[36,44] and 153[44] were obtained from the appropriate starting compounds.



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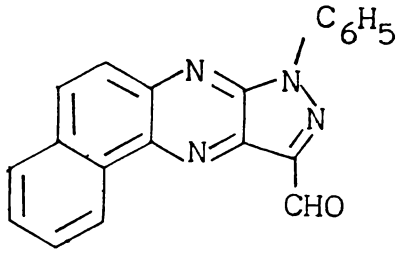
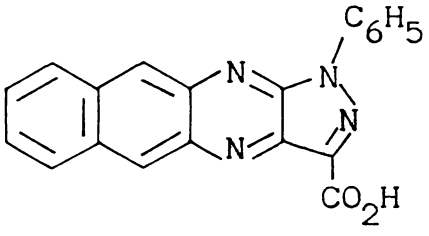


153

The aldehyde 152 has been further characterised as the carboxylic acid, 154 and the phenylhydrazone, 155.



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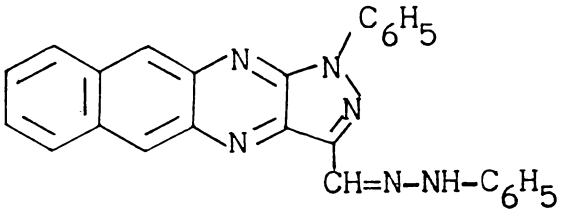
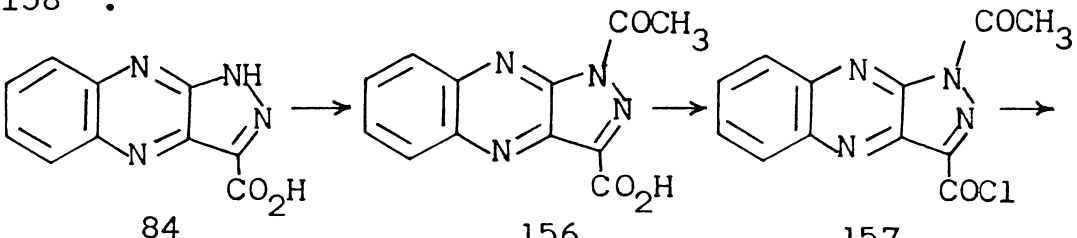
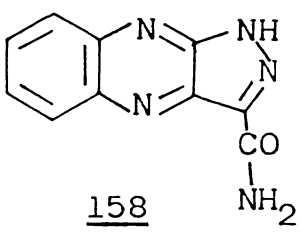


155

The oxidation of 3-(D-erythro-trihydroxypropyl) flavazole (81) with periodic acid to give flavazole-3-carboxaldehyde (82) and its further conversions into 3-hydroxymethylflavazole (83) and flavazole-3-carboxylic acid (84) have already been described[38]. Acetylation of 84 at position 1 to give 156 followed by treatment with thionyl chloride gave the acid chloride, 157 which when reacted with methanolic ammonia yielded the carboxamide, 158[38].

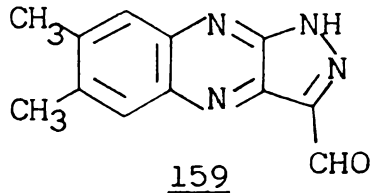


84 156 157



158

3-(D-Erythro-trihydroxypropyl)-6,7-dimethyl-flavazole (93) was also oxidised to the 3-formyl derivative, 159[41]. The conversion of 159 into the oxime, 160, followed by dehydration provided the 3-cyano-6,7-dimethylflavazole (161) which was hydrolysed to the carboxylic acid, 162. The following derivatives,



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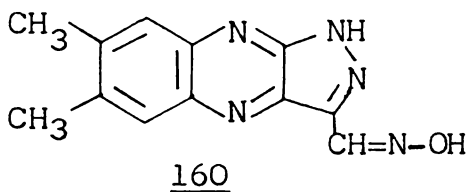

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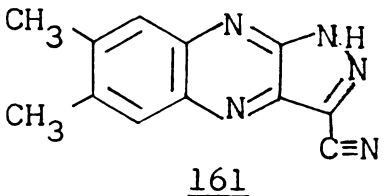

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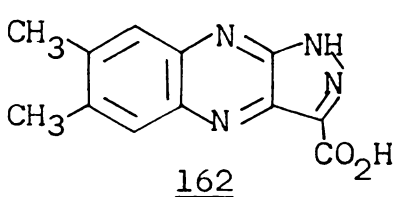
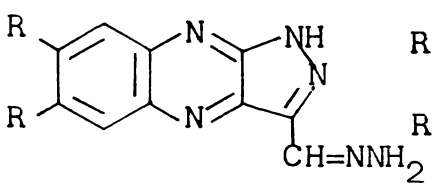
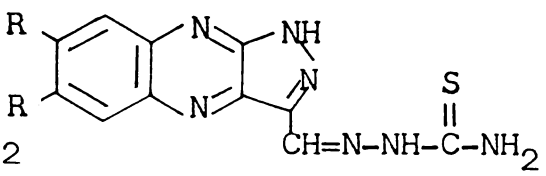


162

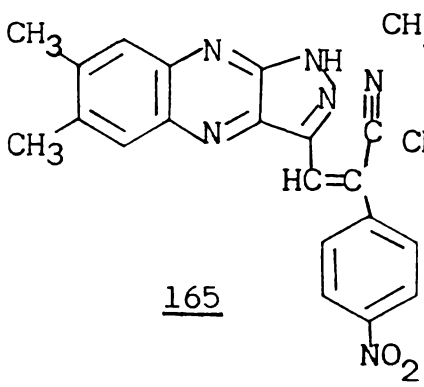
163-167 of 3-formylflavazole were also prepared in order to study their tuberculostatic activity[53].



163, R = H, CH_3



164, R = H, CH_3



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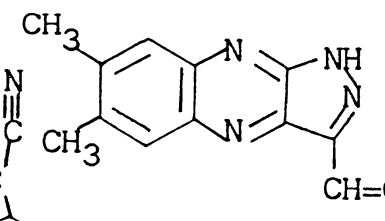

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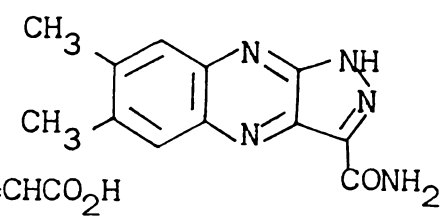
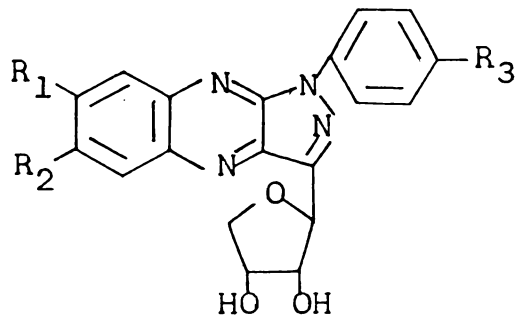


167

In an experiment involving the kinetics of hydrolysis of a glycoside linkage, hydrolysis of 1-phenylflavazole of maltose (28) showed a linear relation between log k and Hammett acidity function (Ho) suggesting unimolecular decomposition of the conjugate acid of 28 without participation of water and absence of intramolecular catalysis[54]. The kinetics of hydrolysis of the 1-phenylflavazole of cellobiose (29) also indicate hydrolysis by the same mechanism. There was no evidence to indicate reverse anomeric effect influencing the hydrolytic behaviour of 28.

2.3.2 Formation of C-nucleosides incorporating flavazoles

As C-nucleosides are gaining importance recently because of their pharmacological properties[55], attempts have been made to prepare new C-nucleosides incorporating flavazoles as the nitrogen heterocyclic system. The first such C-nucleoside 3-(β-D-*erythro*-furanosyl)-1-phenylflavazole (168) was prepared by Sallam[29] by the dehydration of the 1-phenyl-3-(D-*arabino*-tetrahydroxybutyl) flavazole (56).



168, $R_1 = R_2 = R_3 = H$

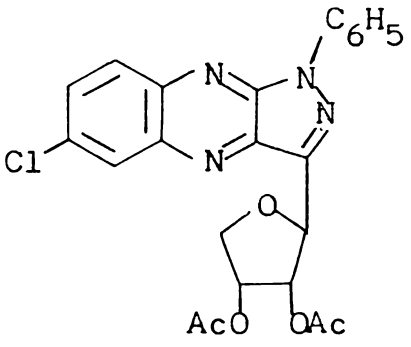
169, $R_1 = R_2 = H$, $R_3 = CH_3$

170, $R_1 = H$, $R_2 = Cl$, $R_3 = H$

171, $R_1 = R_2 = CH_3$, $R_3 = H$

172, $R_1 = R_2 = H$, $R_3 = F$

The structure of the compound and configuration at the anomeric carbon atom were elucidated by periodate oxidation, CD, NMR and mass spectrometry. Sallam, Whistler and Markley prepared similar C-nucleosides with p-tolyl group at position 1 (169) and chlorine at position 6 (170) starting from the appropriate flavazoles[30]. Also preparation of C-nucleosides with two methyl groups at the 6 and 7 positions (171) and the fluorophenyl group at position 1 (172) have been reported by Sallam[31]. The di-O-acetate, 173 and an isopropylidene derivative, 174 of the C-nucleoside, 170 have also been prepared[30].



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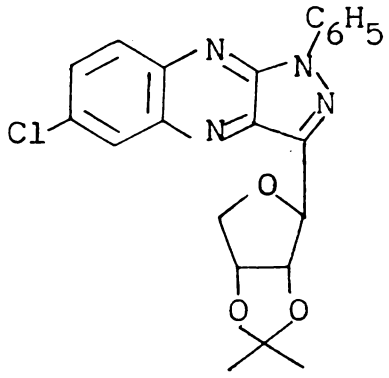
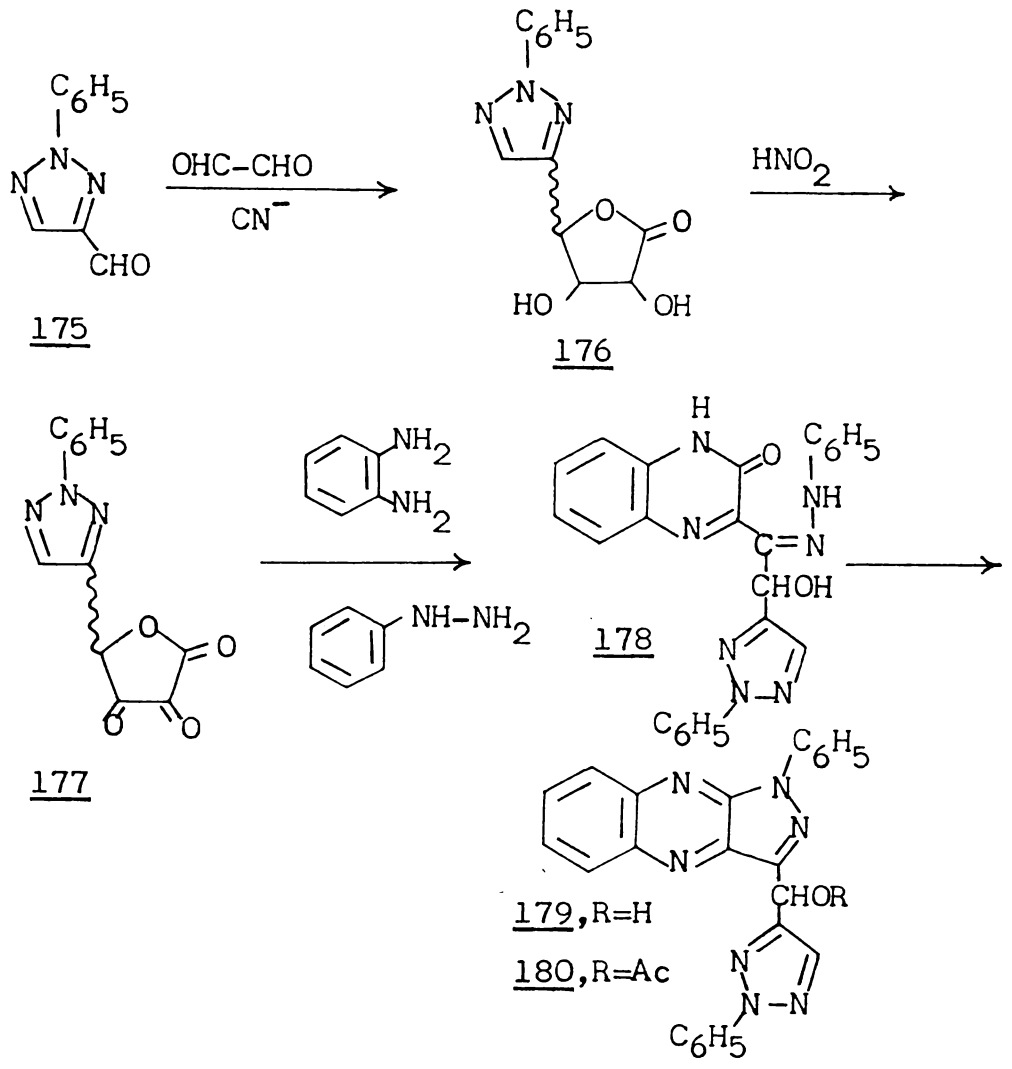


174

A triazolyl C-nucleoside analog of dehydro-L-ascorbic acid was prepared by El Ashry and co-workers and was converted into a flavazole derivative[56,57]. The reaction of 4-formyl-2-phenyl-1,2,3-triazole (175) with glyoxal in the presence of potassium cyanide gave the triazolylfuranone, 176 which with nitrous acid gave 177, a triazolyl C-nucleoside of dehydroascorbic acid. Treatment of 177 with o-phenylene diamine and phenylhydrazine gave the quinoxaline derivative, 178 which was converted into the flavazole, 179 in the usual way by treatment with alkali. The compound 179 was further characterised as the O-acetate, 180.

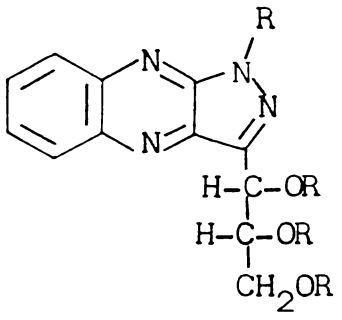


175 176 177 178 179 180

2.3.3 Substitution reactions

Flavazole ring system has been found to undergo substitution reactions at various positions. Thus in the case of a flavazole with position 1 unsubstituted, it undergoes acylation reactions very easily. Both acetylation and benzoylation of 3-(D-erythro-trihydroxypropyl) flavazole (81) under standard conditions, gave the tetraacylated products,

181 and 182 respectively[38]. Similarly, flavazole-3-carboxylic acid (83) undergoes acetylation with acetic



81, R = H

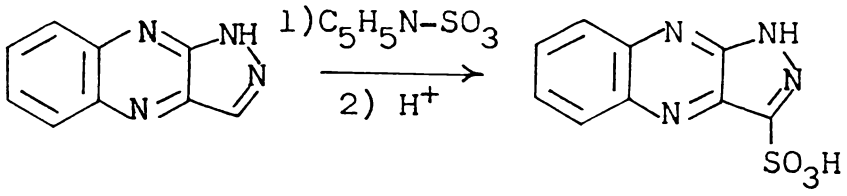
181, R = $COCH_3$

182, R = COC_6H_5

anhydride and pyridine to give the 1-acetylflavazole-3-carboxylic acid[38] (156). Deacetylation also takes place under very mild conditions at position 1 as shown by the fact that when 1-acetylflavazole-3-carbonylchloride (157) was treated with methanolic ammonia, flavazole-3-carboxamide (158) was obtained in good yields[38]. Flavazole 2 did not react with methyliodide even at 100° in sealed tubes in 20 hours or with diazomethane in ether at 20°[38].

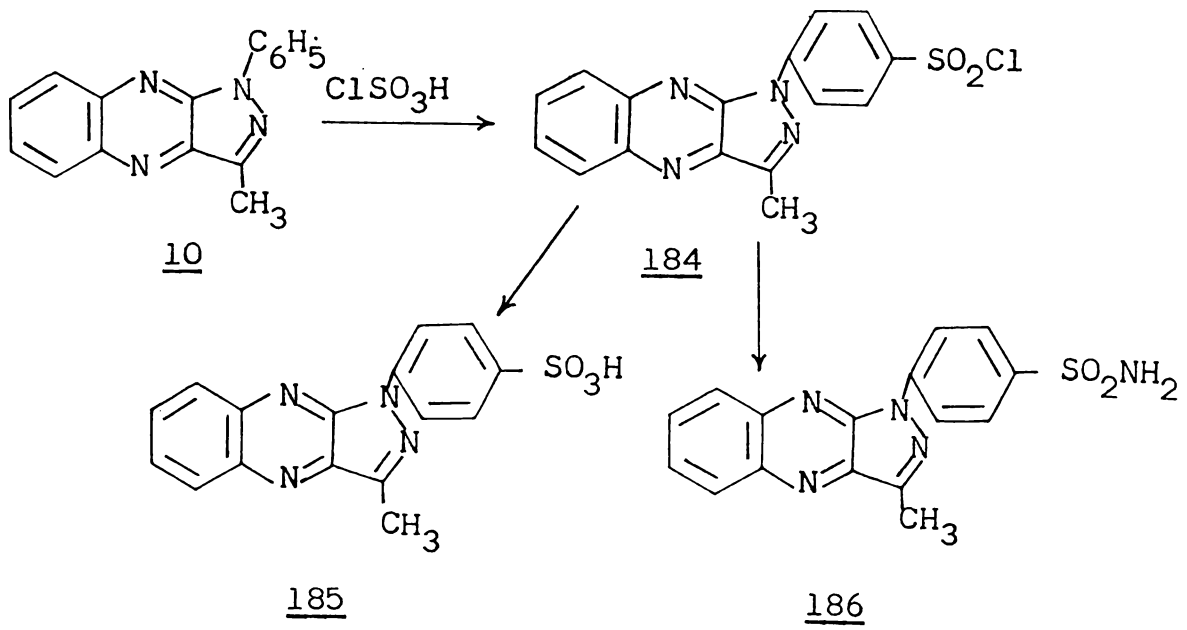
Sulphonation of flavazole 2 with pyridine-sulphur trioxide adduct at 170° followed by treatment of the pyridinium salt with sodium hydroxide and

subsequent liberation of the free acid by ion exchange gave flavazole-3-sulphonic acid[58](183). Treatment of



2 183

1-phenyl-3-methylflavazole (10) with chlorosulphonic acid in chloroform at 60° gave a single product, 184 which was converted into the free sulphonic acid, 185 and the sulphonamide[58], 186.



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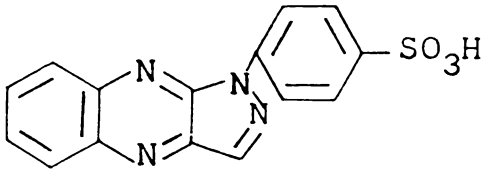
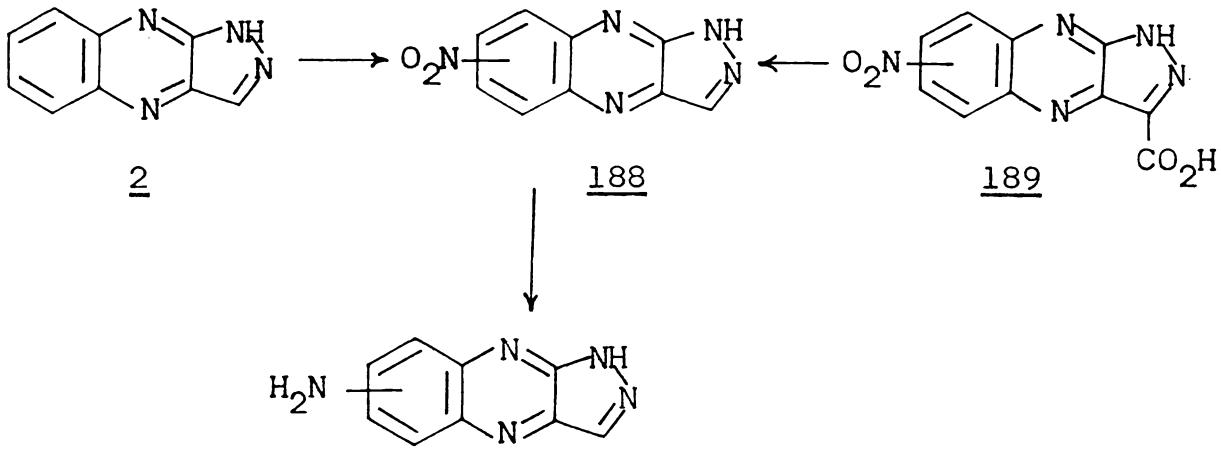
185 186

1-Phenylflavazole (9) was less reactive than 10 and therefore sulphonation took place only when boiled with chlorosulphonic acid without solvent. A mixture of products was obtained in which 187 was found to be the major isomer[58].



187

Nitration of flavazole (2) led to a 1:10 isomeric mixture of mononitro derivatives which were also obtained by the decarboxylation of nitrosubstituted 3-carboxylic acids[59], 189. Nmr spectra showed that the nitro group of the major component was located at position 6 or 7 while that of the minor component was located at position 5 or 8. The exact position of

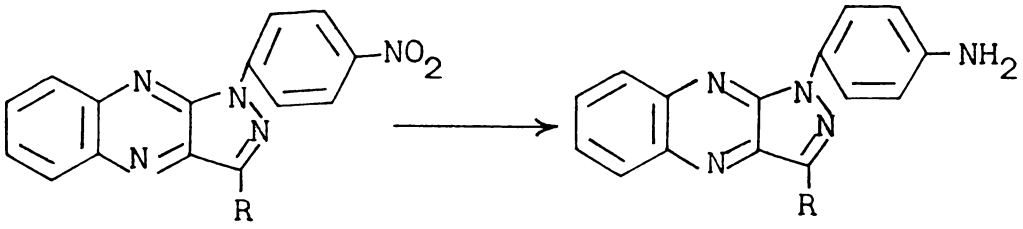


2 188 189

190

the nitro group was not determined[59]. Reduction of the nitro flavazoles produced the corresponding aminoflavazoles (190).

Nitration of 1-phenylflavazole (9) and 3-methyl-1-phenylflavazole (10) gave mixture of isomers with the p-nitro derivatives 191 and 192 predominating[60]. The p-amino derivatives 193 and 194 were obtained by reduction of 191 and 192



191, R = H

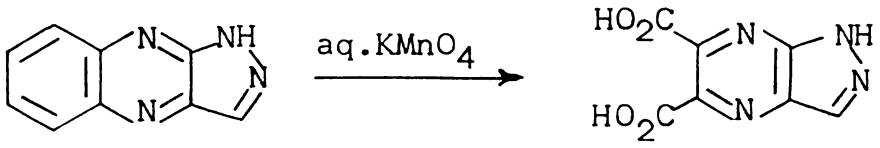
192, R = CH_3

193, R = H

194, R = CH_3

respectively. These amino compounds were characterised as their azo dyes and also as the p-acetylaminosulphonamides. Nitration of 1-phenylflavazoles with a sugar residue having free hydroxyl groups at position 3 proceeded with two side reactions: oxidation and esterification of the sugar residue[60].

Oxidation of flavazole (2) with aqueous potassium permanganate gave pyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid[39](195). No further reactions of 195 were reported.

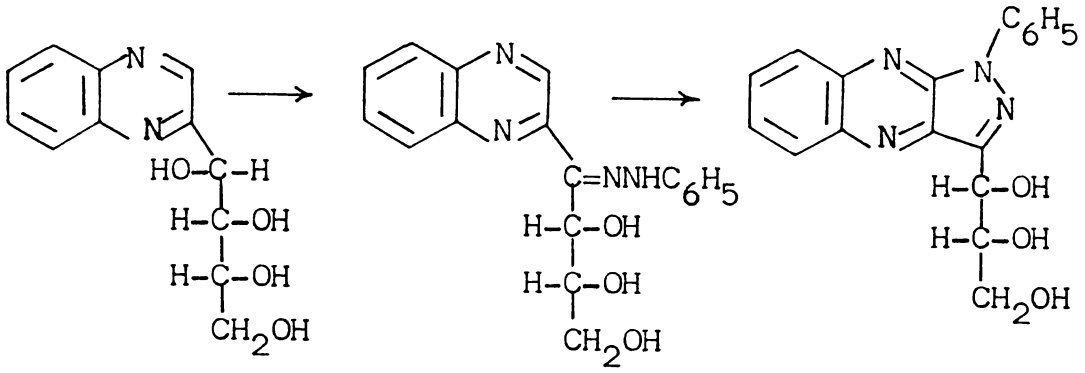


2 195

2.4 Mechanism of formation of flavazoles

Formation of 1-phenylflavazoles by the reaction of phenylhydrazines on a 2-tetrahydroxybutyl quinoxaline derivative, 3 may be considered to take place in two steps. The first step is the conversion of 3 to the phenylhydrazone, 5. In this reaction, C-1 of the side chain reacts with the hydrazine in a way resembling the reactions that occur during osazone formation[1,61]. Ohle and Heilscher[3] has provided some experimental evidence for this concept by the isolation of aniline (11%) and ammonia (18 %) in the reaction of 3 with

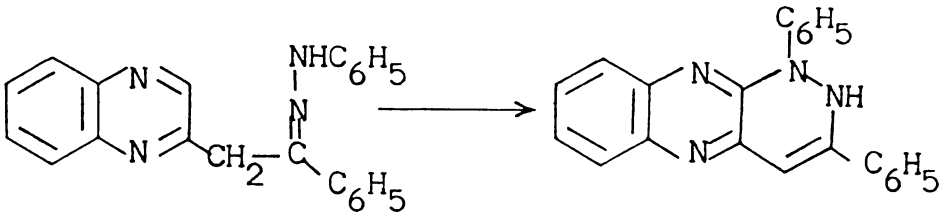
5 mole equivalents of phenylhydrazine in neutral medium producing 9% of 5. The second step is an oxidative cyclisation of 5 to the flavazole, 1. The most common



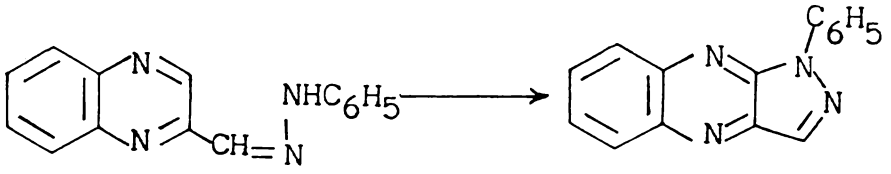
3 5 1

oxidising agent used is phenylhydrazine itself although other mild oxidising/dehydrogenating agents such as copper sulphate, hydrazine and copper powder in acetic acid have also been successfully employed.

Dahn and Fumeaux have studied the cyclisations of 2-phenacylquinoxaline phenylhydrazone (196) and quinoxaline-2-carboxaldehyde phenylhydrazone (10) to 1,3-diphenyl-2H-pyridazino[3,4-b]quinoxaline (197) and 1-phenylflavazole (9) respectively in hot hydrochloric



196 197



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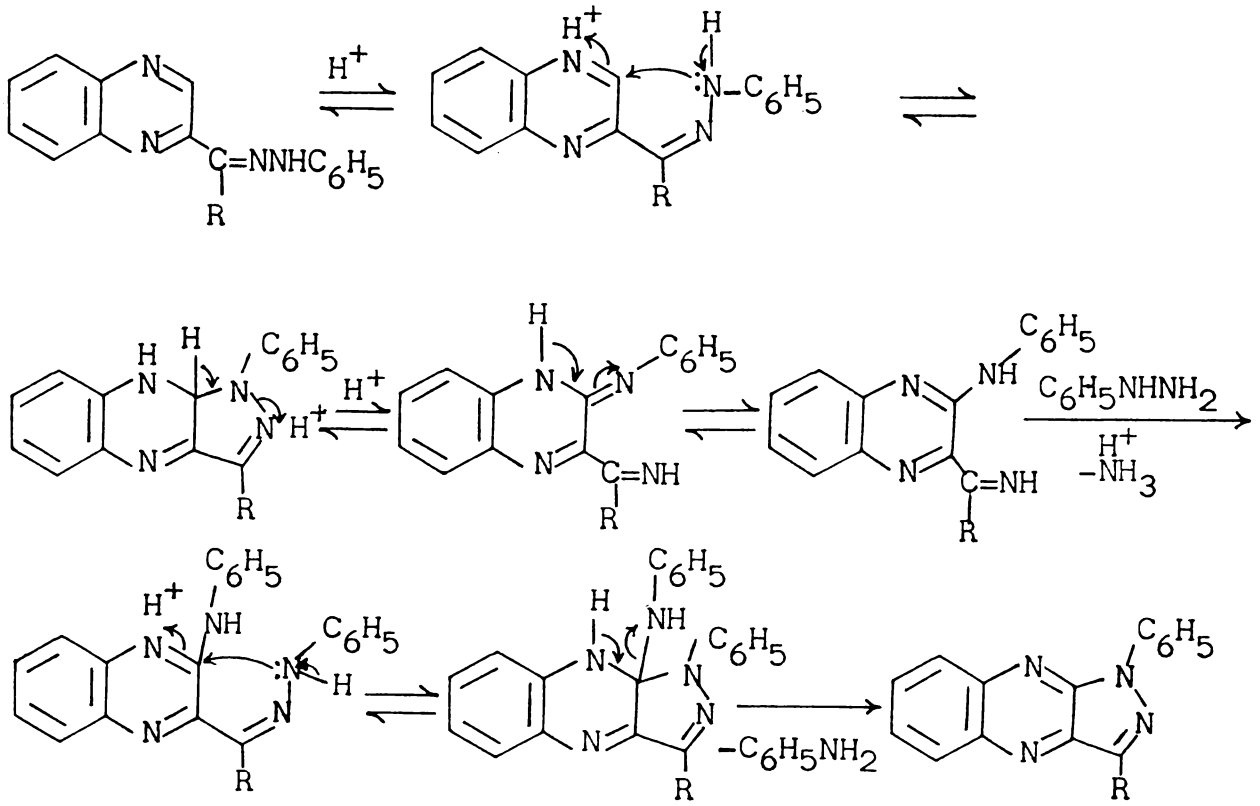
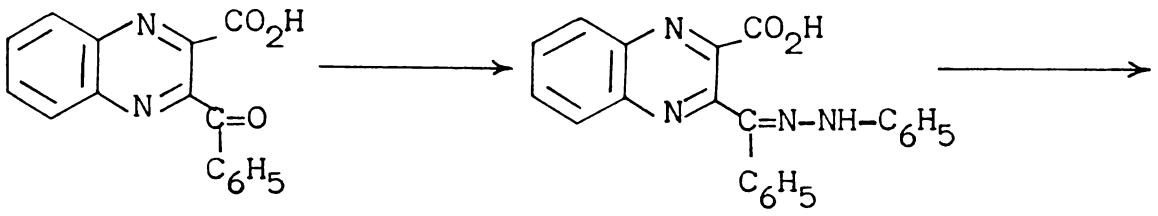
acid in the presence of excess phenylhydrazine[62]. They have proposed a mechanism for this cyclisation similar to the formation of osazones[62-65].



R = H, alkyl, aryl or hydroxyalkyl

A similar mechanism was also suggested[62] for the formation of 197 from 196. The formation of 1,3-diphenylflavazole (200) when 3-benzoylquinoxaline-2-carboxylic acid (198) and its amide, 201 were heated with phenylhydrazine shows that these groups at position 2 can also act as leaving groups[64] as in the case of the benzidine rearrangements[66]. The phenylhydrazones, 199 and 202 are intermediates in these reactions.



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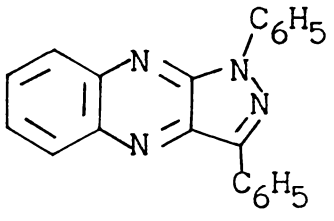
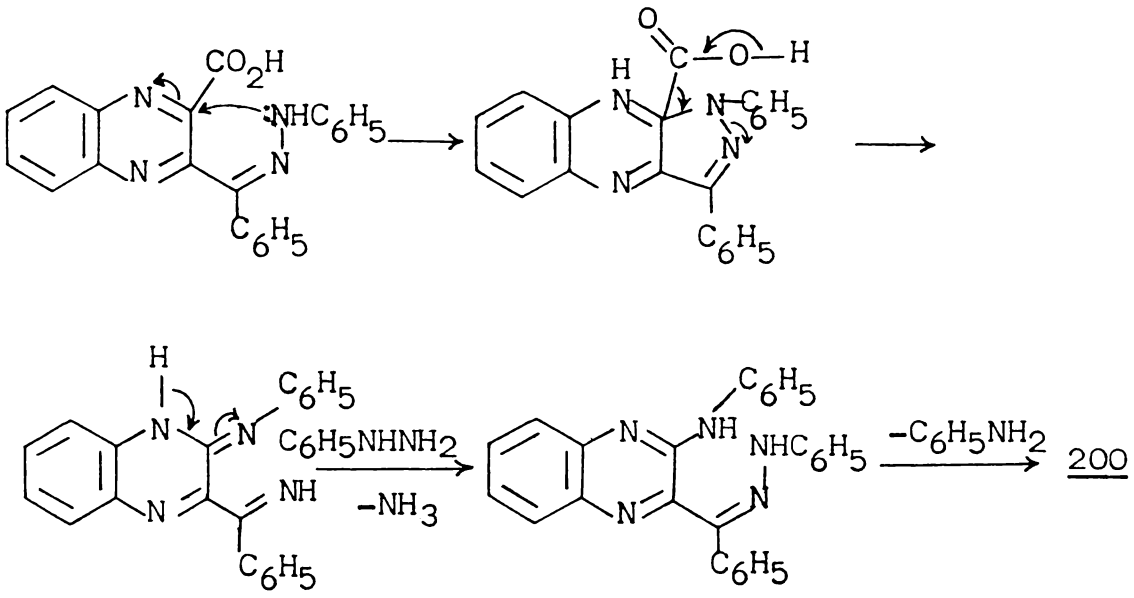
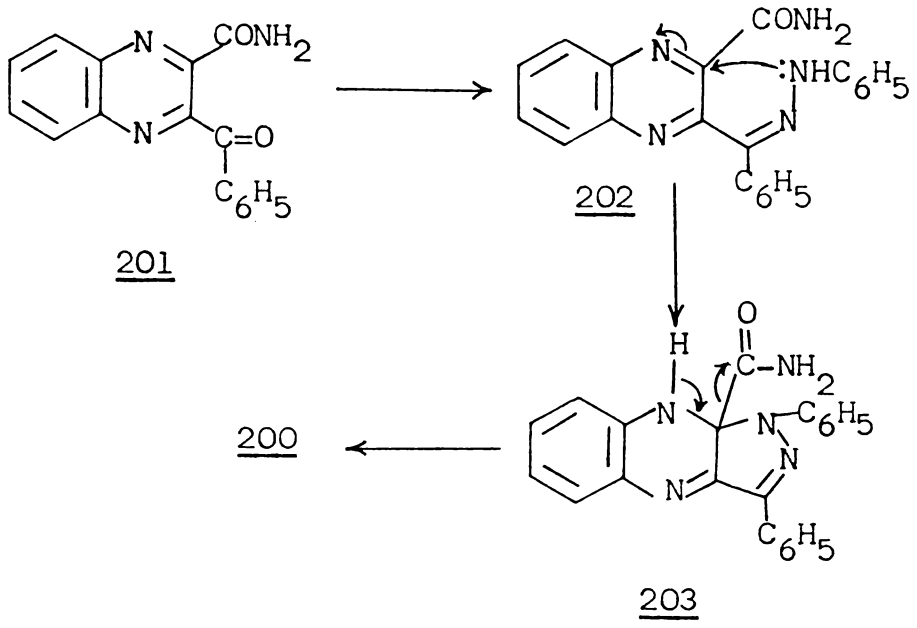


200

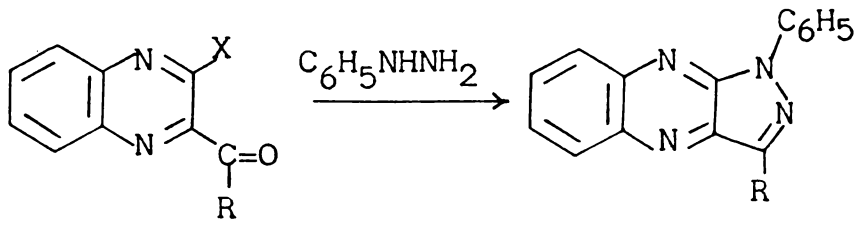
The carboxylic acid group may be easily eliminated as carbon dioxide during cyclisation as shown below:



A different mechanism was proposed by Dahn and Moll for the formation of a flavazole ring with the loss of a carboxamide group at position 2 of the quinoxaline ring[64].

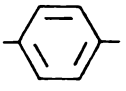


In a more systematic study, Dahn and Nussbaum showed that H, OH, Cl, CN, CO_2H, $CONH_2$, $-CH_2C_6H_5$ and $-COC_6H_5$ act as leaving groups for the formation of flavazoles when a quinoxaline substituted with these groups at position 2 and a benzoyl or a p-methoxybenzoyl group substituted at position 3 were treated with phenylhydrazine[65].



204 205

X = H, OH, Cl, CN, CO_2H, $CONH_2$, $-CH_2C_6H_5$, COC_6H_5

R = C_6H_5 or CH_3O- 

Flavazoles were not formed when X = CH_3 or C_6H_5 under the same experimental conditions. It is possible that groups such as OH, Cl and CN depart as anions as in the case of the amide (see 203), whereas, $-CH_2C_6H_5$ and $-COC_6H_5$ may be eliminated as cations as in the case of hydrogen and carboxylic acid lost as

carbon dioxide. However no experimental evidence can be cited in support of this view.

The fact that 3-acylquinoxalines substituted with CH_3 and C_6H_5 at position 2 do not yield flavazoles when treated with phenylhydrazine may be because they are not good leaving groups either as anions or as cations.

2.5 Physical methods of characterisation

Flavazoles are generally bright yellow crystalline compounds with definite melting points. They often show an intense green fluorescence especially in non-polar solvents[24,25]. Flavazoles may be identified by their melting points and powder X-ray diffraction patterns[5,6]. Among spectrometric methods, nuclear magnetic resonance has been extensively used for their characterisation, especially to understand the substitution pattern of the aromatic system[58,59] and to determine the nature and configurations of the carbohydrate part[22,30].

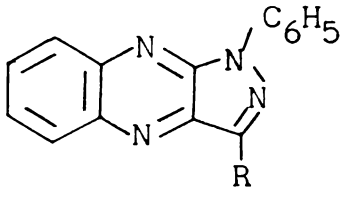
Mass spectral analysis of flavazoles have also been useful in their structural determination[30,49]. A detailed study of the mass spectra of the 1-phenylflavazole

derivatives of monosaccharides showed structurally characteristic and easily interpretable fragmentation patterns[67]. Also the position of the deoxy or methoxy grouping on the sugar residue may be conveniently determined using mass spectrometry[67].

The ultraviolet absorption spectra of flavazole derivatives are quite characteristic. The $\lambda_{max}^{i\text{-}PrOH}$ of glucose 1-phenylflavazole (1) were found to be at 276 nm (ϵ 3.98 x 10^4), 355 nm (ϵ 1.01 x 10^4) and 410 nm (ϵ 3.7 x 10^3). The peak at 410 nm obeyed Beer's law and is useful for the colorimetric or spectrophotometric determination of the flavazole[68]. In water, this absorption was shifted to 405 nm. Maltose 1-phenylflavazole (28) and lactose 1-phenylflavazole (30) also had $\lambda_{max}^{i\text{-}PrOH}$ at 410 nm (ϵ 3.7 x 10^3)[68]. The uv spectrum of the 1-phenylflavazole of amylose showed λ_{max}^{DMSO} at 323 nm, 336 nm and 407 nm[34].

The optical rotation of sugar flavazoles depend on the nature of the carbohydrate residue at position 3 (See Table II) although there is no direct correlation between the optical rotation of the flavazole

and absolute configurations of the sugars. The circular dichroism of a number of 3-polyhydroxyalkyl-1-phenyl-flavazoles (206) was studied by Sallam[69]. In dioxane



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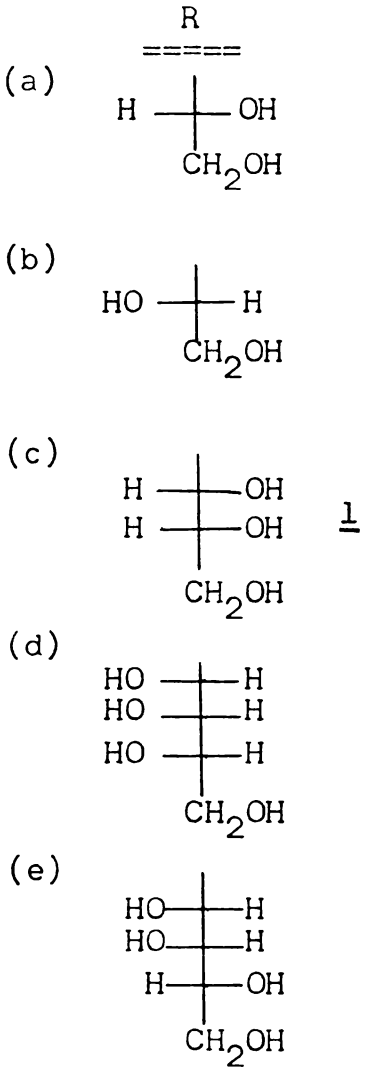
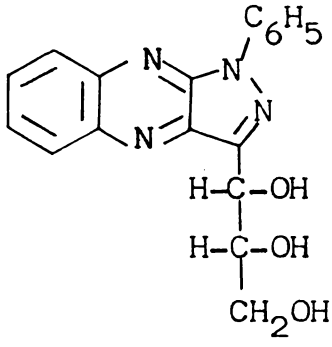
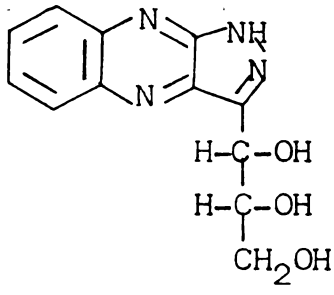


solutions they all showed multiple Cotton effects and a direct correlation was observed between the sign of the Cotton effect at the long wavelength absorption

(442 - 450 nm) and the absolute configuration of C-1'. Thus compounds which have R chirality at C-1' (C-4 of the original sugar) in the Fischer projection formula showed positive Cotton effect. Centres of asymmetry other than at C-1' affected only on the intensity of the band[69]. Chilton and Krahn have also reported that configuration at C-4 of a sugar (C-1' of the flavazole) may be studied from optical rotatory dispersion curves of their 1-phenylflavazole or flavazole derivatives[70]. Thus in the case of glucose, compounds 1 and 81 exhibit negative Cotton effects centred at 410 and 390 nm respectively.



1



81

Preparation and paper chromatographic separation of the 1-phenylflavazole derivatives of glucose and a few other di-and oligosaccharides were reported by Kobayashi

and co-workers[71]. A solvent system consisting of butanol:ethanol:water:ammonia::40:10:49:1 (upper layer) gave the best results for separation by paper chromatography. The R_f values of the 1-phenylflavazole derivatives of the different sugars were as follows: glucose 0.89, isomaltose 0.77, maltose 0.73, isomaltotriose 0.56, a tetraose isolated from dextrin hydrolysate 0.44 and a pentose isolated from the same hydrolysate 0.34.

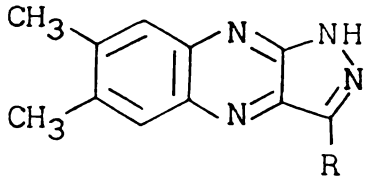
2.6 Biological studies

Biological studies on flavazoles have been rather limited. May be because of the limited solubility of these compounds, their testing has been difficult. However, more soluble derivatives have been prepared recently by increasing the number of hydroxyl groups on the heterocyclic system[42], and by preparing polyhydrogen sulphate salts on the hydroxyl groups of the sugar residue[72,73].

One of the first biological screening experiment for which a flavazole was submitted was the study of the inhibition of multiplication of Staphylococcus

phage by a number of compounds including the 1-phenyl-flavazole of glucose, 1. These studies were carried out with mass lysis and one step growth curve techniques. The growth rate of Staphylococcus aureus was inhibited 5% to 100% when added to growth cultures at concentrations necessary to double lysis time[74]. Similarly flavazole 1 was found to be active against Clostridium septicum infections in mice[75].

Buu-Hoi and co-workers[41] studied the antibacterial, antiinflammatory and analgesic properties of flavazoles, 159 to 162. Also the tuberculostatic

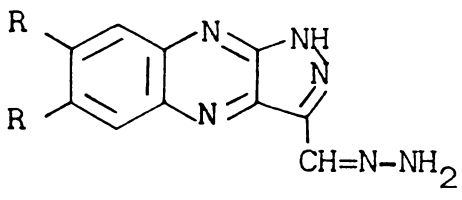


159, R = CHO 160, R = CH=N-OH

161, R = CN 162, R = CO_2H

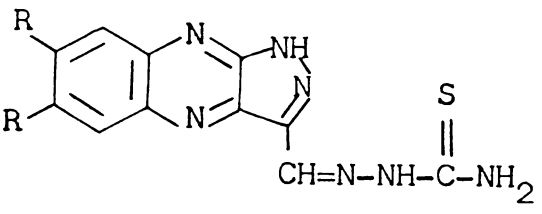
activity of the hydrazone, 163 and semicarbazone, 164 were reported by the same authors[53]. They showed *in vitro* activity as tuberculostatics at 10 to 70 mg/kg

in Middlebrook media. As other related heterocyclic systems did not show any activity, it was suggested that the tuberculostatic activity is intrinsic to the flavazole system[53].



163

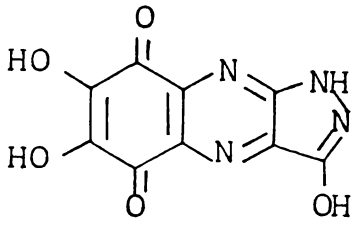
R = H or CH_3



164

R = H or CH_3

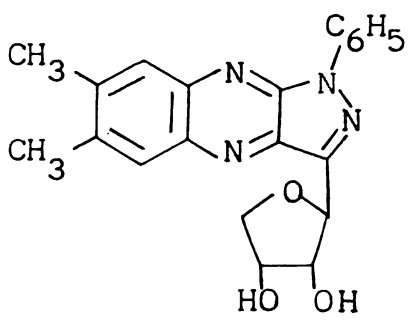
The biological activity of 3,6,7-trihydroxy-flavazole-5,8-dione (96) and its Na, K, Li, ammonium, methylammonium, ethylammonium, n-hexylammonium and benzylammonium salts was reported by Wendt and Ludig in a U.S.Patent[42]. Compound 96 when given 10 mg/kg showed diuretic activity and at 50 mg/kg caused 49% inhibition of edema.



96

Nair and Bernstein prepared the polytrimethylammonium sulphate polysalts of the 1-phenylflavazoles obtained from cellobiose, 29 and maltotriose, 35. While the salt of cellobiose showed *in vitro* complement inhibiting activity[73], that of maltotriose exhibited *in vivo* (guinea pigs) and *in vitro* complement inhibiting activity[72].

Sallam and co-workers[30,31] have shown that C-nucleoside 172 exhibited *in vitro* cytotoxic activity against K_B cells (a human epidermoid carcinoma of the nasopherynx) whereas 171 was inactive. Also cyclisation of the polyhydroxy chain in 58 and 59 to give the C-nucleosides 171 and 172 increased the antileukaemic activity.



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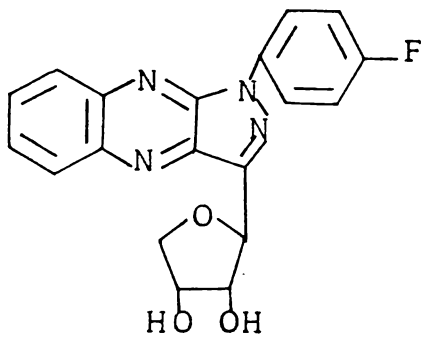


172

A number of immunological studies are being carried out using flavazoles. Thus 1-phenylflavazole of isomaltohexose coupled to chicken gamma globullin induced T-cell dependent anti-α(1 → 6) dextran specific IgM and IgG responses in CBA, BALB/C and A strain mice[76]. The IgG responses were of restricted heterogeneity and belonged mostly to the IgG1 subclass with a minor IgG3 component in the case of BALB/C and CBA mice. All 4 subclasses of IgG were produced in A strain mice[76].

The 1-(m-nitrophenyl) flavazoles prepared from oligosaccharides of isomaltose, maltose and cellobiose series by Teichmann and co-workers[26] were used as model compounds for immunochemical studies. Their m-aminophenyl derivatives had similar properties as the nitro derivative and were useful for the manufacture of immunogen and antigen models and immunoadsorbents with oligosaccharide specific determinant group[77].

Immunogens with oligosaccharide determinant groups were prepared by azo coupling of the 1-(m-aminophenyl) flavazoles (prepared from oligosaccharides) to

proteins[78]. It was seen that unsubstituted hydroxyl groups on positions 2 and 3 adjacent to the reducing end of the sugar were required and the method appeared especially suited for oligosaccharides having a polymerisation degree of 3 to 8. Oligosaccharides-flavazole-azo-edestin conjugates were tested for immunogeneity in rabbits and specific antioligosaccharide antibodies were formed in all cases. High titers of dextran specific antibodies were obtained upon immunisation with an isomaltoheptose-flavazole-azo-edestin conjugate[78].

The 1-(m-nitrophenyl) flavazoles of the isomaltose oligosaccharides were used to study their interaction with human antidextran by the quantitative hepten inhibition and fluorescence quenching techniques[79]. It was found that the m-nitrophenylflavazole heptens inhibited in the dextran-antidextran system in whole serum in the same order as did the isomaltose oligosaccharides. Thus the m-nitrophenylflavazole of isomaltoheptose was the best inhibitor and the inhibitory potency decreased progressively to the m-nitrophenyl-flavazole of isomaltotetraose[79].

The preparation of immuno adsorbents based on cellulose derivatives with the specificities of flavazoles of sugars of the maltose and isomaltose series was reported by Teichmann[80]. Cellulose-m-aminobenzyloxymethylether and p-aminobenzylcellulose were diazotised and coupled with 1-(m-hydroxylphenyl) flavazole and 1-(m-aminophenyl) flavazole of sugars of the maltose and isomaltose series to obtain sugar specific immuno adsorbents for isolation of anti-oligosaccharide antibodies from antiserums[80].

CHAPTER III

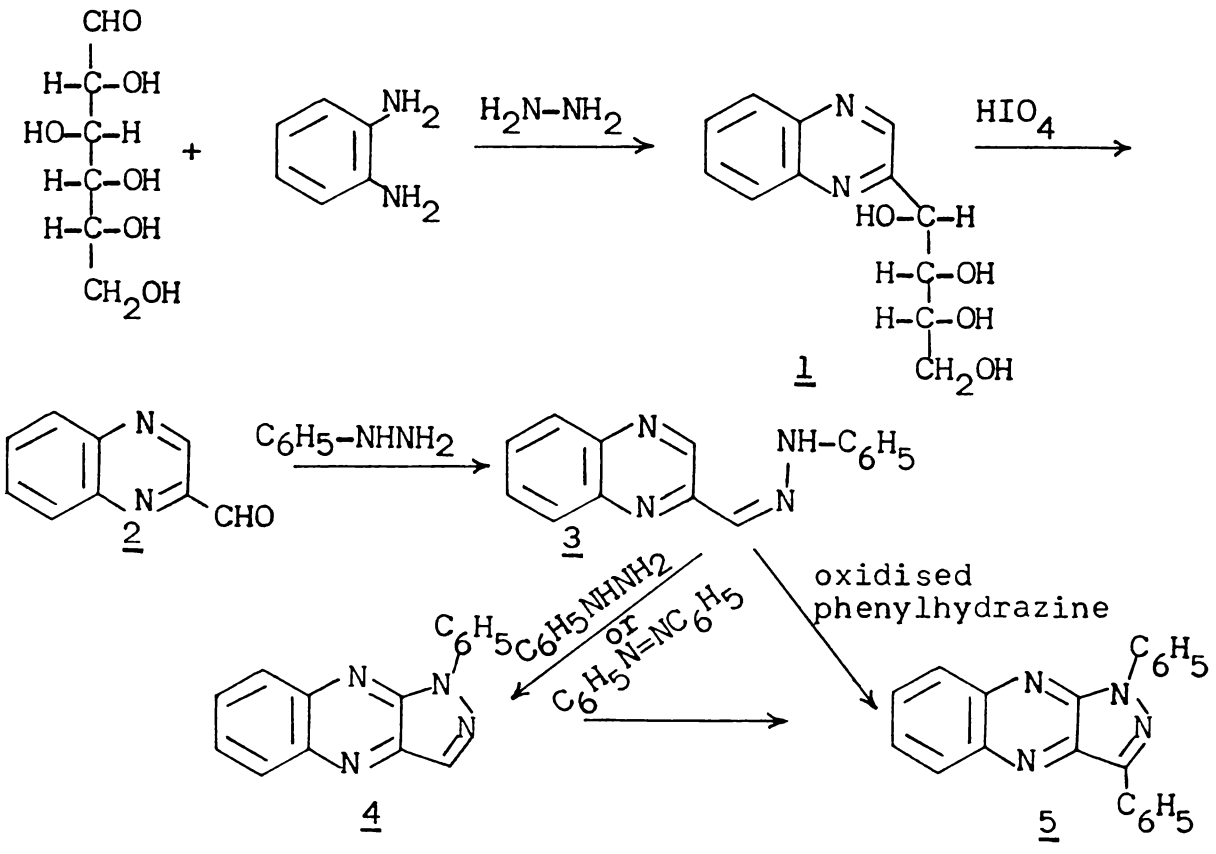
DISCUSSION OF EXPERIMENTAL RESULTS

3.1 Phenylation reactions of 1-phenylflavazole

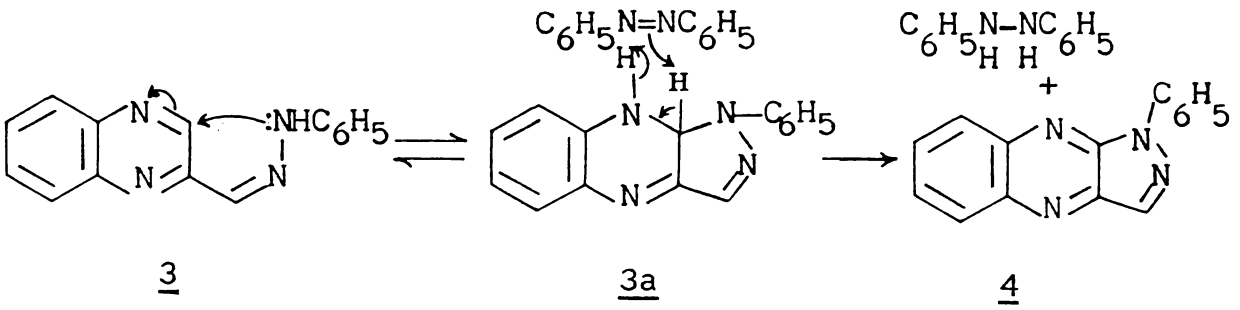
The preparation of 1-phenylflavazole (4) by the oxidative cyclisation of quinoxaline-2-carboxaldehyde phenylhydrazone (3) using phenylhydrazine as the condensing agent in the presence of acetic acid has been reported previously[9,62]. This reaction can also be brought about in one step by the treatment of excess of phenylhydrazine with the carboxaldehyde, 2 in the presence of acid[9]. However, in both these reactions, the use of freshly distilled phenylhydrazine was recommended[9], as otherwise the yield of the product, 4 was found to be very low. We thought it worthwhile to look into the cause of the low yield of the reaction and the results of our investigation are discussed below.

Treatment of D-glucose with o-phenylene diamine in the presence of hydrazine hydrate and acetic acid on a boiling water bath under a carbon dioxide atmosphere provided by the addition of a pinch of sodium bicarbonate, gave 2-(D-*arabino*-tetrahydroxybutyl) quinoxaline[3] (1) in 34% yield. Conversion of 1 into the carboxaldehyde, 2 was carried

out in 63% yield by the oxidation of 1 with sodium metaperiodate in water in the presence of acetic acid at room temperature[81]. The product was isolated by extraction with ether and purified by recrystallisation from petroleum ether. The phenylhydrazone, 3 was prepared in 81% yield from 2 by treatment of 2 with freshly distilled phenylhydrazine in methanol at room temperature for one hour[9,82]. Quinoxaline-2-carboxaldehyde phenylhydrazone (3) was converted into 1-phenylflavazole (4) in 71% yield by heating 3 with freshly distilled phenylhydrazine in the presence of acetic acid and hydrochloric acid in 60% aqueous n-propanol for 24 hours as reported previously by Henseke and Lemke[36].



Although the conversion of 3 to 4 has been explained to take place by a mechanism similar to that of the osazone formation[62] (see page 56), it is in effect an oxidation involving the removal of two hydrogen atoms. It was therefore considered that other mild oxidising agents must be capable of bringing about this conversion. Azobenzene has been reported to act as a dehydrogenating agent in many reactions[83,84]. When azobenzene was used in this reaction, the phenylhydrazone, 3 was converted into 1-phenylflavazole (4) in 93% yield. The reaction conditions involved heating an equivalent amount of azobenzene with 3 in aqueous n-propanol in the presence of acetic acid and hydrochloric acid. The method was also used for the cyclisation of other similar phenylhydrazones to flavazoles (see later). This observation suggests that a mechanism similar to that of the osazone formation is not always necessary for the conversion of 3 to 4, but the cyclic tautomer, 3a formed from 3 may be oxidised by azobenzene as shown below:

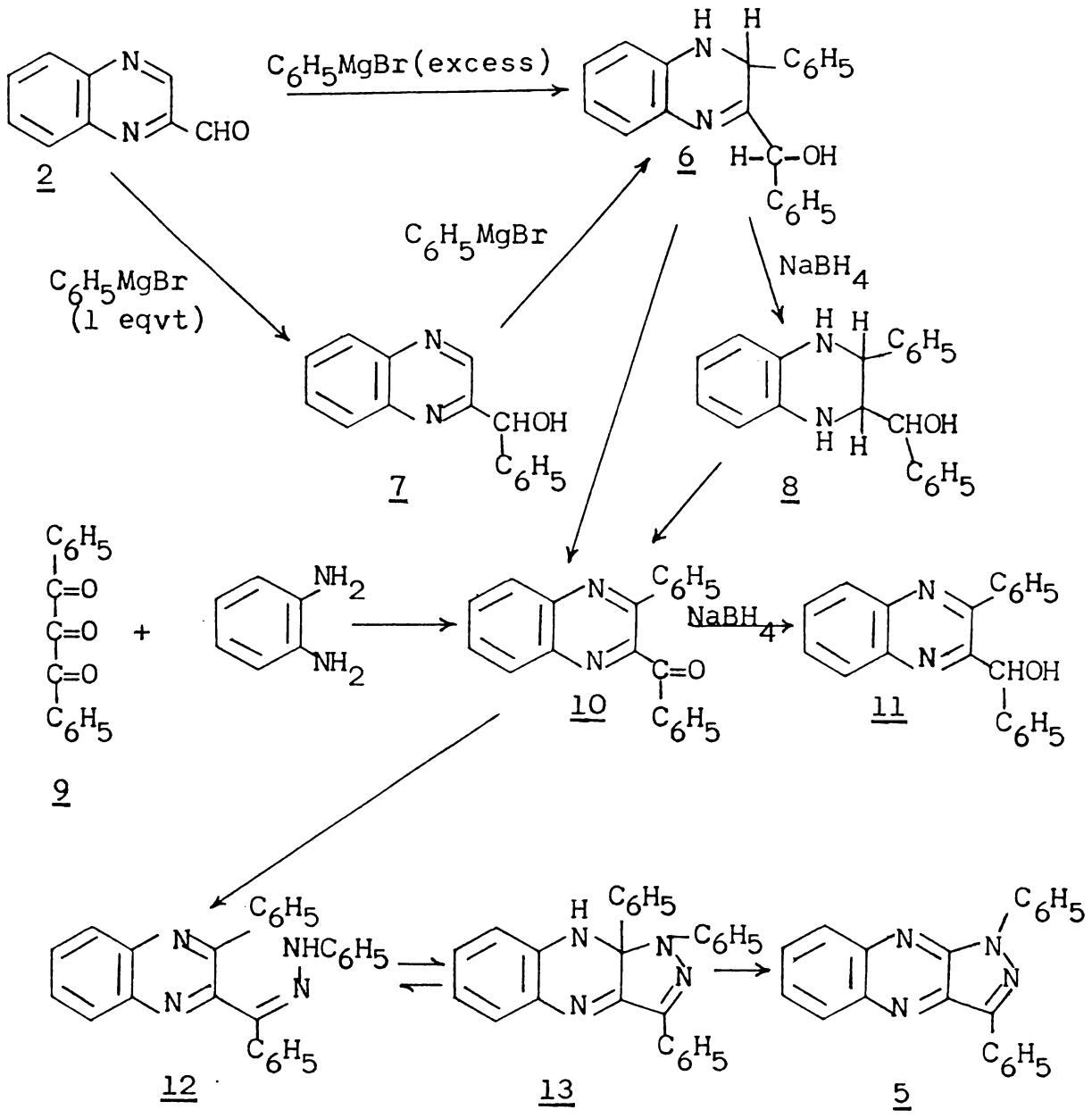


3 3a 4

Another new method by which the oxidative cyclisation of 3 to 4 was brought about, was by heating 3 to a temperature above its melting point in an open atmosphere. Here the oxygen in the atmosphere apparently acts as the oxidising agent. As no attempt was made to refine the conditions of this method to get the maximum yield and as a column chromatography was required to purify the product, the yield of the reaction was only 37%. However as no other reagents or solvents are required for this conversion, this method also is worth further investigation.

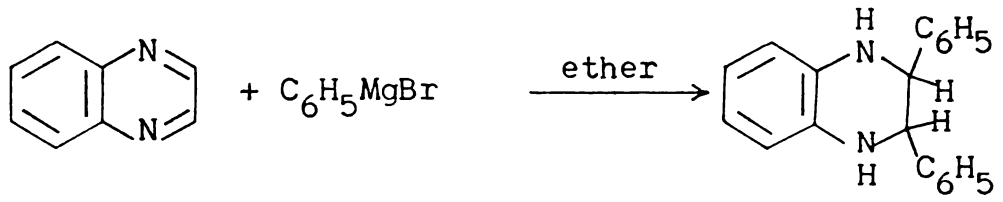
When the cyclisation of 3 to 4 was repeated using oxidised phenylhydrazine (phenylhydrazine through which a slow stream of air was bubbled for two weeks) in the presence of acetic acid instead of freshly distilled phenylhydrazine, a product mixture containing two components was formed. Chromatographic separation of this mixture using a column of silica gel gave only 49% of the expected 1-phenylflavazole (4). A second component was isolated in 16% yield which was subsequently shown to be 1,3-diphenylflavazole[40,64,65] (5) by elemental analysis, mass spectrum and melting point.

In order to prove the structure of 5 unequivocally, it was considered necessary to synthesise it by an independent method such as starting from 2-benzoylquinoxaline[64] (22). The preparation of 2-benzoylquinoxaline (22) itself was proposed as follows: addition of phenylmagnesium bromide to the readily available quinoxaline carboxaldehyde, 2 to yield α-(2-quinoxalinyl)benzyl alcohol (7) followed by mild oxidation must provide the required 2-benzoyl-quinoxaline (22). However, when a solution of quinoxaline-2-carboxaldehyde (2) in ether was added to a solution of phenylmagnesium bromide prepared from bromobenzene and magnesium in ether, the product obtained was not the expected α-(2-quinoxalinyl) benzyl alcohol (7) but α-(2-phenyl-1,2-dihydro-3-quinoxalinyl)benzyl alcohol (6). The structure of 6 was established by its spectral data and elemental analysis. The mass spectrum of 6 shows a weak molecular ion peak at m/e 314, a strong peak at m/e 313 ($M^+ - H$), the base peak at m/e 312 ($M^+ - 2H$) and other strong peaks at m/e 283 (313 - CHOH), m/e 235 (312 - C_6H_5), m/e 207 ($M^+ - C_6H_5CHOH$), etc.



The IR spectrum showed peaks at 3350 cm^{-1} (OH and NH), 1650 cm^{-1} (C=N) and the nmr spectrum was consistent with the structure.

The formation of 6 from 2 may be visualised as the result of the addition of two molecules of phenylmagnesium bromide, one across the 1,2 C=N and the other on the aldehyde group. It may be noted here that Grignard reagents are known to add across the C=N of the quinoxaline molecule[85]. Thus addition of phenylmagnesium bromide to quinoxaline (14) has been reported to give 2,3-diphenyl-1,2,3,4 tetrahydroquinoxaline[85] (15) in 70% yield. The addition



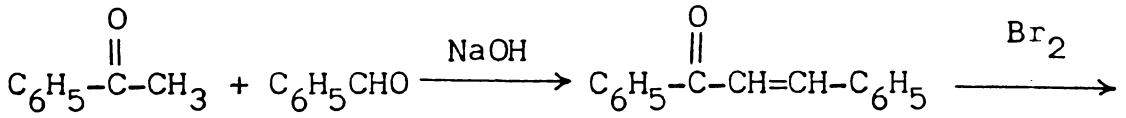
14 15

of phenylmagnesium bromide to 2 might have been stepwise, the first molecule getting added to the more reactive aldehyde group to give 7 followed by addition of a second molecule to the C=N to give 6. The fact that the reaction of 7 with excess of phenylmagnesium bromide gives compound 6 in 66 % yield supports this view. Although the addition of one more molecule

of C_6H_5MgBr to the second C=N is possible, this does not take place probably because the carbon end of that C=N is already substituted by $C_6H_5-\overset{|}{C}H-O^-$ group and thus both the steric effect and the negative charge on the oxygen prevent further addition of the negative end of C_6H_5MgBr to that bond.

Reduction of 6 with sodium borohydride in methanol at room temperature reduced the C=N to give α-(2-phenyl-1,2,3,4-tetrahydro-3-quinoxalinyl)benzyl alcohol(8) in 81% yield. Treatment of 6 with Jones reagent oxidised both the $-\overset{|}{C}H-NH-$ and $-\overset{|}{C}H-OH$ groups to give 2-benzoyl-3-phenylquinoxaline (10) in 91% yield. Although the oxidation of $-\overset{|}{C}H-NH-$ does not ordinarily take place under the above conditions, the facile oxidation of the 1,2 position in this case may be due to the fact that a stable aromatic system is produced. In fact this double oxidation, that is conversion of 6 to 10 also takes place just by heating 6 at 200° in an open atmosphere. The structure of 10 was confirmed by its ir, nmr and mass spectra and also by an independent synthesis from diphenyltriketone (9) and o-phenylene diamine. Diphenyltriketone (9) itself was prepared starting from acetophenone (16) and

benzaldehyde in accordance with procedures given in Organic Synthesis[86-88].



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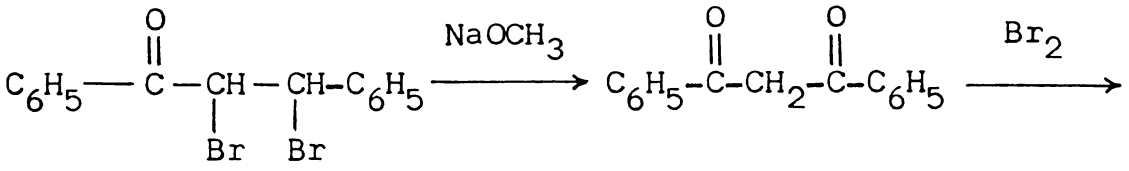

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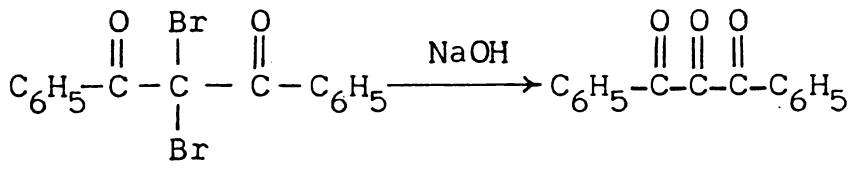


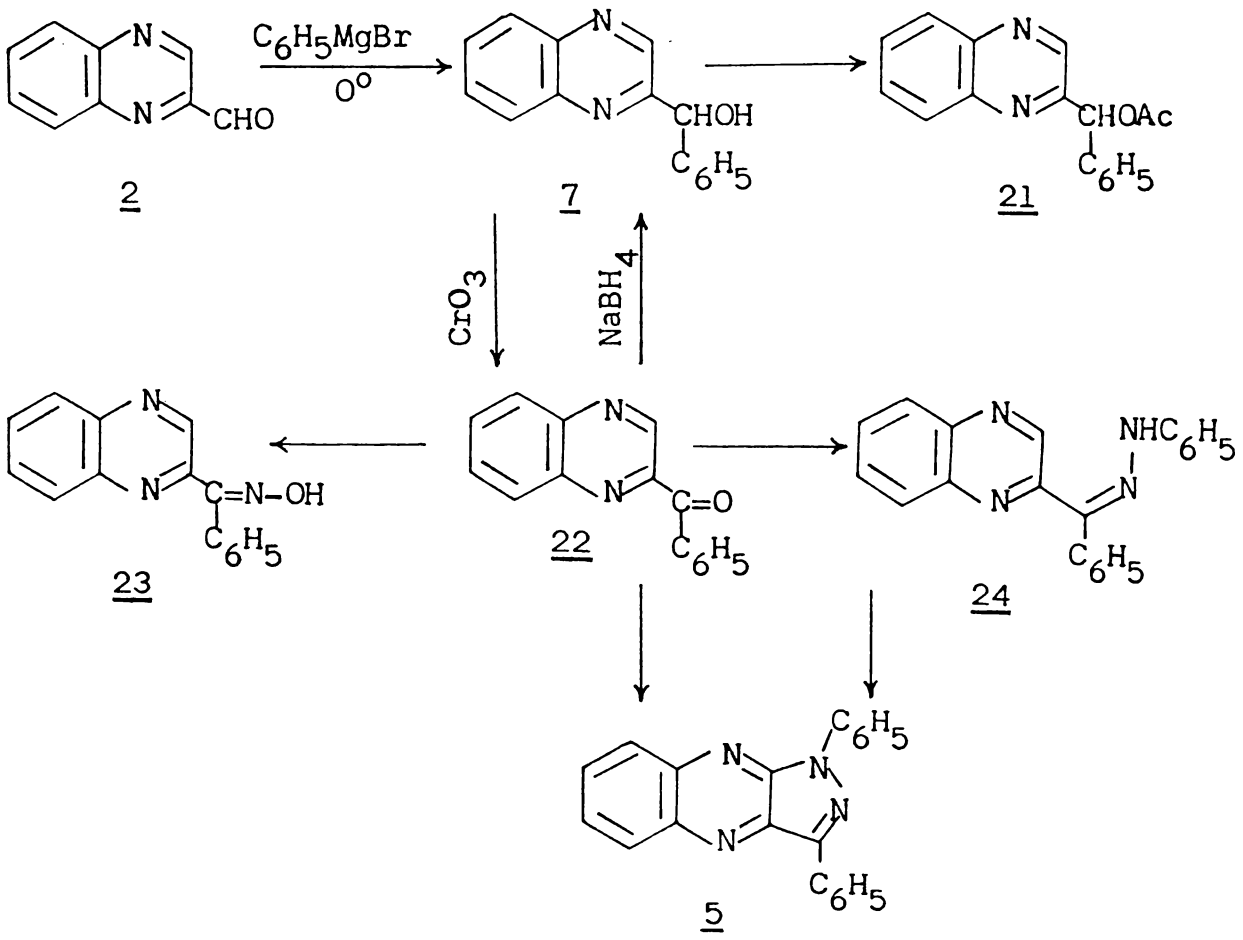
20 9

Reduction of 2-benzoyl-3-phenylquinoxaline (10) with sodium borohydride in methanol gave α-(2-phenyl-3-quinoxalinyl)benzyl alcohol (11) in 83% yield. Also treatment of the ketone, 10 with phenylhydrazine in 60% aqueous n-propanol in the presence of acetic acid gave 83% of the phenylhydrazone, 12. This

2-benzoyl-3-phenylquinoxaline phenylhydrazone (12) was also obtained in 70% yield by the treatment of the alcohol 6 with excess of phenylhydrazine under the same conditions. In this reaction, phenylhydrazine has apparently worked as an oxidising agent first to produce the heteroaromatic ketone, 10 which was then converted into the phenylhydrazone derivative, 12. When this compound 12 was heated to 250° for 5 hours (it melted at 215° with decomposition) it was converted into 1,3-diphenylflavazole (5) apparently by the loss of a molecule of benzene or some oxidised form of it as the pyrolysis was carried out in a system open to atmosphere. Although the mechanism of this reaction is not clear, the loss of the elements of a benzene molecule may be taking place from the cyclic form 13. It may be recalled here that Dahn and co-workers have earlier reported that cyclisation to flavazole takes place if groups such as CO_2H, Cl, CN, $CONH_2$, OH, COC_6H_5 and $CH_2C_6H_5$ are present at this position[64,65]. These groups get eliminated during the cyclisation process. However, it has been specifically mentioned that groups such as CH_3 and C_6H_5 do not depart and flavazole formation does not take place if these groups

are present. Our observation apparently contradicts their finding and shows that the stable flavazole system will be formed even by the elimination of a phenyl group if the starting material is heated to sufficiently high temperatures. The yield of this conversion after purification by chromatography and recrystallisation from hexane was 59%.

Addition of one equivalent of phenylmagnesium bromide in ether to an ether solution of quinoxaline-2-carboxaldehyde (2) cooled in an ice-salt mixture followed by addition of water, extraction with ether and recrystallisation from chloroform-hexane gave 76% of α-(2-quinoxalinyl)benzyl alcohol (7), characterised also as its O-acetate, 21. Oxidation of 7 with Jones reagent at 0° gave 89% of the known 2-benzoylquinoxaline[64], (22). This conversion of 7 to 22 was also achieved in 51% yield by heating 7 at 160° (above its melting point) for 2 hours and purification of the product by column chromatography. 2-Benzoylquinoxaline (22) was characterised as its oxime, 23 and also by reduction with sodium borohydride in methanol back to the alcohol, 7. Treatment of the ketone 22 with phenylhydrazine in boiling methanol for one hour gave 2-benzoylquinoxaline phenylhydrazone (24) in 74% yield. This phenylhydrazone,



24 was converted into 1,3-diphenylflavazole (5) by two methods. The first method involved oxidative cyclisation using phenylhydrazine as the condensing agent in presence of acetic acid and hydrochloric acid. The conversion took place in 88% yield. In the second method, the phenylhydrazone, 24 was heated in a test tube at 240° for one hour when 1,3-diphenyl-flavazole (5) was obtained in 47% yield after

purification by column chromatography. The alcohol 7 was also converted into the flavazole derivative, 5 in one step by heating it with excess of phenylhydrazine in 60% n-propanol in the presence of acetic acid and hydrochloric acid for 2 hours. In this reaction, the oxidation of the alcohol to the ketone, the conversion of the ketone to the phenylhydrazone and oxidative cyclisation of the phenylhydrazone to 1,3-diphenyl-flavazole (5) were all brought about by phenylhydrazine.

Having established the structure of the byproduct formed when stored phenylhydrazine was used for the conversion of 3 to 4 as 1,3-diphenylflavazole (5) we wanted to find out the mechanism by which this product was formed. Previous reports indicate that oxidised phenylhydrazine can act as a source of phenyl radical[89,90] as shown below:

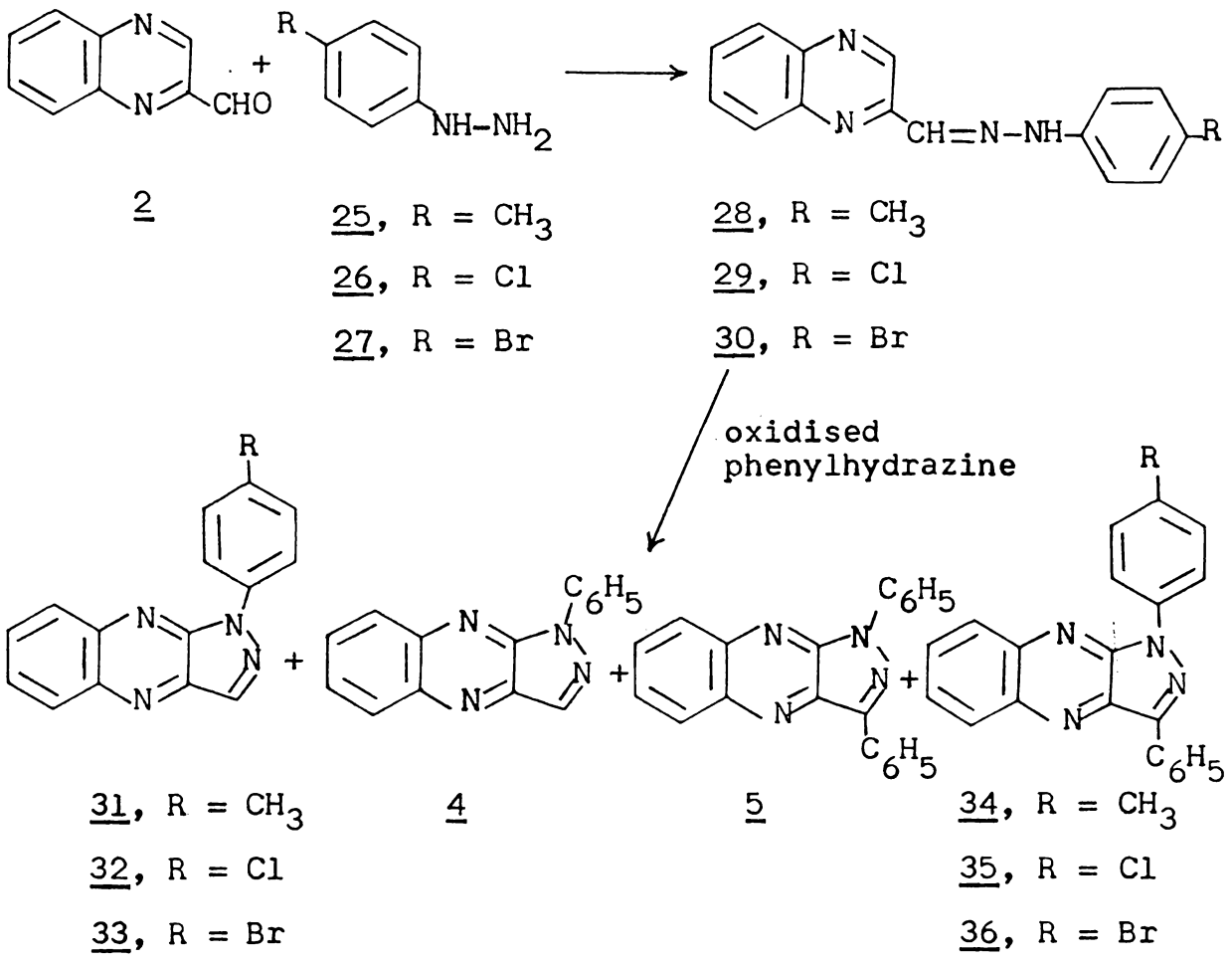
$$C_6H_5NHNH_2 \xrightarrow[-H_2O]{(O)} C_6H_5N{=}NH \xrightarrow{-H\cdot} C_6H_5{-}N{=}N\cdot \xrightarrow{-N_2} C_6H_5\cdot$$

This may mean that a free radical phenylation reaction takes place at position 3 when stored or oxidised phenylhydrazine is used as the condensing agent.

Treatment of oxidised phenylhydrazine with both quinoxaline-2-carboxaldehyde phenylhydrazone (3) and 1-phenylflavazole (4) produced a mixture of 4 and 1,3-diphenylflavazole (5) although a slightly greater amount of 5 was produced when the phenylhydrazone, 3 was treated with oxidised phenylhydrazine. Phenylation reactions may be taking place in both the quinoxaline-2-carboxaldehyde phenylhydrazone (3) and 1-phenylflavazole (4) by a free radical mechanism. In order to establish that other substances producing phenyl radicals also give rise to phenylation at position 3 of 1-phenylflavazole (4), it was treated with excess of benzoylperoxide in n-propanol. The product after chromatographic separation yielded 67% of the starting material, 4 and 23% of 1,3-diphenylflavazole (5). Also, reaction of quinoxaline-2-carboxaldehyde phenylhydrazone (3) with benzoylperoxide both in the presence of phenylhydrazine hydrochloride and in the absence of phenylhydrazine hydrochloride produced a mixture of 4 and 5. In the case of the reaction of 3 with benzoylperoxide in the absence of phenylhydrazine hydrochloride, the yield of products were very low probably due to side reactions.

In order to show that this unusual phenylation reaction is general with different substituted

phenylhydrazones of quinoxaline-2-carboxaldehyde, p-tolyl,p-bromophenyl, and p-chlorophenylhydrazines were prepared and characterised according to known procedures[91]. Also the substituted phenylhydrazones 28, 29 and 30 were prepared by the treatment of quinoxaline-2-carboxaldehyde (2) with the appropriate phenylhydrazines. When quinoxaline-2-carboxaldehyde p-tolylhydrazone (28) was treated with oxidised



2

25, R = CH_3
26, R = Cl
27, R = Br

28, R = CH_3
29, R = Cl
30, R = Br

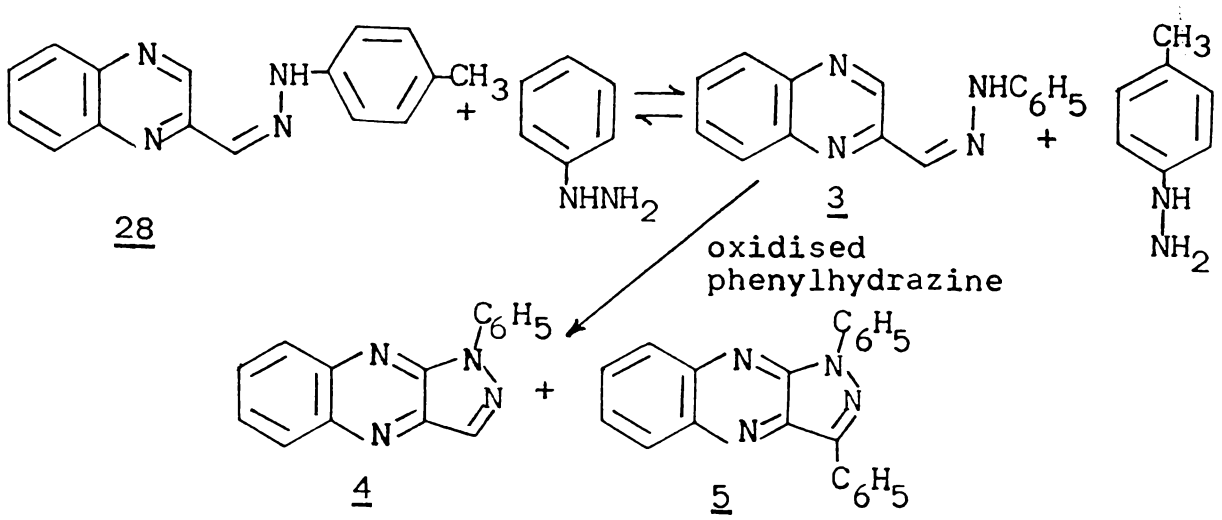
31, R = CH_3
32, R = Cl
33, R = Br

4

5

34, R = CH_3
35, R = Cl
36, R = Br

phenylhydrazine in aqueous n-propanol in the presence of acetic acid a mixture containing 50% of 1-p-tolylflavazole (31), 12% of 1,3-diphenylflavazole (5), 10% of 1-p-tolyl-3-phenylflavazole (34) and a small amount of 1-phenylflavazole (4) was obtained. The percentages are based on the starting quinoxaline-2-carboxaldehyde p-tolylhydrazone (28). The formation of 34 may be explained as being formed by phenylation of either 28 and/or 31 at the 3 position as has been observed in the case of 1-phenylflavazole. The formation of 1-phenylflavazole, 4 and 1,3-diphenylflavazole (5), may be explained as follows: An exchange of phenylhydrazines must have taken place between 28 and phenylhydrazine to form quinoxaline-2-carboxaldehyde phenylhydrazone (3). This type of exchange is an expected phenomenon in the presence of acid. Phenylhydrazone 3 then goes on to form 1-phenylflavazole (4) and 1,3-diphenylflavazole (5) which are obtained as minor components.

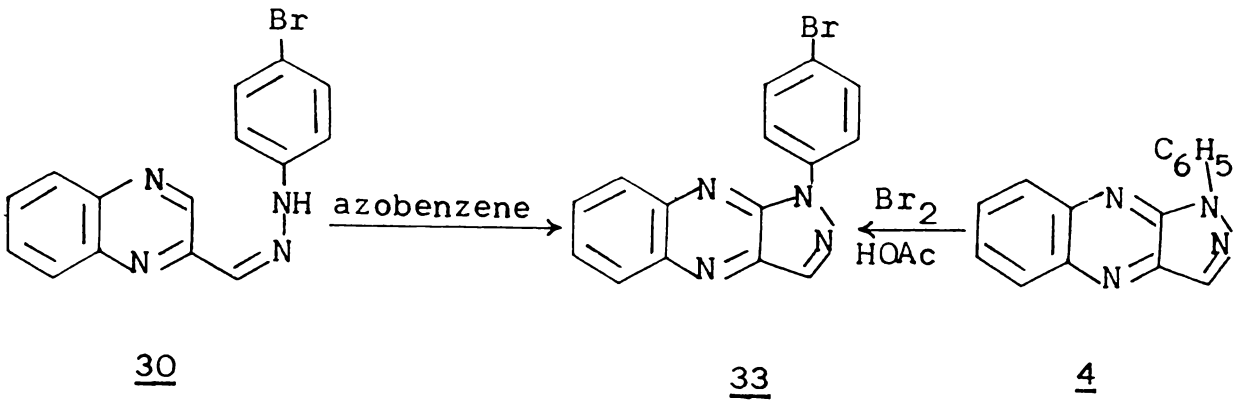


28 3 4 5

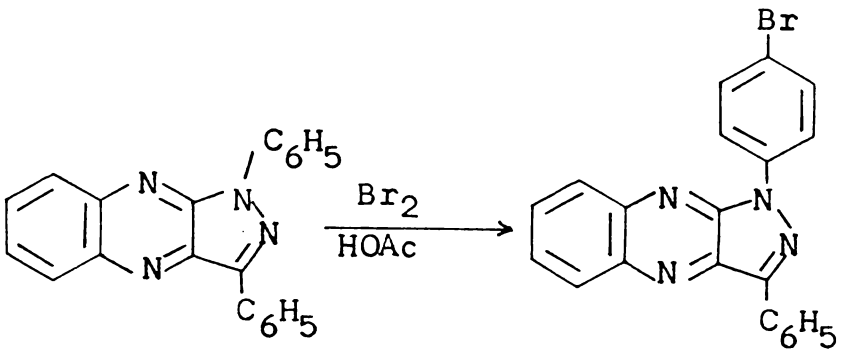
The p-tolylhydrazone, 28 can be cyclised to 1-p-tolylflavazole (31) in 91% yield and without the formation of any byproducts if the reaction is carried out using azobenzene as the condensing agent. The structure of 31 was established by ultraviolet spectrum and elemental analysis.

The reactions of the other para-substituted phenylhydrazones, 29 and 30 also lead to similar mixtures of products. Thus quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (29) when treated with oxidised phenylhydrazine in n-propanol in the presence of acetic acid gave a mixture of 52% of 1-p-chlorophenylflavazole (32), 14% of 1,3-diphenylflavazole (5) 12% of 1-p-chlorophenyl-3-phenylflavazole (35) and a small amount of 1-phenylflavazole (4). The formation of 35 shows that phenylation at position 3 has taken place because of the oxidised phenylhydrazine. Also, exchange of phenylhydrazine with p-chlorophenylhydrazine in 29 in the presence of acid has been shown by the formation of 4 and 5. Cyclisation of 29 without any exchange with phenylhydrazine has been brought about by the use of azobenzene as the condensing agent when 90% of 1-p-chlorophenylflavazole (32) was obtained.

The reaction of quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30) with oxidised phenylhydrazine also underwent oxidative cyclisation, phenylation at position 3 and exchange of the p-bromophenylhydrazine with phenylhydrazine followed by cyclisation and phenylation to produce 46% of 1-p-bromophenylflavazole (33), 11% of 1-p-bromophenyl-3-phenylflavazole (36), 16% of 1,3-diphenylflavazole (5) and a little 1-phenylflavazole (4). Here also use of azobenzene as the condensing agent for the cyclisation of 30 gave only 1-p-bromophenylflavazole (33) in 78% yield. Bromination of 1-phenylflavazole (4) using bromine in glacial acetic acid provided 92% of 1-p-bromophenylflavazole (33). This is the first time a halogenation reaction of a flavazole has been reported. Although nitration and sulphonation of 1-phenylflavazoles have been reported previously under electrophilic substitution reaction conditions, they have led to the formation of mixtures of products. Treatment of 1,3-diphenylflavazole (5) with bromine in acetic acid also causes bromination at the *para* position of the 1-phenyl group producing a single product 36 in 94% yield.

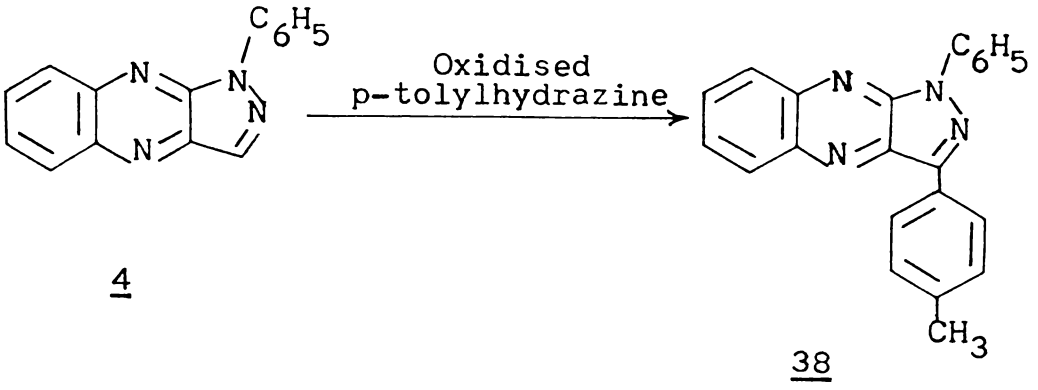


30 33 4



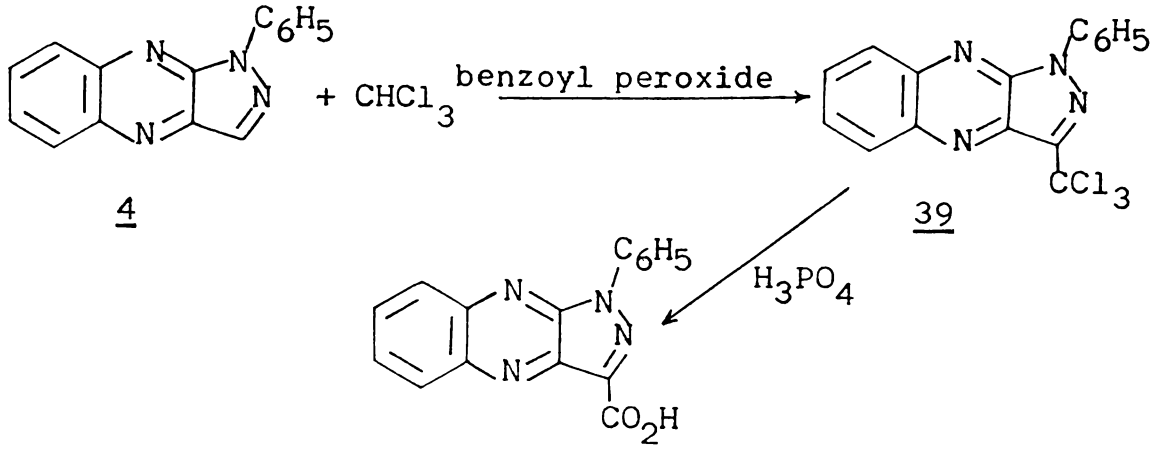
5 36

In order to show that the phenylation reaction at position 3 of 1-phenylflavazole can occur when treated with oxidised para-substituted phenylhydrazines, flavazole 4 was treated with oxidised p-tolylhydrazine (25) in propanol in the presence of acetic acid. A mixture of the 1-phenyl-3-p-tolylflavazole (38) and the starting material, 4 was obtained which was separated by column chromatography to yield 12% of 38 and 41% of 4.



4 38

As in the reactions of 1-phenylflavazole (4) with both oxidised phenylhydrazine and benzoylperoxide phenylation takes place only at position 3, that position appears to be the most vulnerable point for free radical attack. This has been confirmed by the treatment of 4 with chloroform in the presence of benzoylperoxide, when 1-phenyl-3-trichloromethylflavazole (39) was obtained in 50% yield. Under these conditions a trichloromethyl group is known to be substituted by

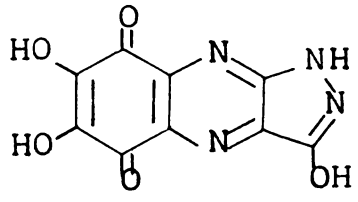


4 39 40

a free radical mechanism[92]. The structure of 39 was established by its hydrolysis to the known 1-phenylflavazole-3-carboxylic acid[3,4] (40) in 86% yield.

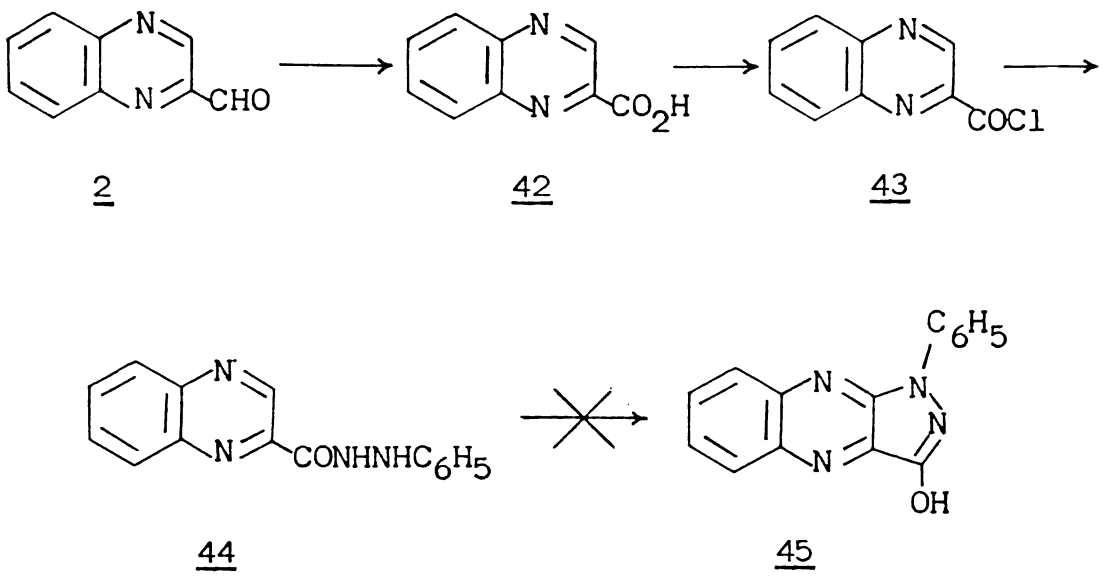
3.2 Preparation of 1-phenylflavazoles substituted at position 3

Although 1-phenylflavazoles substituted at position 3 with groups such as phenyl, methyl, aldehyde, carboxylic acid and sugar residues (all with a carbon atom attached to the 3 position) have been prepared, 1-phenylflavazoles with chloro, amino, and hydroxyl groups at position 3 have not been reported so far. 3-Amino and 3-hydroxy substituted 1-phenylflavazoles are especially important as a polyoxygenated flavazole, 41 has shown significant diuretic and anti-inflammatory properties[42].



41

The preparation of 3-hydroxy-1-phenylflavazole (45) was first attempted by the cyclisation of quinoxaline-2-carbonyl phenylhydrazide (44). Compound 44 was prepared starting



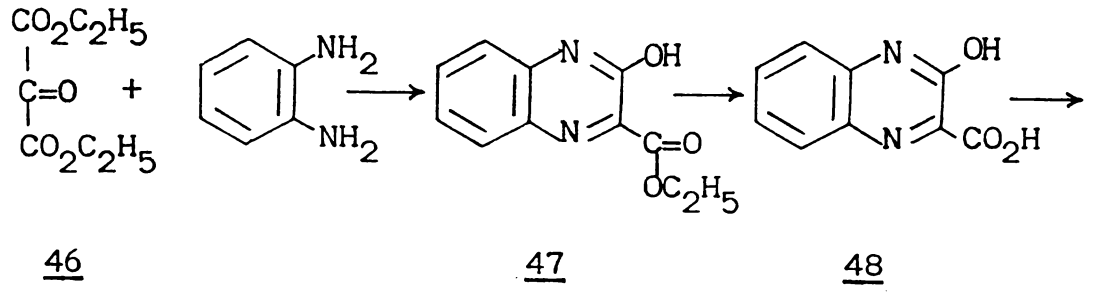
2 42 43

44 45

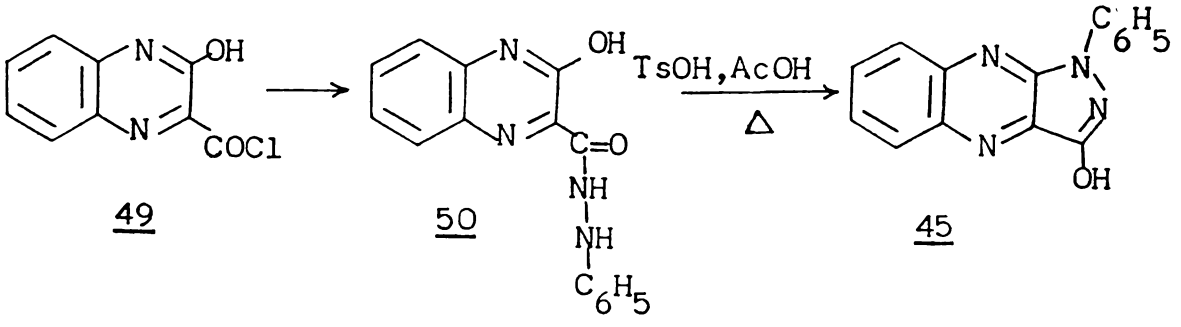
from the known quinoxaline-2-carboxaldehyde (2). Oxidation of 2 with potassium permanganate solution gave 65% of the carboxylic acid[93], 42. This acid was also prepared in 68% yield by the direct oxidation of 2-(D-arabino-tetrahydroxybutyl)quinoxaline (1). Conversion of 42 to the acid chloride[93], 43 followed by treatment with phenylhydrazine provided the phenylhydrazide, 44. However this compound (44) could not be converted into 45 under a variety of oxidative cyclisation conditions such as treating with excess of phenylhydrazine in the presence of acetic acid, treatment with azobenzene, oxidation with benzoylperoxide and oxidation with lead

tetraacetate. These methods have been successful for the cyclisation of quinoxaline-2-carboxaldehyde phenylhydrazone (3) to 1-phenylflavazole (4) in excellent yields as described in the previous section. One reason for the noncyclisation of 44 may be the reduced nucleophilicity of the nitrogen due to the presence of the C=O group at the β-position.

Another approach to obtain 3-hydroxy-1-phenylflavazole (45) was to prepare 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50) and to cyclise it to 45 by dehydration. Compound 50 was prepared starting from diethyl malonate which was oxidised with selenium



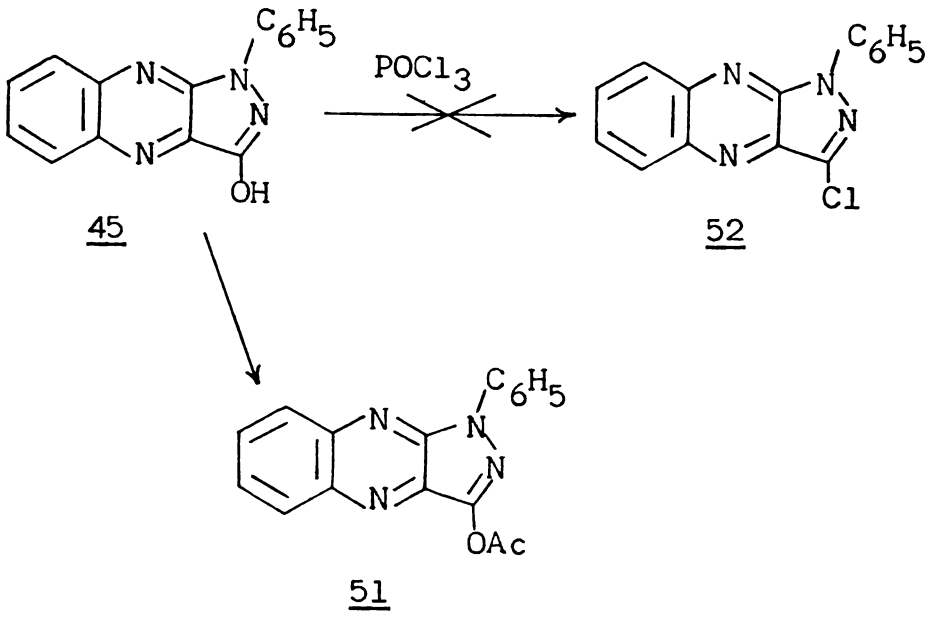
46 47 48



49 50 45

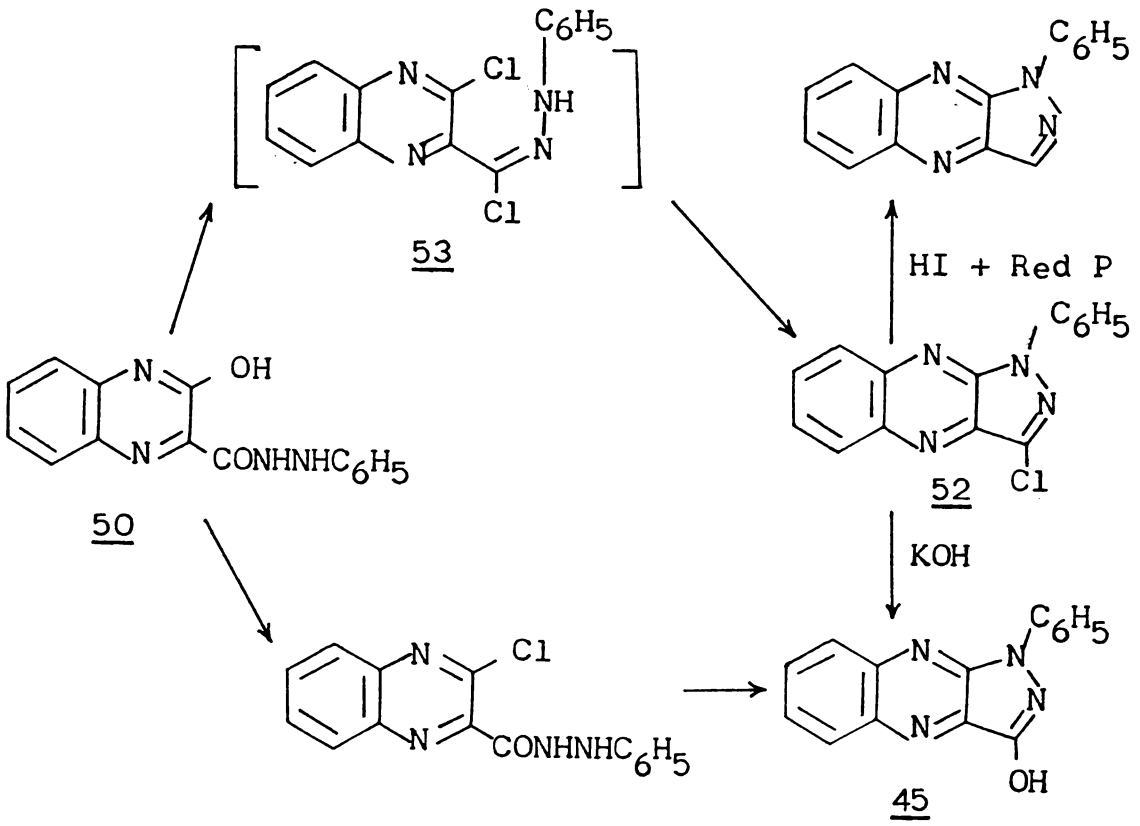
dioxide to give 32.3% of diethylmesoxalate (46) as its hydrate[94]. Condensation of 46 with o-phenylene diamine in dilute hydrochloric acid gave ethyl 2-hydroxy-3-quinoxaline carboxylate[95] (47) in 93% yield. Hydrolysis of the ester 47 to the carboxylic acid[96], 48 followed by treatment with thionyl chloride gave the acid chloride, 49 which was converted into the phenylhydrazide, 50 by treatment with phenylhydrazine. The phenylhydrazide, 50 was also obtained in 82% yield by the reaction of phenylhydrazine with the ester, 47. The attempted cyclisation of 50 to the 3-hydroxy-1-phenylflavazole (45) by boiling an acetic acid solution of 50 for 10 hours was not successful although these conditions have been reported for the cyclisation of 2-hydroxy-3-benzoylquinoxaline phenylhydrazone to 1,3-diphenylflavazole[40] (5). However, heating a solution of 50 in acetic acid in the presence of p-toluenesulphonic acid as a catalyst for 4 hours on a boiling water bath provided 81% of 3-hydroxy-1-phenylflavazole (45). The infrared spectrum of 45 showed a broad absorption at 3300 - 2800 cm^{-1} for a highly hydrogen bonded -OH group, no absorption above 1600 cm^{-1} for any C=O group. The uv absorption

showed λ_{max}^{MeOH} 283.8 nm (ϵ 1.4x10^5) which shifted to λ_{max}^{MeOH} 300 nm (ϵ 1.5x10^5) in the presence of alkali showing clearly the presence of a phenolic -OH group. Also, acetylation of 45 with acetic anhydride in pyridine gave the 3-acetoxy-1-phenylflavazole (51). Now that the 3-hydroxy derivative, 45 was in hand, conversion of 45 to 3-chloro-1-phenylflavazole (52) was considered rather easy. However treatment of 45 with phosphorylchloride alone or mixed with phosphorous pentachloride under refluxing conditions did not produce the 3-chloro derivative, 52 for some reason which is not clear to us at the moment.



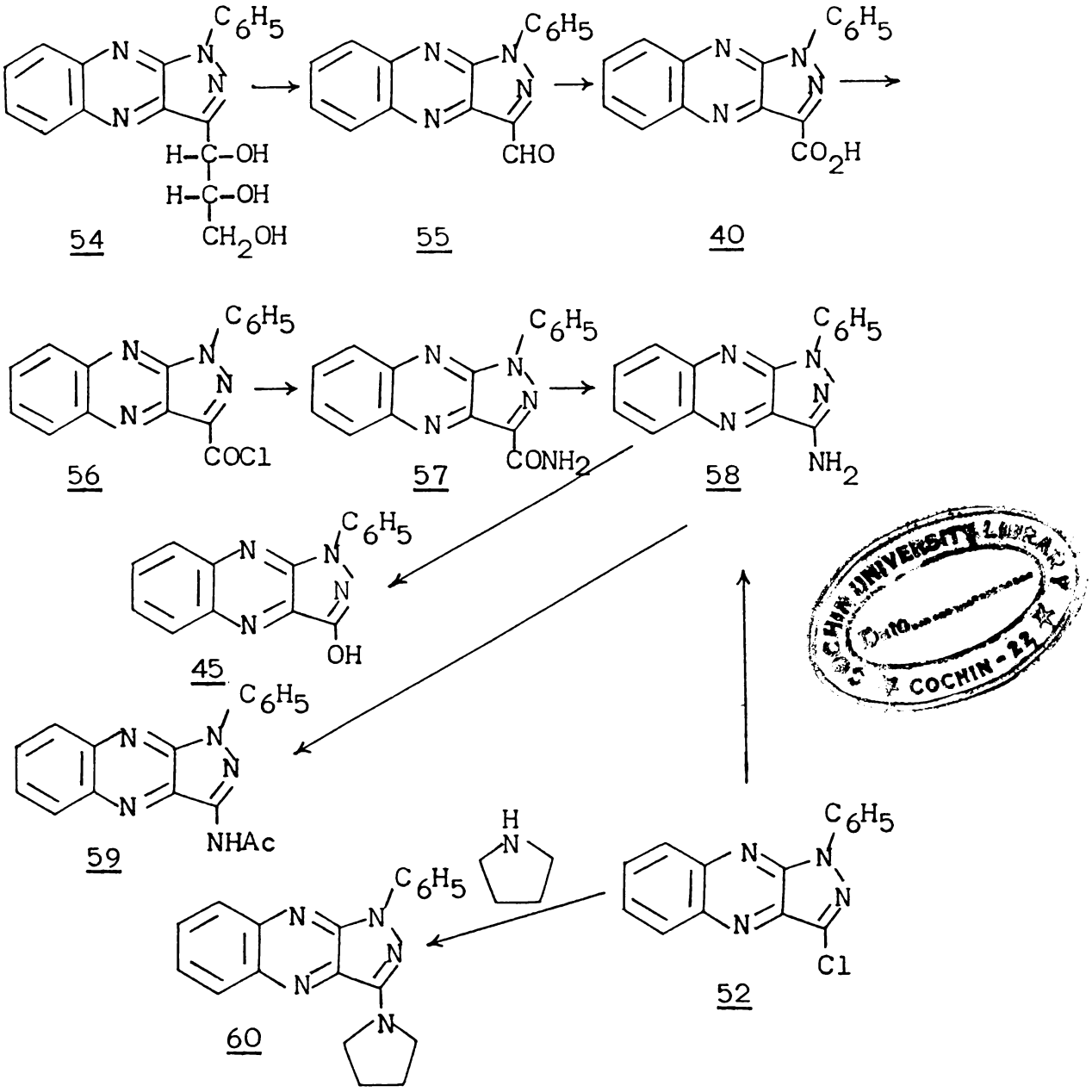
45 52 51

Preparation of the chloro derivative, 52 was achieved by the treatment of phenylhydrazide, 50 with phosphorylchloride when a mixture consisting 71% of 52 and 16% of 45 was obtained. Using larger amount of phosphorylchloride or carrying out the reaction for a longer period did not improve the yield of 52. As 45 cannot be converted into 52 under these conditions, 45 is not an intermediate in the conversion of 50 to 52. May be that the acylphenylhydrazide, 50 is first converted into the dichloro derivative, 53 which then cyclises to form 52. The structure 52 was confirmed by its reaction with alcoholic potassium hydroxide



at 150° for 65 hours in a sealed tube when 3-hydroxy-1-phenylflavazole (45) was obtained in 70% yield. Also reduction of 52 with hydroiodic acid in the presence of red phosphorous at 130-140° for 3 hours provided 1-phenylflavazole (4) in 73% yield.

Treatment of 52 with liquor ammonia in ethanol at 200° for 70 hours in a sealed tube gave 91% of 3-amino-1-phenylflavazole (58). The same amine, 58 was also obtained by a Hofmann reaction of 1-phenylflavazole-3-carboxamide (57) which was prepared by a series of reactions starting from 3-(D-*erythro*-trihydroxypropyl)-1-phenylflavazole (54) obtained by the condensation of D-glucose with o-phenylene diamine and phenylhydrazine[3,4]. The oxidation of flavazole, 54 with lead tetraacetate in benzene gave 87% of 1-phenylflavazole-3-carboxaldehyde[3] (55) which was further oxidised with chromium trioxide in acetic acid to give the carboxylic acid[4], 40 in 86% yield. Conversion of the carboxylic acid to the acidchloride, 56 by treatment with thionylchloride followed by reaction with ammonia gave the carboxamide, 57. Conversion of 57 to the amine, 58 was carried out in 88% yield by a Hofmann reaction using sodium hypochlorite.



54 55 40

56 57 58

45

59

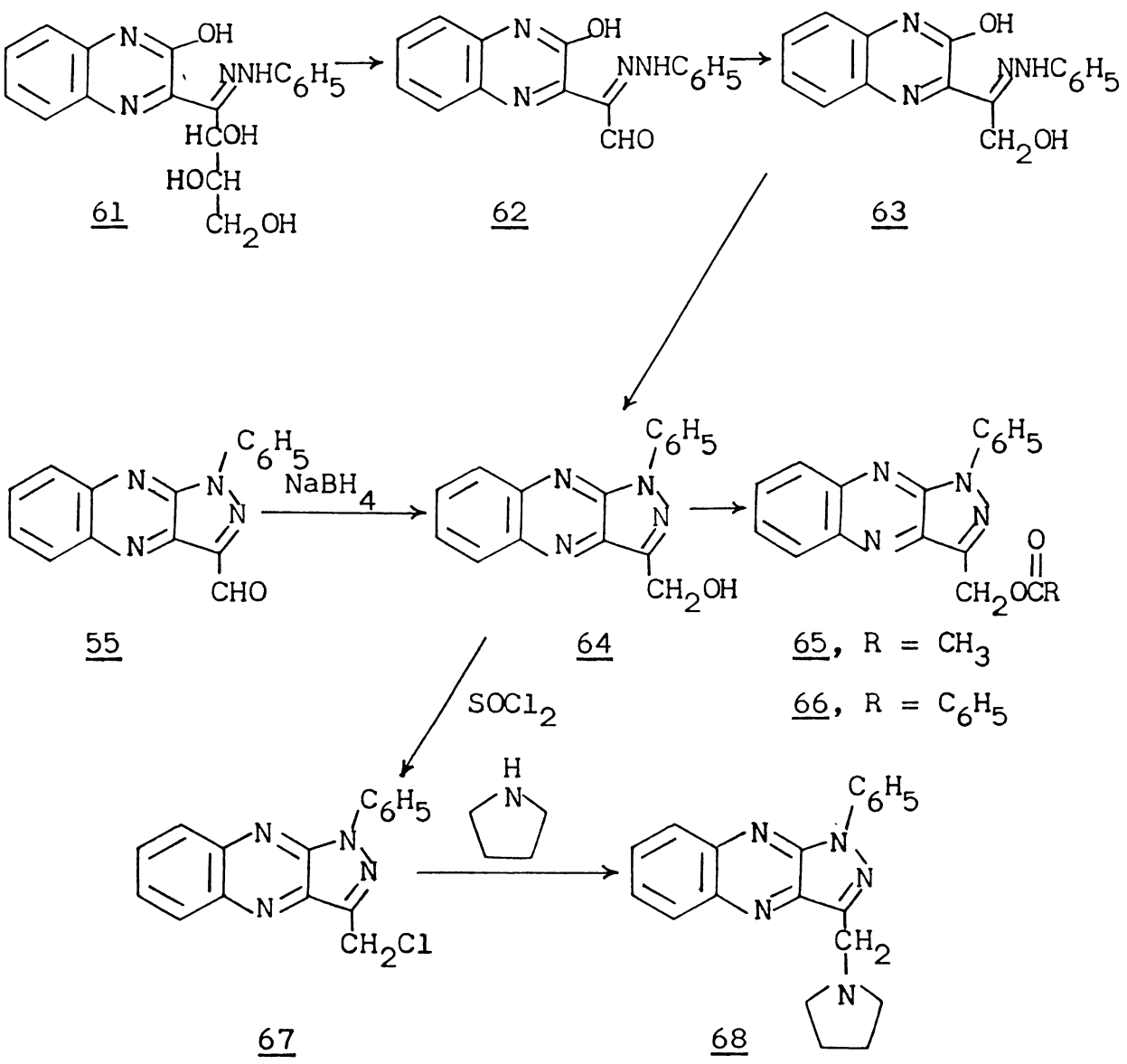
52

60

The two samples of amine obtained by the two different methods were identical in all respects. The ir spectrum (CCl_4) of 58 showed two absorptions at 3460 cm^{-1} and 3380 cm^{-1} for the NH_2 and uv spectrum had λ_{max}^{MeOH} 290.6 nm (ϵ 1.35×10^5) and the mass spectrum was consistent with

the structure. The N-acetyl derivative, 59 was obtained by acetylation of 58 with acetic anhydride in pyridine. Also treatment of the amine 58 with sodium nitrite and hydrochloric acid gave 3-hydroxy-1-phenylflavazole (45) thus confirming the structure of 58. Another derivative with a nitrogen substituted at position 3, the 3-(N-pyrrolidyl)-1-phenylflavazole (60) was also prepared by displacement of the chlorine atom in 52 with pyrrolidine.

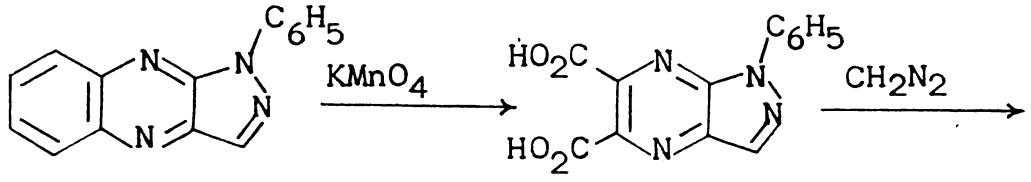
Reduction of 1-phenylflavazole-3-carboxaldehyde (55) with sodium borohydride gave the known 3-hydroxymethyl-1-phenylflavazole[18] (64) in 91% yield. The compound 64 was also obtained starting from 3-(1-phenylhydrazono-L-threo-2,3,4-trihydroxybutyl)-2-quinoxalinone (61). Oxidation of 61 with sodium meta-periodate to give 62 followed by reduction with sodium borohydride gave the hydroxymethyl derivative, 63 which was cyclised with sodium hydroxide in the presence of a few drops of n-butanol to provide[45] 64. This compound was also characterised as its acetyl derivative, 65 and benzoyl derivative[45], 66. Treatment of 64 with thionyl chloride provided 85% of the 3-(chloromethyl)-1-phenylflavazole (67). The chlorine in 67 was displaced



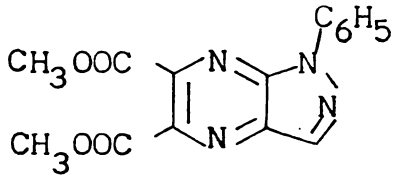
with pyrrolidine to give 3-(N-pyrrolidylmethyl)-1-phenylflavazole (68) in 88% yield. Compound 68 was fully characterised by its spectral data and elemental analysis.

3.3 Reactions of 1-phenylflavazole with oxidising, reducing and brominating agents

Although the oxidation of flavazole (2) to pyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid has been carried out[39], the oxidation reaction of 1-phenylflavazole (4) has not been reported. We, therefore carried out the oxidation of 1-phenylflavazole (4) with a neutral solution of potassium permanganate at 120° to give 62% of the dicarboxylic acid, 1-phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid (69). This acid was also characterised as its dimethyl ester, 70 which was formed by treating 69 with diazomethane in methanol at low temperatures.

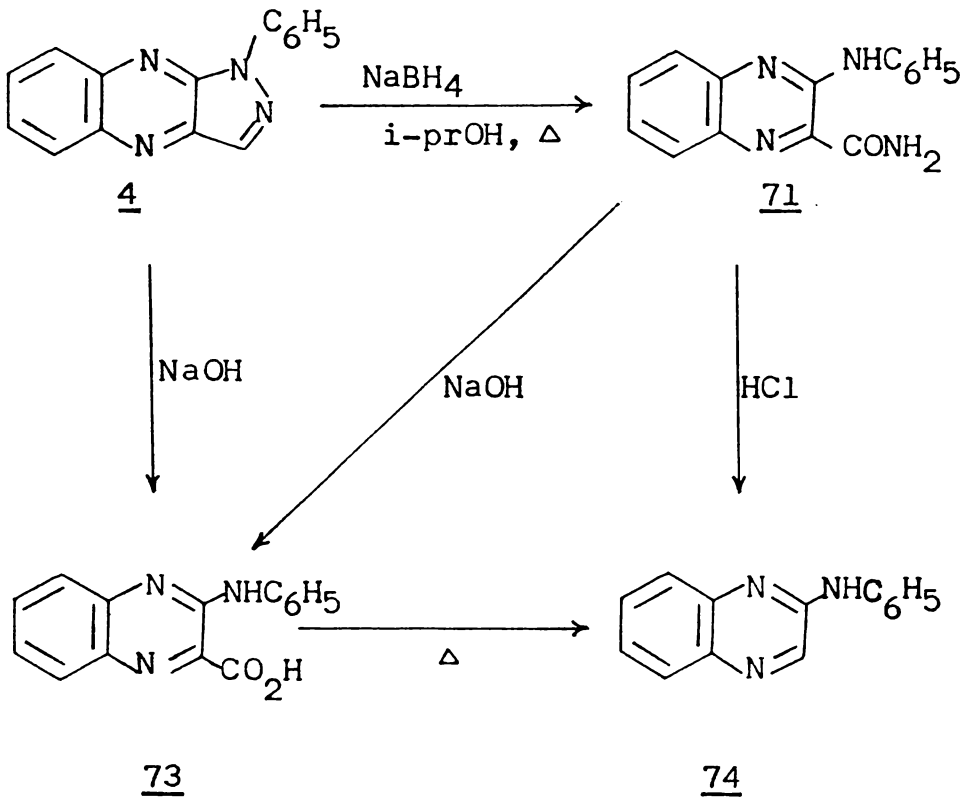


4 69



70

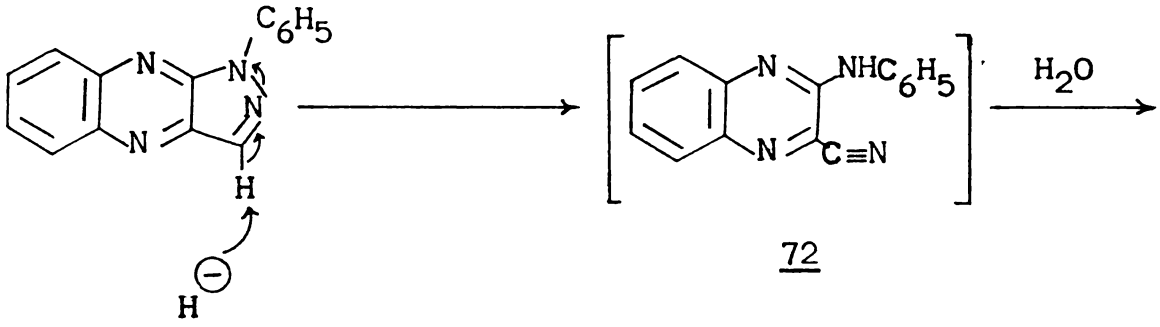
1-Phenylflavazole (4) being a stable aromatic system is not easily reduced. Thus attempts to reduce 4 with sodium borohydride in methanol and lithium aluminium hydride in ether at room temperature were not successful. However treatment of 4 with sodium borohydride in isopropanol and heating the solution under reflux for a long time produced 58% of a new compound, which was subsequently shown to be 2-anilino-quinoxaline-3-carboxamide (71) on the basis of its spectral data, elemental analysis and chemical reactions.



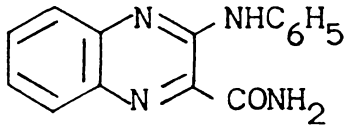
4 71 73 74

The ir spectrum (KBr) of 71 showed strong peaks at 3410 and 3320 cm^{-1} for NH_2 and 1680 cm^{-1} for C=O. The mass spectrum had strong molecular ion peak at m/e 264, peaks at m/e 248($M^+ - NH_2$), m/e 220($M^+ - CONH_2$) and m/e 77($C_6H_5^+$).

The formation of 71 from 4 by the reaction with sodium borohydride may be explained as follows: As the system is not easily reduced, the hydride ion act as a base under the strenuous reaction conditions and the flavazole, 4 is converted into the nitrile, 72 which undergoes partial hydrolysis to the carboxamide, 71 by the action of the limited amount of water present in the isopropyl alcohol. In support of this view, the following points may be cited. First, a small



72



71

amount of the carboxylic acid[101], 73 was also isolated from the reaction mixture. Secondly, alkaline hydrolysis of flavazole 4 in the presence of boiling 10% sodium hydroxide solution also provided the carboxylic acid, 73 in 83% yield. This reaction may also be taking place through the same intermediate, 72.

The alkaline hydrolysis of 71 gave 85% of the carboxylic acid, 73. However hydrolysis of 71 using concentrated hydrochloric acid gave only 2-anilinoquinoxaline[102] (74) as the acid, 73 formed first may undergo decarboxylation under the same reaction conditions. 2-Anilinoquinoxaline (74) was also formed when the acid, 73 was decarboxylated by heating at 200°.

The bromination reactions of 1-phenylflavazole (4) to 1-(p-bromophenyl)flavazole (33) and 1,3-diphenylflavazole to 1-(p-bromophenyl)-3-phenylflavazole (36) have already been discussed.

3.4 Spectral characteristics of 1-phenylflavazoles

As a number of new flavazole derivatives have been prepared, the correlation of their spectral

features, especially those of uv and mass spectra was considered to be useful. As these compounds contain only aromatic hydrogens, a detailed analysis and correlation of nmr spectra appears to have only limited applications.

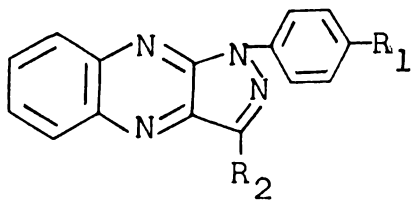
3.4.1 Ultraviolet absorptions

Flavazoles with an aromatic group substituted at position 1 show two characteristic absorptions, one around 265 nm with a high molar absorptivity and the other at around 332 nm with relatively low intensity of absorption. In the case of flavazoles substituted with aromatic groups at both 1 and 3 positions, there are three absorptions, namely at 262-266 nm, 285-290 nm and 331-337 nm, the absorption at 285-290 nm being the weakest. The effect of substituents at the para position of the phenyl group either at position 1 or at position 3 of the flavazole group is not very clear.

1-Phenylflavazoles with substituted methyl group at position 3 show absorptions at 267-270 nm (high intensity) and 334-340 nm (low intensity). A basic nitrogen at position 3 leads to a significant

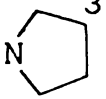
Table III

Ultraviolet absorptions of 1-phenylflavazole derivatives



Sl.No.	Compound No.	R_1	R_2	λ_{max}^{MeOH} nm (log ε)
1.	4	H	H	263.8(5.37), 334.0(4.73)
2.	31	CH_3	H	266.0(5.63), 332.8(4.02)
3.	32	Cl	H	269.8(5.18), 331.6(4.54)
4.	33	Br	H	271.0(5.03), 333.0(4.35)
5.	5	H	C_6H_5	262.4(5.31), 285.0(sh), 337.2(4.56)
6.	34	CH_3	C_6H_5	261.8(4.77), 285.0(sh), 336.2(4.24)
7.	35	Cl	C_6H_5	264.0(5.31), 287.8(5.23), 337.0(4.63)

(contd...)

Sl.No.	Compound No.	R_1	R_2	λ_{max}^{MeOH} nm (log ε)
8.	36	Br	C_6H_5	261.2(5.53), 289.4(5.48), 336.6(4.89)
9.	38	H	CH_3-C_6H_4(p)	266.2(5.54), 285.0(sh), 337.0(4.84)
10.	39	H	CCl_3	270.0(5.32), 340.0(4.62)
11.	67	H	CH_2Cl	267.0(5.04), 333.8(4.50)
12.	68	H	CH_2-N 	267.6(4.78), 336.6(4.15)
13.	58	H	NH_2	290.6(5.13), 335.0(sh)
14.	59	H	$NHCOCH_3$	264.2(4.71), 336.0(4.16)
15.	60	H	N 	300.0(5.30), 340.0(sh)
16.	45	H	OH	283.8(5.16), 335.0(sh)
17.	45 + NaOH	H	O^-Na^+	300.0(5.18)
18.	51	H	OAc	241.0(5.15)
19.	52	H	Cl	267.8(4.89), 334.2(4.27)
20.	57	H	$CONH_2$	249.8(5.69), 338.4(5.06)

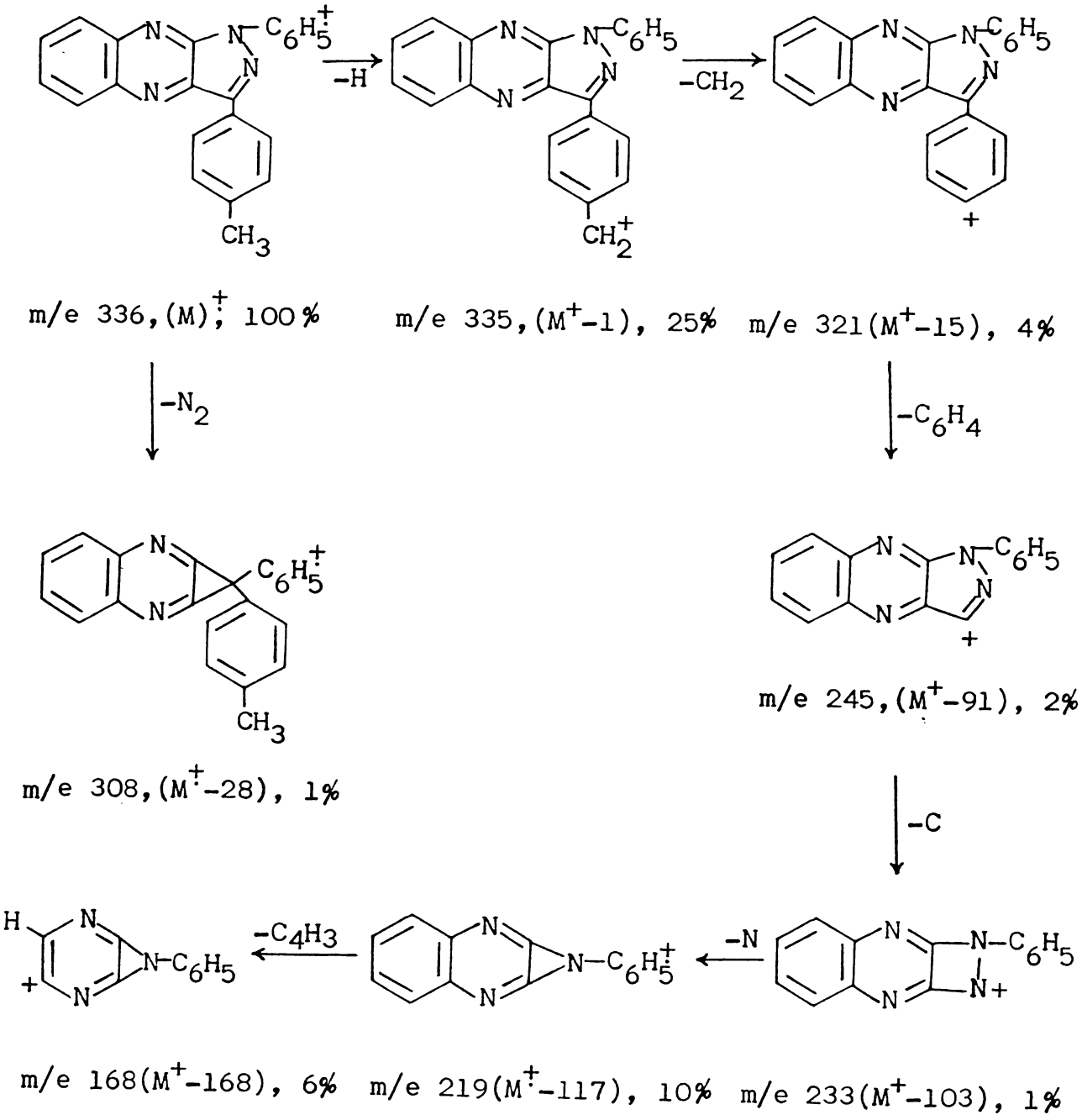
bathochromic shift in the absorption at the short wavelength region, as also is the case for OH and O^- at the same position. The effect of a Cl or a $NHCOCH_3$ group at position 3 is about the same as that of a substituted methyl group whereas a $CONH_2$ or OAc group leads to a hypsochromic shift of the absorption at the short wavelength region.

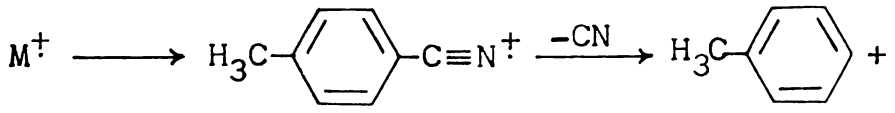
In general the uv spectra of the flavazole ring system is quite characteristic as seen from Table III.

3.4.2 Mass spectral correlations

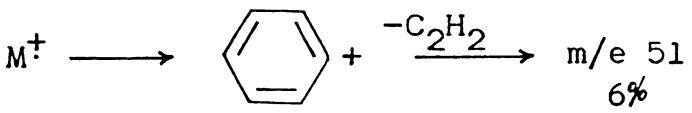
The mass spectra of 1-phenylflavazoles show distinct molecular ion peaks[67]. In most cases the molecular ion peak is also the base peak. Other peaks arise from the loss of substituent at position 3 and subsequent loss of $^+C{\equiv}N$. 1-Phenylflavazoles also give rise to peaks characteristic of compounds containing phenyl group, namely at m/e 77 and m/e 51. A peak corresponding to $M^{\dagger}$ - 28 is seen by the loss of N_2 and the peak at m/e 28 may be due to $(N_2)^{\dagger}$.

A typical mass spectrum of a 3-substituted 1-phenylflavazole, 38 is presented below:

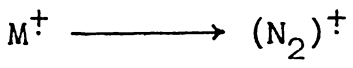




m/e 117(M^+-219), 2% m/e 91, 7%



m/e 77, 15%



m/e 28, 3%

Similar fragmentation pattern is observed in the mass spectra of other 1-phenylflavazole derivatives also. The masses of the important fragments are given under each compound in the chapter on Experimental Procedures.

CHAPTER IV

EXPERIMENTAL PROCEDURES

All melting points were taken using capillary tubes on a melting point bath containing liquid paraffin or silicon oil and are not corrected. Thin layer chromatography was performed on 5 x 20 cm glass plates coated with silica gel G. Chloroform was used as the developing solvent unless otherwise mentioned. Compounds were detected either by their colour or by developing with iodine. Mass spectra were recorded on a Varian MAT CH 7 Mass spectrometer. Nmr spectra were run in deuterio chloroform using Hitachi R-600 FT NMR spectrometer or a Varian FT 80A Spectrometer with tetramethylsilane as an internal standard. Infrared spectra were obtained on a Perkin Elmer Model 682 grating spectrophotometer. Ultraviolet spectra were obtained using a Hitachi Model 200 spectrophotometer in methanol. Elemental analyses were performed at the Hindustan Ciba-Geigy Research Centre, Bombay, National Chemical Laboratory, Pune or Kerala State Drugs and Pharmaceuticals Limited, Alleppey.

4.1 2-(D-Arabino-tetrahydroxybutyl)quinoxaline[3](1)

A solution of 36.0 g (0.2 mole) of D-glucose in 54.0 ml of water was mixed with 6.0 ml of glacial acetic acid, 21.6 g (0.2 mole) of o-phenylene diamine,

5.0 ml (0.1 mole) of hydrazine hydrate and a pinch of sodium bicarbonate and the mixture was heated under reflux for 5.0 hours on a boiling water bath. The solution was cooled in ice and the precipitated product was filtered and washed with water. It was recrystallised from hot water and dried to give 17.0 g (34%) of 2-(D-arabino-tetrahydroxybutyl) quinoxaline (1), mp 192° (decomp) (lit.[3] mp 192°).

4.2 Quinoxaline-2-carboxaldehyde[81] (2)

A mixture of 5.0 g (0.02 mole) of 2-(D-arabino-tetrahydroxybutyl)quinoxaline (1) and 13.0 g (0.06 mole) of sodium metaperiodate in 300 ml of water and 10 ml of glacial acetic acid was kept at room temperature with occasional shaking for 16 hours. The mixture was filtered and the filtrate neutralised with sodium bicarbonate. The neutral solution was extracted with ether, the ether extract was dried with anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was recrystallised from petroleum ether (60 - 80°) to give 2.0 g (63%) of quinoxaline-2-carboxaldehyde (2) mp 107° (lit.[81] mp 107 - 108°).

4.3 Quinoxaline-2-carboxaldehyde phenylhydrazone[82] (3)

A solution of 1.6 g (0.01 mole) of quinoxaline-2-carboxaldehyde (2) and 1.1 g (0.01 mole) of phenylhydrazine in 20 ml of methanol was stirred at room temperature for 1 hour using a magnetic stirrer. Yellow crystals of quinoxaline-2-carboxaldehyde phenylhydrazone were formed. The mixture was cooled in ice, filtered and washed with a small amount of ice cold methanol and the product recrystallised from methanol to give 2.0 g (81%) of quinoxaline-2-carboxaldehyde phenylhydrazone (3) mp 230° (lit.[82] mp 229 - 230°).

4.4 1-Phenylflavazole (4)

a) Using phenylhydrazine as condensing agent[36]

A mixture of 0.5 g (0.002 mole) quinoxaline-2-carboxaldehyde phenylhydrazone (3), 0.5 ml of freshly distilled phenylhydrazine, 0.5 ml of 1 N HCl, 0.5 ml of glacial acetic acid and 50 ml of 60% aqueous 1-propanol was heated under reflux for 24 hours on a boiling water bath and then cooled in a refrigerator. The yellow

crystals formed were filtered and recrystallised from 50% acetic acid to give 0.35 g (71%) of 1-phenylflavazole (4) mp 152° (lit.[36] mp 152°).

b) Using azobenzene as condensing agent

A mixture of 0.5 g (0.002 mole) of quinoxaline-2-carboxaldehyde phenylhydrazone (3), 0.37 g (0.002 mole) of azobenzene, 50 ml of 60% aqueous 1-propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath and then refrigerated overnight. The product was filtered, washed with water followed by a small quantity of cold 50% aqueous n-propanol, dried, and recrystallised from 50% acetic acid to give 0.46 g (93%) of 1-phenylflavazole (4) mp 152°. A mixed mp determination of this material with the sample of 1-phenylflavazole prepared previously was undepressed.

c) By heating quinoxaline-2-carboxaldehyde phenylhydrazone (3) at 240 - 250°

Compound 3 (250 mg, 0.001 mole) was heated in a test tube at 240-250° for 3 hours in an oil bath. It

was cooled, dissolved in 25 ml of chloroform and purified by passing over a column of silica gel and the product was recrystallised from methanol to give 90 mg (37%) of 1-phenylflavazole (4), mp 152°. The products obtained by the above three methods were identical in all respects.

4.5 Reaction of quinoxaline-2-carboxaldehyde phenylhydrazone (3) with oxidised phenylhydrazine

A mixture of 0.5 g (0.002 mole) of quinoxaline-2-carboxaldehyde phenylhydrazone (3), 50 ml of 60% aqueous n-propanol, 10 ml of 1 N HCl and 0.5 ml of glacial acetic acid was heated under reflux on a boiling water bath. To the hot mixture, 0.5 ml of oxidised phenylhydrazine (phenylhydrazine through which a slow stream of air was passed for two weeks) was added and boiling continued for 5 hours. Again 0.5 ml of oxidised phenylhydrazine was introduced into the boiling mixture. Heating was continued for another 20 hours and the reaction mixture refrigerated overnight. The separated material was filtered and washed with water. This product was a mixture of two components as shown by tlc. The two

compounds were separated by chromatographing over a column of silica gel. Elution with carbon tetrachloride provided the first component, 100 mg (16%), mp 234°. Further elution of the column with chloroform gave 240 mg (49%) of 1-phenylflavazole (4) mp 152°.

Component one, which showed an intense green fluorescence in carbon tetrachloride was shown to be 1,3-diphenylflavazole (5) by its mass spectrum (molecular weight 322), melting point (lit.[40] mp 234°) and elemental analysis.

UV: λ_{max}^{MeOH} 262.4 nm (ϵ 2.05 x 10^5), 337.2 nm (ϵ 3.87 x 10^4)

Anal. Calcd. for $C_{21}H_{14}N_4$: C, 78.24; H, 4.38;N, 17.38

Found: C, 78.43; H, 4.31; N, 17.15.

4.6 Reaction of 1-phenylflavazole (4) with oxidised phenylhydrazine

A mixture of 250 mg (0.001 mole) of 1-phenylflavazole (4),50 ml of 60% aqueous n-propanol, 0.5 ml of glacial acetic acid, 1.0 ml of oxidised phenylhydrazine

and 10 ml of 1 N HCl was heated under reflux on a boiling water bath for 50 hours. At this time, another 1.0 ml of oxidised phenylhydrazine was added and the reaction continued for another 15 hours. The reaction mixture was concentrated to 25 ml and kept overnight in a refrigerator. The precipitated material was filtered and washed with water to give a sticky product which showed two components corresponding to 1-phenylflavazole (4) and 1,3-diphenylflavazole (5) by tlc. The mixture was separated by column chromatography on silica gel. Elution with carbon tetrachloride gave 30 mg (9%) of 1,3-diphenylflavazole (5) mp 234° and further elution with chloroform yielded 210 mg (84%) of 1-phenylflavazole (4) mp 152°. Both compounds were characterised by mixed mp determinations with authentic samples.

4.7 Reaction of quinoxaline-2-carboxaldehyde phenylhydrazone (3) with benzoylperoxide

A mixture of 0.5 g (0.002 mole) of quinoxaline-2-carboxaldehyde phenylhydrazone (3), 50 ml of 60% aqueous n-propanol, 0.5 ml of glacial acetic acid and 0.5 g of

benzoyl peroxide was heated under reflux on a boiling water bath. After 3 hours another 0.5 g of benzoyl peroxide was added and refluxing continued for an additional 21 hours. The reaction mixture was diluted with 100 ml of water, heated for a few minutes and then cooled overnight in a refrigerator. The sticky material was filtered, washed with water and dried. A tlc examination indicated that the product contained small quantities of 1-phenylflavazole (4) and 1,3-diphenylflavazole (5) among other impurities. Starting material was not detected in the mixture. Chromatography on a silica gel column using carbon tetrachloride as solvent yielded 50 mg (8%) 1,3-diphenylflavazole (5) mp 234° and 45 mg (9%) of 1-phenylflavazole (4) mp 152°. No other product was obtained.

4.8 Reaction of 1-phenylflavazole (4) with benzoylperoxide

A mixture 300 mg (0.0012 mole) of 1-phenylflavazole (4), 50 ml of 60% aqueous n-propanol and 0.5 ml of glacial acetic acid was heated under reflux on a boiling water bath. To the boiling solution was

added 100 mg each of benzoyl peroxide at 1 hour intervals. Thus a total of 4.7 g of benzoyl peroxide was added and the mixture was refluxed for 46 hours. The mixture was concentrated to 20 ml and cooled. A saturated solution of sodium bicarbonate (50 ml) was added and stirred for 1 hour. The gummy product was filtered, washed with water and dried. A tlc examination indicated that the product contained both 1-phenylflavazole (4) and 1,3-diphenylflavazole (5). These compounds were separated by column chromatography on silica gel as described earlier to give 90 mg (23%) of 1,3-diphenylflavazole (5) mp 234° and 200 mg (67%) of 1-phenylflavazole (4) mp 152°.

4.9 α-(1,2-Dihydro-2-phenyl-3-quinoxalinyl) benzyl alcohol (6)

a) From the reaction of quinoxaline-2-carboxaldehyde (2) with excess of phenylmagnesium bromide

A solution of 4.7 g (0.03 mole) of quinoxaline-2-carboxaldehyde (2) in 200 ml of dry ether was added dropwise to a stirred, cooled (freezing mixture) solution of phenylmagnesium bromide[97] prepared from 17.5 ml of freshly distilled bromobenzene and 5.0 g of magnesium

turnings in ether. After the completion of addition, the mixture was stirred for 30 minutes and 100 ml of water was added dropwise, stirred for 2 hours and kept overnight at room temperature. The ether layer was separated, washed with water and dried with anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the residue recrystallised from hexane to give 6.2 g (66%) of α-(1,2-dihydro-2-phenyl-3-quinoxalinyl)benzyl alcohol (6) mp 130° (decomp) as blood red crystals.

MS : m/e 314, ($M^{+\cdot}$), 313, 312, 283 (M^+ - H, CHOH), 235 (M^+ - 2H, C_6H_5), 207 (M^+ - C_6H_5CHOH) etc.

IR : KBr, 3350 cm^{-1}(OH and NH), 1650 cm^{-1}(C=N).

UV : λ_{max}^{MeOH} 269.2 nm (ε 1.05 x 10^5).

Anal. Calcd. for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91.

Found: C, 80.45; H, 5.80; N, 8.74.

b) From α-(2-quinoxalinyl)benzyl alcohol (7) and phenylmagnesium bromide

A solution of 2.4 g (0.01 mole) of α-(2-quinoxalinyl) benzyl alcohol (7) in 300 ml of dry ether was cooled in a

freezing mixture and to the cooled solution was added dropwise with stirring, a solution of phenylmagnesium bromide[97] prepared from 7.0 ml of freshly distilled bromobenzene and 1.6 g of magnesium turnings. After the completion of addition, the mixture was stirred 30 minutes, 200 ml of water was added slowly with stirring and kept overnight at room temperature. The ether layer was separated, dried with anhydrous sodium sulphate and evaporated under reduced pressure to dryness. The residue was recrystallised from hexane to give 2.1 g (67%) of α-(1,2-dihydro-2-phenyl-3-quinoxalinyl)benzyl alcohol (6) mp 130°. The products obtained by (a) and (b) above were identical in all respects.

4.10 α-(2-Quinoxalinyl)benzyl alcohol (7)

To a cooled (freezing mixture) solution of 12.5 g (0.08 mole) of quinoxaline-2-carboxaldehyde (2) was added dropwise with stirring a solution of phenyl-magnesium bromide[97] prepared from 12.0 ml of freshly distilled bromobenzene and 2.5 g of magnesium turnings under a nitrogen atmosphere. After the completion of addition, the mixture was stirred 30 minutes and 400 ml

of cold water was added slowly with stirring and the mixture was kept overnight at room temperature. The ether layer was separated, dried with anhydrous sodium sulphate and concentrated to dryness under reduced pressure. The residue was leached with petroleum ether to remove any unreacted starting material and the residue recrystallised from chloroform-hexane (1:9) to give 14.3 g (76%) of α-(2-quinoxalinyl)benzyl alcohol (7), mp 138° (decomp).

MS : m/e 236 (M^+), 217(M^+ - OH), 159(M^+ - C_6H_5), 129(M^+ - C_6H_5CHOH), 103[M^+ - C_6H_5-CH(OH)-C=N] etc.

NMR($CDCl_3$): δ 5.0(1,d,J=5Hz,OH), 5.95(1,d,J=5Hz,Benzylic H) 7.3(5,s,phenyl H), 7.7 (2,m, 6H+7H), 8.1(2,m,5H+8H) and 8.7(1,s,3H).

IR(KBr): 3250 cm^{-1}(broad,OH).

UV: λ_{max}^{MeOH} 237.2 nm(ε 1.18 x 10^5),319 nm(ε 2.9 x 10^4).

Anal. Calcd. for $C_{15}H_{12}N_2O$: C, 76.25; H, 5.12; N, 11.86

Found: C, 75.96; H, 4.85; N, 11.95.

4.11 α-(2-Phenyl-1,2,3,4-tetrahydro-3-quinoxalinyl) benzyl alcohol (8)

To a solution of 160 mg (0.0005 mole) of α-(1,2-dihydro-2-phenyl-3-quinoxalinyl)benzyl alcohol (6)

in 10 ml of methanol was added 10 mg of sodium borohydride and stirred for 30 minutes. The colour of the solution was changed from blood red to yellow. It was concentrated to 5.0 ml, diluted to 100 ml with water, stirred well, filtered, washed with water, dried and recrystallised from hexane-chloroform (9:1) to give 130 mg (81%) of α-(2-Phenyl-1,2,3,4-tetrahydro-3-quinoxalinyl)benzyl alcohol (8), mp 80°.

MS : m/e 316(M^+), 209($M^+ - C_6H_5CHOH$), 132($M^+ - C_6H_5CHOH$ and C_6H_5), 77 (C_6H_5) etc.

IR(KBr): 3400 cm^{-1}(broad, OH+NH), 1600 cm^{-1}(aromatic).

UV : λ_{max}^{MeOH} 219 nm(ε 2.4 x 10^5), 316.4 nm (ε 4.0 x 10^4)

Anal. Calcd. for $C_{21}H_{20}N_2O$: C, 79.72; H, 6.37; N, 8.86.

Found: C, 79.99; H, 6.50; N, 8.74.

4.12 Benzalacetophenone[86] (17)

To a solution of 110 g (2.75 moles) of sodium hydroxide in 1 l of water and 600 ml of 95% alcohol in a 3 l bottle fitted with a mechanical stirrer, was added 260 g (2.15 moles) of pure acetophenone (16), the bottle

was rapidly surrounded with cracked ice, the stirrer started and 230 g (2.15 moles) of pure benzaldehyde added at once. The temperature of the mixture was maintained between 15° and 30° during the reaction by cooling in ice. After 3 hours, the mixture became so thick that stirring was not possible. The stirrer was then removed and the mixture left in an ice box for about 10 hours. The mixture was cooled in a freezing mixture, filtered, washed with water until free from base, followed by about 100 ml of cold 95% alcohol, dried and recrystallised from 95% alcohol to give 385 g (85%) of benzalacetophenone (17) as light yellow material, mp 55° (lit.[86] mp 55-57°).

4.13 Benzalacetophenone dibromide[87] (18)

A solution of 104 g (0.5 mole) of benzal-acetophenone (17) in 300 ml of carbon tetrachloride was cooled in an ice bath and 80 g (25.6 ml, 0.5 mole) of bromine was added with stirring. After the reaction was complete, the product was filtered off, washed with hot alcohol and dried to give 155 g (84%) of benzal-acetophenone dibromide (18), mp 156°(lit.[87] mp 156-157°).

4.14 Dibenzoylmethane[87] (19)

To a mixture of 92 g (0.25 mole) of benzal-acetophenone dibromide (18) in 85 ml of absolute methyl alcohol was added rapidly with stirring, a solution of sodium methoxide prepared from 11.5 g of sodium and 115 ml of absolute methyl alcohol, and the mixture was heated under reflux for one hour. The reaction mixture was cooled, made acidic with concentrated hydrochloric acid and heated for 5 minutes. It was diluted with 80 ml of cold water and the flask was cooled in ice bath with rapid stirring when small crystals were formed. The product was filtered, washed with a small amount of cold 50% methanol followed by water until free of acid and dried. The crude material was recrystallised from 75 ml of hot methyl alcohol to give 39 g (69%) of dibenzoylmethane (19), mp 78° (lit.[87] mp 78°).

4.15 Dibenzoyldibromomethane[88] (20)

To a solution of 28 g (0.125 mole) of dibenzoylmethane (19) in 14 ml of chloroform was added slowly a solution of 44 g (0.28 mole) of dry bromine

in 110 ml of chloroform while the mixture was cooled in an ice bath. The temperature of the mixture was maintained below 15° during the bromination. After all the bromine had been added, stirring was continued for about 15 minutes. The solvent was removed under diminished pressure and the residue was recrystallised from 95% ethyl alcohol to give 35.75 g (75%) of dibenzoyldibromomethane (20), mp 94° (lit.[88] mp 94°).

4.16 Diphenyltriketone hydrate[88] (9)

To a solution of 17.15 g (0.21 mole) of fused sodium acetate in 71.0 ml of hot glacial acetic acid was added 36.2 g (0.095 mole) of dibenzoyl-dibromomethane (20) and the mixture was heated under reflux until the precipitation of sodium bromide was complete. The mixture was then cooled to room temperature and diluted with 100 ml of water with constant shaking. The separated crystals were filtered, washed well with water and dried to give 20.5 g (85%) of diphenyltriketone hydrate (9), mp 70° (lit.[88] mp 65-90°).

4.17 2-Benzoyl-3-phenylquinoxaline (10)

a) From diphenyltriketone hydrate (9)

A mixture of 130 mg (0.0005 mole) of diphenyltriketone hydrate (9), 55 mg (0.0005 mole) of o-phenylene diamine, 20 ml of ethanol and 1.0 ml of glacial acetic acid was heated under reflux for 24 hours on a boiling water bath. The solution was concentrated to 5 ml, poured into 50 ml of ice cold water and stirred well. After 1 hour the crystals were filtered, washed with water and dried. The product was purified by column chromatography on silica gel using chloroform as solvent and recrystallised from hexane-chloroform (9:1) to give 90 mg (64%) of 2-benzoyl-3-phenylquinoxaline (10), mp 153°.

MS : m/e 310(M^+), 282(M^+ - CO), 233(M^+ - C_6H_5), 205(M^+ - C_6H_5CO), 179(M^+ - C_6H_5CO-C=N)

NMR($CDCl_3$): absorption only at δ 7.7(m).

IR(KBr): 1670 cm^{-1}(C=O), 1595 cm^{-1}(aromatic).

UV : λ_{max}^{MeOH} 250 nm(ϵ 3.54 x 10^5), 334 nm(ϵ 8.9 x 10^4).

Anal. Calcd. for $C_{21}H_{14}N_2O$: C, 81.27; H, 4.55; N, 9.03.
Found: C, 81.20; H, 4.76; N, 9.13.

b) By the oxidation of α-(1,2-dihydro-2-phenyl-3-quinoxalinyl)benzyl alcohol (6)

A solution of 940 mg (0.003 mole) of 6 in 50 ml of acetone was cooled in an ice bath. To the cooled solution 1.5 ml of Jones reagent (prepared from 26.72 g of CrO_3 and 100 ml of sulphuric acid obtained by diluting 23 ml of conc. acid) was added dropwise with constant stirring at 0^o. After the addition was complete the mixture was stirred 30 minutes at 0^o, 20 ml of ice cold water was added and the mixture extracted with ether. The ether extract was washed with 5% sodium bicarbonate solution followed by water, dried with anhydrous sodium sulphate and concentrated to dryness under reduced pressure. The residue was recrystallised from hexane to give 0.81 g (91%) of 2-benzoyl-3-phenylquinoxaline (10) mp 153^o.

c) By heating α-(2-phenyl-1,2,3,4-tetrahydro-3-quinoxalinyl)benzyl alcohol (8)

Compound 8 (100 mg; 0.00033 mole) was heated in a test tube at 200^o for 2 hours in an oil bath. It was cooled, dissolved in 10 ml of chloroform and

purified by column chromatography on silica gel. The product was recrystallised from hexane to give 68 mg (67%) of 2-benzoyl-3-phenylquinoxaline (10) mp 153°.

d) By heating α-(2-phenyl-1,2-dihydro-3-quinoxalinyl) benzyl alcohol (6) at 200°

α-(2-Phenyl-1,2-dihydro-3-quinoxalinyl) benzyl alcohol (6) (100 mg; 0.00033 mole) was heated in a test tube at 200° for 2 hours in an oil bath. It was cooled and the product was dissolved in 10 ml of chloroform. The chloroform solution was passed over a column of silica gel and the product obtained was recrystallised from hexane to give 70 mg (71%) of 2-benzoyl-3-phenylquinoxaline (10) mp 153°.

e) By heating α-(2-phenyl-3-quinoxalinyl)benzyl alcohol (11) at 200°

Compound 11 (100 mg; 0.00033 mole) was heated in a test tube at 200° for 2 hours in an oil bath. It was cooled, dissolved in 10 ml of chloroform and purified by passing over a column of silica gel and the product obtained was recrystallised from hexane to

give 65 mg (64%) of 2-benzoyl-3-phenylquinoxaline (10), mp 153°.

The products obtained by (a), (b), (c), (d) and (e) above were identical in all respects.

4.18 α-(2-Phenyl-3-quinoxalinyl)benzyl alcohol (11)

To a solution of 310 mg (0.001 mole) of 2-benzoyl-3-phenylquinoxaline (10) in 100 ml of methanol was added 30 mg of sodium borohydride and the mixture was stirred at room temperature for 2 hours. The solution was concentrated to 20 ml under reduced pressure and diluted to 100 ml with water. The mixture was cooled overnight in a refrigerator, the crystals were filtered, washed with water, dried and recrystallised from hexane to give 250 mg (83%) of α-(2-phenyl-3-quinoxalinyl)benzyl alcohol (11) mp 125°.

MS : m/e 312(M^+), 310 (M^+ - 2H), 295(M^+ - OH), 282(M^+ - CHOH), 235(M^+ - C_6H_5), 205(M^+ - C_6H_5CHOH), 180[M^+ - $C_6H_5CH(OH)C{=}N$].

NMR($CDCl_3$): δ 5.5(1,d,J=7Hz, OH), 6.1(1,d,J=7Hz, benzylic H), 7.5(14, complex m, aromatic).

IR(KBr): 3310 cm^{-1}(broad, OH), 1600 cm^{-1}(aromatic).

UV : λ_{max}^{MeOH} 241.8 nm(ϵ 1.5 x 10^5), 326.6 nm(ϵ 4.0 x 10^4).

Anal. Calcd. for $C_{21}H_{16}N_2O$: C, 80.74; H, 5.16; N, 8.97.

Found: C, 80.96; H, 5.03; N, 8.65.

4.19 2-Benzoyl-3-phenylquinoxaline phenylhydrazone (12)

a) From 2-benzoyl-3-phenylquinoxaline (10)

A mixture of 300 mg (0.001 mole) of 2-benzoyl-3-phenylquinoxaline (10), 50 ml of 60% aqueous n-propanol, 0.3 ml of glacial acetic acid and 1 ml of 1 N HCl was heated under reflux for 4 hours on a boiling water bath and cooled overnight in a refrigerator. The crystals formed were filtered, washed with water followed by a small amount of cold 60% aqueous n-propanol, dried and recrystallised from chloroform-hexane (1:3) to give 320 mg (83%) of 2-benzoyl-3-phenylquinoxaline phenylhydrazone (12) mp 215° (decomp).

MS : m/e 400(M^+), 323($M^+ - C_6H_5$), 308($M^+ - C_6H_5NH$), 294($M^+ - C_6H_5NHN$), 217(323 - C_6H_5NHN), 205($M^+ - C_6H_5C{=}NNHC_6H_5$), 77($C_6H_5^+$), etc.

IR(KBr): 3280 cm^{-1}(NH).

UV : λ_{max}^{MeOH} 249.8 nm(ϵ 1.34 x 10^5).

Anal. Calcd. for $C_{27}H_{20}N_4$: C,80.97; H, 5.03; N, 13.99.
Found: C, 81.09; H, 5.23; N, 14.02.

b) From α-(1,2-dihydro-2-phenyl-3-quinoxalinyl) benzyl alcohol (6)

A mixture of 310 mg (0.001 mole) of α-(1,2-dihydro-2-phenyl-3-quinoxalinyl)benzyl alcohol (6), 0.5 ml of phenylhydrazine, 50 ml of 60% aqueous n-propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath. The mixture was cooled in a refrigerator overnight and the crystals were filtered, washed with water followed by a small amount of cold 60% aqueous n-propanol and dried. It was recrystallised from chloroform-hexane (1:3) to give 280 mg (70%) of 12, mp 215° (decomp). A mixed mp determination of the two samples from a and b above was undepressed.

4.20 α-(2-Quinoxalinyl)benzyl acetate (21)

A mixture of 470 mg (0.002 mole) of α-(2-quinoxalinyl)benzyl alcohol (7) in 5 ml of dry

pyridine and 0.3 ml (0.003 mole) of acetic anhydride was stirred 16 hours at room temperature under a $CaCl_2$ guard tube. The reaction mixture was poured into ice cold water with vigorous stirring and the colourless crystals formed were filtered, washed with water, dried and recrystallised from hexane to give 480 mg (86%) of α-(2-quinoxalinyl)benzyl acetate (21), mp 90°.

NMR($CDCl_3$): δ 2.3(3,s,CH_3), 7.15(1,s,benzylic H), 9.05(1,s,3H), 7.7(9,complex m, other Hs).

IR(KBr): 1745 cm^{-1}(C=O).

UV : λ_{max}^{MeOH} 237.2 nm(ε 1.68 x 10^5), 319.2 nm(ε 3.9 x 10^4).

Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C,73.36; H, 5.07; N, 10.07.
Found: C, 73.63; H, 4.85; N, 10.28.

4.21 2-Benzoylquinoxaline[64] (22)

A solution of 1.2 g (0.005 mole) of α-(2-quinoxalinyl)benzyl alcohol (7) in 40 ml of acetone was cooled in an ice bath. To this solution was added dropwise 1.5 ml of Jones reagent (prepared from 26.72 g of CrO_3 and 100 ml of sulphuric acid obtained by diluting

23 ml of conc. H_2SO_4) with stirring. After the completion of addition the mixture was stirred for 30 minutes at 0^o, 20 ml of ice cold water was added and extracted with ether. The extract was washed with 5% sodium bicarbonate solution followed by water, dried with anhydrous sodium sulphate, and concentrated to dryness under reduced pressure. The residue was recrystallised from hexane to give 1.04 g (89%) of 2-benzoylquinoxaline (22), mp 80^o (lit.[64] mp 80^o).

IR(KBr): 1660 cm^{-1}(C=O).

UV : λ_{max}^{MeOH} 249.8 nm(ϵ 2.5 x 10^5).

Anal. Calcd. for $C_{15}H_{10}N_2O$: C,76.91; H, 4.30; N, 11.96.

Found: C,76.77; H, 4.51; N, 12.23.

Heating 240 mg (0.001 mole) of 7 in a test tube at 160^o for 2 hours in an oil bath followed by purification using column chromatography on silica gel and recrystallisation from hexane also gave 120 mg (51%) of 2-benzoylquinoxaline (22) mp 80^o.

Reduction of 130 mg (0.0005 mole) of 2-benzoylquinoxaline (22) in 5 ml of methanol with 10 mg of sodium borohydride for 1 hour followed by the

usual work up and recrystallisation of the product from hexane-chloroform (9:1) gave 100 mg (78%) of α(2-quinoxalinyl)benzyl alcohol (7) mp 138°.

4.22 2-Benzoylquinoxaline oxime[64] (23)

A mixture of 230 mg (0.001 mole) of 2-benzoylquinoxaline (22), 200 mg of hydroxylamine hydrochloride and 20 ml of 50% aqueous n-propanol was refluxed for 2 hours on a boiling water bath. The mixture was cooled, diluted to 100 ml with cold water and stirred well. It was cooled, filtered, washed with water and recrystallised from 50% aqueous n-propanol to give 205 mg (82%) of 2-benzoylquinoxaline oxime (23) mp 208° (lit.[64] mp 208°).

4.23 2-Benzoylquinoxaline phenylhydrazone (24)

A mixture of 120 mg (0.0005 mole) of 2-benzoylquinoxaline (22) in 10 ml of methanol and 60 mg (0.0005 mole) of phenylhydrazine was heated under reflux for 1 hour. The solution was concentrated to 3 ml and cooled in an ice bath. The precipitate formed was filtered, washed with a little amount of ice cold methanol and recrystallised from methanol to give

120 mg (74%) of 2-benzoylquinoxaline phenylhydrazone (24) mp 160° (decomp).

Anal. Calcd. for $C_{21}H_{16}N_4$: C, 77.75; H, 4.97; N, 17.27.

Found: C, 78.07; H, 4.89; N, 17.40.

4.24 1,3-Diphenylflavazole[40] (5)

a) From 2-benzoylquinoxaline phenylhydrazone (24) using phenylhydrazine as condensing agent

A mixture of 320 mg (0.001 mole) of 2-benzoylquinoxaline phenylhydrazone (24), 50 ml of 60% aqueous n-propanol, 0.5 ml of phenylhydrazine, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 2 hours on a boiling water bath. The reaction mixture was cooled overnight in a refrigerator and crystals formed were filtered, washed with water and a small amount of cold 60% aqueous n-propanol, dried and recrystallised from 80% acetic acid to give 280 mg (88%) of 1,3-diphenylflavazole (5) mp 234° (lit.[40] mp 234°).

b) From 2-benzoylquinoxaline phenylhydrazone (24) by heating at 240°

Compound 24 (160 mg; 0.0005 mole) was heated in a test tube at 240° for 1 hour in an oil bath. It

was cooled, dissolved in 10 ml of chloroform and purified by passing over a column of silica gel and the product was recrystallised from hexane to give 75 mg (47%) of 1,3-diphenylflavazole (5), mp 234°.

c) By the reaction of α-(2-quinoxalinyl)benzyl alcohol (7) with excess of phenylhydrazine

A mixture of 240 mg (0.001 mole) of α-(2-quinoxalinyl)benzyl alcohol (7), 0.5 ml of phenylhydrazine, 50 ml of 60% aqueous propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 2 hours on a boiling water bath and cooled overnight in a refrigerator. The crystals formed were filtered, washed with water and recrystallised from 90% acetic acid to give 240 mg (75%) of 1,3-diphenylflavazole (5) mp 234°. Mixed melting point with an authentic sample was undepressed.

d) From 2-benzoyl-3-phenylquinoxaline phenylhydrazone (12) by heating at 250°

2-Benzoyl-3-phenylquinoxaline phenylhydrazone (12) (400 mg; 0.001 mole) was heated at 250° for 5 hours in a test tube on an oil bath. The colour of the product

was changed to black. It was cooled and dissolved in 50 ml of carbon tetrachloride and purified by passing over a column of silica gel. The product was recrystallised from hexane to give 190 mg (59%) of 1,3-diphenylflavazole (5) mp 234°. Mixed melting point with an authentic sample of 1,3-diphenyl-flavazole (5) was undepressed.

4.25 Preparation of substituted phenylhydrazines[91,98,99]

A suspension of 0.2 mole of finely powdered substituted aniline (p-bromo, p-chloro or p-methyl) in 70 ml of conc. hydrochloric acid was warmed to about 60° for 1 hour and was then cooled in a freezing mixture. An ice-cold solution of 20 g of sodium nitrite in 50 ml of water was added dropwise with vigorous stirring. The diazonium salt formed was filtered and added slowly to a solution of sodium sulphite (prepared by passing SO_2 into a solution of 45 g of sodium hydroxide in 300 ml of water until the solution turned acidic as indicated by phenol-phthalein). The resulting solution was warmed to 60°, made acidic to litmus by the addition of about 20 ml

of conc. hydrochloric acid and heated for about an hour. About 100 ml of conc. hydrochloric acid was added and the mixture was allowed to cool. The p-substituted phenylhydrazine hydrochloride crystallised as a lump of small needless, which were filtered and redissolved in minimum quantity of hot water and cooled. After neutralisation with 50% sodium hydroxide solution, the mixture was cooled in freezing mixture and the crystals formed were filtered and recrystallised from hot water.

By this method, p-tolylhydrazine[98] (25) (86%) mp 61°, p-chlorophenylhydrazine[99] (26) (81%) mp 86° and p-bromophenylhydrazine[98,99] (27) (75%) mp 108° were prepared.

4.26 Quinoxaline-2-carboxaldehyde p-tolylhydrazone (28)

A solution of 3.9 g (0.025 mole) of quinoxaline-2-carboxaldehyde (2) and 3.0 g (0.025 mole) p-tolylhydrazine (25) in 50 ml of methanol was stirred for 1 hour at room temperature. The mixture was diluted to 250 ml with water, stirred for another 2 hours and kept overnight in the refrigerator. The precipitate

was filtered, washed with water, dried and recrystallised from methanol to give 5.2 g (79%) of quinoxaline-2-carboxaldehyde p-tolylhydrazone (28) mp 192° (decomp).

Anal. Calcd. for $C_{16}H_{14}N_4$: C,73.26; H, 5.38; N, 21.36

Found: C, 73.04; H, 5.53; N, 21.64.

4.27 Quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (29)

A solution of 3.9 g (0.025 mole) of quinoxaline-2-carboxaldehyde (2) in 50 ml of methanol and 3.6 g (0.025 mole) of p-chlorophenylhydrazine (26) was stirred at room temperature for 1 hour. The mixture was diluted to 250 ml with water, stirred 2 hours more and kept overnight in a refrigerator. The precipitated material was filtered, washed with water, dried and recrystallised from methanol to give 5.8 g (83%) of quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (29) mp 236° (decomp).

Anal. Calcd. for $C_{15}H_{11}N_4Cl$: C, 63.72; H, 3.92; N, 19.82.

Found: C, 63.57; H, 4.10; N, 19.98.

4.28 Quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30)

A solution of 3.9 g (0.025 mole) of quinoxaline-2-carboxaldehyde (2) in 50 ml of methanol and 4.7 g (0.025 mole) of p-bromophenylhydrazine (27) was stirred at room temperature for 1 hour. The mixture was diluted to 250 ml with water and stirred for 2 hours more and kept overnight in a refrigerator. The solid was filtered, washed with water, dried and recrystallised from methanol to give 6.0 g (73%) of quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30) mp 225° (decomp).

Anal. Calcd. for $C_{15}H_{11}N_4Br$: C,55.06; H, 3.39; N, 17.13.

Found: C, 55.33; H, 3.65; N, 17.37.

4.29 1-p-Tolylflavazole (31)

A mixture of 520 mg (0.002 mole) of quinoxaline-2-carboxaldehyde p-tolylhydrazone (28), 370 mg (0.002 mole) of azobenzene, 50 ml of 60% aqueous n-propanol, 0.5 ml of glacial acetic acid and 5 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath and then kept

in a refrigerator overnight. The product was filtered, washed with water followed by few ml of cold 50% n-propanol and recrystallised from 60% acetic acid to give 470 mg (91%) of 1-p-tolylflavazole (31) mp 176°.

UV : λ_{max}^{MeOH} 266 nm (ϵ 4.25 x 10^4), 332.8 nm (ϵ 1.05 x 10^4).

Anal. Calcd. for $C_{16}H_{12}N_4$: C,73.82; H, 4.65; N, 21.53.

Found: C, 73.51; H, 4.50; N, 21.84.

4.30 1-p-Chlorophenylflavazole (32)

A mixture of 560 mg (0.002 mole) of quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (29), 370 mg (0.002 mole) of azobenzene, 50 ml of 60% aqueous 1-propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath. The reaction mixture was cooled and kept in a refrigerator overnight. The precipitated material was filtered, washed with water followed by a few ml of cold 50% aqueous 1-propanol and recrystallised from 5:3 acetic acid - water mixture to give 500 mg (90%) of 1-p-chlorophenylflavazole (32) mp 198°.

UV : λ_{max}^{MeOH} 269.8 nm(ϵ 1.5 x 10^5), 331.6 nm (ϵ 3.5 x 10^4).

Anal. Calcd. for $C_{15}H_9N_4Cl$: C, 64.17; H, 3.23; N, 19.96.

Found: C, 64.35; H, 3.33; N, 20.12.

4.31 1-p-Bromophenylflavazole (33)

a) From quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30)

A mixture of 650 mg (0.002 mole) of quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30), 370 mg (0.002 mole) of azobenzene, 50 ml of 60% aqueous n-propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath. The mixture was kept overnight in a refrigerator, filtered, washed with water followed by small quantities of cold 50% aqueous n-propanol and dried. It was recrystallised from 10:3 acetic acid-water mixture to give 510 mg (78%) 1-p-bromophenylflavazole (33) mp 212°.

UV : λ_{max}^{MeOH} 271 nm(ε 1.07 x 10^5), 333 nm(ε 2.26 x 10^4).

Anal. Calcd. for $C_{15}H_9N_4Br$: C,55.40; H, 2.79; N, 17.23.

Found: C, 55.20; H, 2.98; N, 17.07.

b) From 1-phenylflavazole (4) by bromination

To a solution of 0.5 g (0.002 mole) of 1-phenylflavazole (4) in 100 ml of glacial acetic acid

was added 3 ml of a 21% (v/v) bromine in glacial acetic acid and the mixture was stirred for 48 hours at room temperature. Completion of the reaction was checked by tlc. The mixture was diluted to 250 ml with water, the precipitate was filtered, dried and recrystallised from 75% acetic acid to give 600 mg (92%) of 1-p-bromophenylflavazole (33) mp 212°. The compound obtained by the two methods has the same R_f value and a mixed melting point determination was undepressed.

4.32 1-p-Tolyl-3-phenylflavazole (34)

A mixture of 525 mg (0.002 mole) of quinoxaline-2-carboxaldehyde p-tolylhydrazone (28), 50 ml of 60% aqueous 1-propanol, 0.5 ml of glacial acetic acid, 1 ml of oxidised phenylhydrazine and 10 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath. After this point another 1 ml of oxidised phenylhydrazine was added and refluxed for an additional 10 hours. Again 1 ml of oxidised phenylhydrazine was added and refluxing continued for 10 hours more. The mixture was concentrated to 20 ml, diluted to 70 ml with water, cooled overnight in a

refrigerator, filtered, washed with water and dried. The sticky material contained four components as shown by tlc. These compounds were separated by column chromatography on silica gel using carbon tetrachloride as solvent to give after recrystallisation from hexane, 65 mg (10%) of 1-p-tolyl-3-phenylflavazole (34) mp 224°, 80 mg (12%) of 1,3-diphenylflavazole (5) mp 234° 260 mg (50%) of 1-p-tolylflavazole (31) mp 176° and 40 mg (8%) of 1-phenylflavazole (4) mp 152°.

UV : λ_{max}^{MeOH} 261.8 nm(ϵ 5.88 x 10^4), 285.0 nm(sh). 236.2 nm(ϵ 1.73 x 10^4).

Anal. Calcd. for $C_{22}H_{16}N_4$: C, 78.55; H, 4.79; N, 16.66.
Found: C, 78.83; H, 4.52; N, 16.89.

4.33 1-p-Chlorophenyl-3-phenylflavazole (35)

A mixture of 560 mg (0.002 mole) of quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (29), 50 ml of 60% aqueous 1-propanol, 0.5 ml of glacial acetic acid, 1 ml of oxidised phenylhydrazine and 10 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath. At this point 1 ml more of oxidised phenylhydrazine was added and refluxed for an additional 10 hours. Again

1 ml of oxidised phenylhydrazine was added and refluxing continued for 10 hours more. The mixture concentrated to 20 ml, diluted to 70 ml with water, cooled overnight in a refrigerator, filtered, washed with water and dried. The sticky material contained four components as shown by tlc (silica gel G plates using chloroform as mobile phase). These compounds were separated by column chromatography on silica gel using carbon tetrachloride as eluent to give after recrystallisation from hexane, 85 mg (12%) of 1-p-chlorophenyl-3-phenylflavazole (35) mp 215°, 90 mg (14%) of 1,3-diphenylflavazole (5) mp 234°, 35 mg (7%) of 1-phenylflavazole (4) mp 152° and 280 mg (52%) of 1-p-chlorophenylflavazole (32) mp 198° were obtained.

MS : m/e 356(M^+), 358(M+2), 279($M^+ - C_6H_5$), 244($M^+ - C_6H_4Cl$),etc.

UV : λ_{max}^{MeOH} 264 nm(ε 2.04 x 10^5), 287.8 nm(ε 1.6 x 10^5), 337 nm(ε 4.26 x 10^4).

Anal. Calcd. for $C_{21}H_{13}N_4Cl$: C, 70.68; H, 3.67; N, 15.71.

Found: C, 70.51; H, 3.58; N, 15.98.

4.34 1-p-Bromophenyl-3-phenylflavazole (36)

a) By the reaction of quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30) with oxidised phenylhydrazine

A mixture of 650 mg (0.002 mole) of

quinoxaline-2-carboxaldehyde p-bromophenylhydrazone (30), 50 ml of 60% aqueous n-propanol, 1 ml of oxidised phenylhydrazine, 10 ml of 1 N HCl and 0.5 ml of glacial acetic acid was heated under reflux for 8 hours on a boiling water bath. At this time 1 ml of oxidised phenylhydrazine and 10 ml of 1 N HCl were added and heating continued for 20 hours more. The reaction mixture was concentrated to 30 ml, diluted with 50 ml of water, refrigerated overnight, filtered, washed with water and dried to give a mixture of four compounds as shown by tlc. This mixture of compounds was separated by column chromatography on silica gel using carbon tetrachloride as eluent to give 90 mg (11%) of 1-p-bromophenyl-3-phenylflavazole (36) mp 246° after recrystallisation from petroleum ether, 100 mg (16%) of 1,3-diphenylflavazole (5) mp 234°, 40 mg (8%) of 1-phenylflavazole (4) mp 152° and 300 mg (46%) of 1-p-bromophenylflavazole (33) mp 212°.

MS : m/e 400 and 402(M^{+}), 321(M^{+}- Br), etc.

UV : λ_{max}^{MeOH} 261.2 nm(ϵ 3.4×10^5), 289.4 nm ($\epsilon 3.06 \times 10^5$), 336.6 nm($\epsilon$ 7.38×10^4).

Anal. Calcd. for $C_{21}H_{13}N_4Br$: C, 62.85; H, 3.27; N, 13.97.

Found: C, 63.15; H, 3.29; N, 14.04.

b) By the reaction of 1,3-diphenylflavazole (5) with bromine

A solution of 320 mg (0.001 mole) of 1,3-diphenylflavazole (5) in 100 ml of glacial acetic acid and 2 ml of a 21% (v/v) bromine in glacial acetic acid was mixed and stirred at room temperature for 24 hours. A tlc of the reaction mixture (silica gel G plates - hexane:benzene - 3:2 solvent) indicated that bromination was complete. The mixture was diluted to 250 ml with water, cooled overnight in a refrigerator, filtered, washed with water, dried and recrystallised from 90% acetic acid to give 380 mg (94%) of 1-p-bromophenyl-3-phenylflavazole (16) mp 246°. A mixed melting point determination of the products from (a) and (b) was undepressed.

4.35 1-Phenyl-3-p-tolylflavazole (38)

A mixture of 0.5 g (0.002 mole) of 1-phenylflavazole (4), 75 ml of n-propanol solution containing 1.5 g of oxidised p-tolylhydrazine (Oxidised p-tolylhydrazine was prepared by the following method. Air was passed slowly through a solution of 2.5 g of p-tolylhydrazine (25) in 150 ml of n-propanol for 7 days at

room temperature. The volume of this solution was reduced to 120 ml during that period), 0.5 ml of glacial acetic acid, 30 ml of water and 10 ml of 1 N HCl was heated under reflux for 24 hours on a boiling water bath. At this time 45 ml of n-propanol solution containing 1.0 g of oxidised p-tolylhydrazine and 10 ml of 1 N HCl were added and heating continued for 24 hours more. The solution was concentrated to 50 ml and was diluted with 50 ml of water, heated for 10 minutes on a boiling water bath and kept overnight in a refrigerator. The precipitated material which was filtered, washed with water and dried was shown to be a mixture of two compounds by tlc. The two compounds were separated by column chromatography over silica gel. Elution with carbon tetrachloride gave 80 mg (12 %) of 1-phenyl-3-p-tolylflavazole (38) mp 202° and further elution of the column with chloroform provided 200 mg (41%) of starting material, 1-phenylflavazole (4) mp 152°, identical with an authentic sample. Compound 38 was recrystallised from hexane to get an analytical sample.

MS : m/e 336(M^{+}), 321(M^{+} - CH_3), 245(M^{+} - $C_6H_4CH_3$), 219(M^{+} - $CH_3C_6H_4C{=}N$), 77($C_6H_5^{+}$), etc.

UV : λ_{max}^{MeOH} 266.2 nm(ϵ 3.48 x 10^5), 337 nm(ϵ 6.86 x 10^4).

Anal. Calcd. for $C_{22}H_{16}N_4$: C, 78.55; H, 4.79; N, 16.66.
Found: C, 78.31; H, 4.98; N, 16.87.

4.36 1-phenyl-3-trichloromethylflavazole (39)

A solution of 1.2 g (0.005 mole) of 1-phenylflavazole (4) and 500 mg of benzoyl peroxide in 500 ml of chloroform was heated under reflux for 24 hours on a heating mantle[92]. Portions of 500 mg benzoyl peroxide were added after each 24 hours and the reaction continued for 120 hours. The solution was cooled to room temperature, and neutralised with 200 ml of a saturated sodium bicarbonate solution by stirring vigorously for 12 hours. The chloroform layer was separated, washed with water, dried with anhydrous sodium sulphate and concentrated to 50 ml. The product was purified by column chromatography on silica gel using chloroform as solvent and was recrystallised from hexane-chloroform (9:1) to give 900 mg (50%) of 1-phenyl-3-trichloromethylflavazole (39) mp 216°.

UV : λ_{max}^{MeOH} 270 nm(ϵ 2.08 x 10^5), 340 nm(ϵ 4.26 x 10^4).

Anal. Calcd. for $C_{16}H_9N_4Cl_3$: C, 52.84; H, 2.49; N, 15.41.
Found: C, 52.97; H, 2.70; N, 15.25.

4.37 1-Phenyl-3-(D-*erythro*-trihydroxypropyl) flavazole[5] (54)

A solution of 1.8 g (0.01 mole) of D-glucose and 1.1 g (0.01 mole) of o-phenylene diamine in 100 ml of water was mixed with 7.2 g (0.05 mole) of phenylhydrazine hydrochloride and 2.4 g (0.04 mole) of glacial acetic acid and the mixture was heated under reflux for 12 hours on a boiling water bath under a carbon dioxide atmosphere. The reaction mixture was filtered, the solid mass was washed with a little water and ethanol and the product was recrystallised from 1-propanol after decolourisation with norit. The material was again recrystallised from glacial acetic acid to give 2.0 g (60%) of 1-phenyl-3-(D-*erythro*-trihydroxypropyl)flavazole (54) mp 218° (lit.[5] mp 218°).

4.38 1-Phenylflavazole-3-carboxaldehyde[3] (55)

A solution of 3.2 g (0.01 mole) of 1-phenyl-3-(D-*erythro*-trihydroxypropyl)flavazole (54) in 300 ml of thiophene free dry benzene was mixed with 13.3 g (0.03 mole) of freshly prepared lead tetraacetate and the mixture was heated under reflux for 5 hours under a calcium chloride guard tube on a boiling water bath.

An examination of the reaction mixture by tlc showed that the reaction was complete. After cooling the mixture, 5.0 ml of water was added and stirred well to decompose unreacted lead tetraacetate. The inorganic materials filtered off, washed with few ml of benzene and the benzene layer after drying with anhydrous sodium sulphate was concentrated to dryness. The residue was recrystallised from ethanol to give 2.4 g (87%) of 1-phenylflavazole-3-carboxaldehyde (55) mp 144° (lit.[3] mp 144°).

4.39 1-Phenylflavazole-3-carboxylic acid (40)

a) By the oxidation of 1-phenylflavazole-3-carboxaldehyde[4], (55)

To a solution of 540 mg (0.002 mole) of 1-phenylflavazole-3-carboxaldehyde (55) in 10 ml of glacial acetic acid was added dropwise with stirring a solution of 5 g of chromium trioxide in 90 ml of glacial acetic acid until oxidation was complete. The excess chromic acid was decomposed by adding few drops of isopropanol. The reaction mixture was extracted with chloroform, the chloroform extract washed with

water, dried with anhydrous sodium sulphate and evaporated to dryness. The residue was recrystallised from ethanol to give 500 mg (86%) of 1-phenylflavazole-3-carboxylic acid (40) mp 244° (lit.[4] mp 244°).

b) By hydrolysis of 1-phenyl-3-trichloromethyl flavazole (39)

A mixture of 180 mg (0.0005 mole) of 39 and 20 ml of orthophosphoric acid (88-93%) was heated with stirring at 150° for 30 hours in an oil bath. It was cooled and poured into 100 ml of cold water and kept overnight at room temperature. The crystallised yellow product was filtered, washed with water, dried and recrystallised from 50% acetic acid to give 110 mg (76%) of 1-phenylflavazole-3-carboxylic acid (40) mp 244°. Mixed melting point of the samples obtained by the two methods was undepressed.

4.40 Quinoxaline-2-carboxylic acid[93] (42)

a) By oxidation of quinoxaline-2-carboxaldehyde (2)

To a solution of 360 mg (0.004 mole) of quinoxaline-2-carboxaldehyde (2) in 30 ml of acetone was added dropwise with vigorous stirring, 7.0 ml

of 5% potassium permanganate solution. After the reaction was complete the excess permanganate was decomposed by adding few drops of ethanol. The precipitated manganese dioxide was filtered off, the acetone was removed under reduced pressure, the remainder was diluted with 30 ml of water, cooled and acidified with conc. hydrochloric acid. The colourless crystals formed were cooled in ice, filtered, washed with cold water and dried to give 450 mg (65%) of quinoxaline-2-carboxylic acid (42) mp 197° (decomp) (lit.[93] mp 197°).

b) By oxidation of 2-(D-arabino-tetrahydroxybutyl)quinoxaline (1)

A solution of 1.0 g (0.004 mole) of 2-(D-arabino-tetrahydroxybutyl)quinoxaline (1) in 50 ml of acetone was oxidised with 80 ml of 5% potassium permanganate solution as described above to give 470 mg (68%) of quinoxaline-2-carboxylic acid (42) mp 197°.

4.41 Quinoxaline-2-carbonylchloride[93] (43)

A mixture of 350 mg (0.002 mole) of quinoxaline-2-carboxylic acid (42) and 5.0 ml of thionyl chloride was heated under reflux for 1 hour on a boiling water bath.

It was cooled and the excess thionyl chloride was removed under reduced pressure. The solid was recrystallised from dry petroleum ether to give 300 mg (78%) of quinoxaline-2-carbonylchloride (43) mp 113° (decomp) (lit.[93] mp 113°).

4.42 Quinoxaline-2-carbonylphenylhydrazide (44)

A mixture of 430 mg (0.004 mole) of phenylhydrazine in 50 ml of ether and 50 ml of saturated sodium bicarbonate solution was stirred and 760 mg (0.004 mole) of quinoxaline-2-carbonylchloride (43) dissolved in 200 ml of dry ether was added dropwise. After the addition was complete, the mixture was stirred for 1 hour. The ether layer was evaporated under reduced pressure. The precipitated material was filtered, washed with water, dried and recrystallised from ethanol to give 810 mg (77%) of quinoxaline-2-carbonylphenylhydrazide (44) mp 187°.

IR(KBr): 3260 and 3300 cm^{-1}(NH-NH), 1670 cm^{-1}(C=O).

UV : λ_{max}^{MeOH} 242.6 nm(ϵ 1.1 x 10^5).

Anal. Calcd. for $C_{15}H_{12}N_4O$: C, 68.67; H, 4.58; N, 21.20.

Found: C, 68.42; H, 4.49; N, 21.42.

Hydrolysis of 520 mg (0.002 mole) of 44 with 25 ml of 1 M sodium hydroxide solution gave 270 mg (78%) of quinoxaline-2-carboxylic acid (42) mp 197°. A mixture melting point determination with an authentic sample of 42 was not depressed.

4.43 Attempted cyclisation of quinoxaline-2-carbonyl-phenylhydrazide (44)

a) Using phenylhydrazine

A mixture of 520 mg (0.002 mole) of quinoxaline-2-carbonylphenylhydrazide (44), 0.5 ml of freshly distilled phenylhydrazine, 5.0 ml of 1 N HCl, 0.5 ml of glacial acetic acid and 50 ml of 60% aqueous n-propanol was heated under reflux for 24 hours on a boiling water bath. An examination of the reaction mixture by tlc indicated that no new product was formed. On cooling, the starting material crystallised out which was recrystallised from ethanol to give 460 mg (88%) of 44, mp 187° and mixed mp 187°.

b) Using lead tetraacetate

To a solution of 520 mg (0.002 mole) of quinoxaline-2-carbonylphenylhydrazide (44) in 50 ml of

thiophene free dry benzene cooled in ice was added 900 mg (0.002 mole) of lead tetraacetate with vigorous stirring. The mixture was stirred for 1 hour at 0^{o}. Tlc did not indicate the formation of any new product. Decomposition of the excess lead tetraacetate with water followed by separation and evaporation of benzene layer did not yield any clean product.

c) Using azobenzene

A mixture of 520 mg (0.002 mole) of quinoxaline-2-carbonylphenylhydrazide (44), 0.37 g (0.002 mole) of azobenzene, 5.0 ml of 1 N HCl, 0.5 ml of glacial acetic acid and 50 ml of 60% aqueous n-propanol was heated under reflux for 20 hours on a boiling water bath. Tlc indicated that no reaction took place. The mixture was cooled, filtered, washed with water and recrystallised from ethanol to give 450 mg (85%) of the starting material, 44, mp 187^{o}.

d) Using benzoyl peroxide

A mixture of 520 mg (0.002 mole) of quinoxaline-2-carbonylphenylhydrazide (44), 50 ml of 60% aqueous n-propanol, 0.5 ml of glacial acetic acid and 0.5 g of

benzoyl peroxide was heated under reflux for 24 hours on a boiling water bath. An examination of the reaction mixture by tlc indicated that no new product was formed. On cooling, the starting material crystallised out which was recrystallised from ethanol to give 450 mg (87%) of 44, mp 187° and mixed mp 187°.

4.44 Ethyl mesoxalate[94] (46)

A mixture of 40 g (2 mole) of diethyl malonate and 14 g (1 mole) of selenium dioxide was heated at 120-130° for 2 hours. The precipitated selenium was removed by decantation. The liquid was distilled under vacuum to give the fractions (A) upto 80°/45 mm, 2 ml; (B) 80-130°/36 mm, 25 ml and (C) 130-230°/36 mm, 4 ml. Fraction (C) was a complex, garlic smelling mixture of selenium containing compounds and was rejected. Fraction (B) was extracted with water (7 x 10 ml) and the extracts were quickly evaporated separately until they became viscous and yellow. On cooling in ice, they crystallised slowly giving 7.75 g (32.3%) of white ethyl mesoxalate hydrate, mp 56° (lit.[94] mp 56°). On drying the liquid remaining from the aqueous extract, 12 ml of diethyl malonate, bp 193-198° was recovered.

4.45 Ethyl 2-hydroxy-3-quinoxaline carboxylate[95,96] (47)

A solution of 1.1 g (0.01 mole) of o-phenylene diamine in 30 ml of 1 N HCl and 1.75 g (0.01 mole) of ethyl mesoxalate (46) was stirred 30 minutes at room temperature. Crystals of ethyl 2-hydroxy-3-quinoxaline carboxylate (47) were formed. The mixture was cooled in a refrigerator overnight, filtered, washed with a little of ice cold water and recrystallised from hot water to give 1.96 g (93%) of ethyl 2-hydroxy-3-quinoxaline carboxylate (47) mp 176° (lit.[95,96] mp 175.5-176.5°).

4.46 2-Hydroxy-3-quinoxaline carboxylic acid[96] (48)

A mixture of 1.0 g (0.005 mole) of ethyl 2-hydroxy-3-quinoxaline carboxylate (47) and 10 ml of 5% sodium hydroxide solution was heated 30 minutes on a boiling water bath. It was cooled and acidified with conc. hydrochloric acid. The mixture was cooled overnight in a refrigerator, filtered, washed with a little ice cold water and recrystallised from hot water to give 0.75 g (78%) of 2-hydroxy-3-quinoxaline carboxylic acid (48) mp 264° [lit.[96] mp 263-265° (decomp)].

4.47 2-Hydroxyquinoxaline-3-carbonylchloride (49)

A mixture of 0.5 g (0.0025 mole) of 2-hydroxy-3-quinoxaline carboxylic acid (48) and 5.0 ml of thionyl chloride was heated under reflux for 1 hour under a $CaCl_2$ guard tube. The excess thionyl chloride was removed under reduced pressure and the residue was recrystallised from petroleum ether to give 400 mg (77%) of 2-hydroxyquinoxaline-3-carbonylchloride (49) mp 210°(decomp).

Anal. Calcd. for $C_9H_5N_2O_2Cl$: C, 51.81; H, 2.42; N, 13.43.

Found: C, 52.09; H, 2.28; N, 13.65.

4.48 2-Hydroxyquinoxaline-3-carbonylphenylhydrazide (50)

a) From ethyl 2-hydroxy-3-quinoxaline carboxylate (47)

A mixture of 1.0 g (0.005 mole) of ethyl 2-hydroxy-3-quinoxaline carboxylate (47) and 4.0 ml of freshly distilled phenylhydrazine was heated 2 hours on a boiling water bath. The mixture was cooled, 200 ml of 1 N HCl was added and shaken well to dissolve the unreacted phenylhydrazine. The suspended dark brown

product was filtered, washed with water, dried and recrystallised from methanol to give 1.15 g (82%) of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50) mp 250° (decomp).

Anal. Calcd. for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99.
Found: C, 64.10; H, 4.64; N, 19.85.

b) From 2-hydroxyquinoxaline-3-carbonylchloride (49)

To a mixture of a solution of 1.0 ml of phenylhydrazine in 40 ml of ether and 40 ml of saturated sodium bicarbonate solution was added dropwise a solution of 520 mg (0.0025 mole) of 2-hydroxyquinoxaline-3-carbonylchloride (49) in 250 ml of dry ether with vigorous stirring. After the addition was complete stirring was continued for 1 hour. The ether layer was evaporated from the mixture under reduced pressure, the dark brown crystals formed were filtered, washed with water and a little of ice cold methanol and recrystallised from methanol to give 550 mg (79%) of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50) mp 250° (decomp). Mixed melting point with an authentic sample from (a) was undepressed.

4.49 Attempted cyclisation of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50) using glacial acetic acid

A mixture of 1.4 g (0.005 mole) of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50) and 10 ml of glacial acetic acid was heated under reflux for 10 hours at 140° in an oil bath. An examination of the reaction mixture by tlc indicated that no new product was formed. The mixture was cooled and poured into 100 ml of water, cooled, filtered, washed with water and dried. Recrystallised from methanol to give 1.2 g (86%) of the starting material, (50) mp 250°.

4.50 3-Hydroxy-1-phenylflavazole (45)

a) From 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50)

A mixture of 560 mg (0.002 mole) of 50 in 25 ml of glacial acetic acid and 200 mg of p-toluenesulphonic acid was heated 4 hours on a boiling water bath. The reaction mixture was cooled and poured into about 200 gm of ice with stirring. When the ice melted, the solution was neutralised

with sodium bicarbonate, cooled, filtered, washed with a little of ice cold water and recrystallised from 2:1 methanol-water to give 425 mg (81%) of 3-hydroxy-1-phenylflavazole (45) mp 259° (decomp).

MS : m/e 262(M^+), 245(M^+ - OH), 233(M^+ - COH), 171(M^+ - C_6H_5N), 77($C_6H_5^+$), etc.

IR(KBr): 3300-2800 cm^{-1}(broad OH).

UV : λ_{max}^{MeOH} 283.8 nm(ε 1.44 x 10^5) shifted to λ_{max}^{MeOH} 300 nm(ε 1.5 x 10^5) in the presence of sodium hydroxide.

Anal. Calcd. for $C_{15}H_{10}N_4O$: C, 68.69; H, 3.84; N, 21.37.
Found: C, 68.50; 4.05; N, 21.67.

b) From 3-amino-1-phenylflavazole (58)

A mixture of 130 mg (0.0005 mole) of 3-amino-1-phenylflavazole (58) and 4.0 ml of conc. hydrochloric acid was heated for 1 hour on a water bath at 60° and then cooled and maintained at -5° using a freezing mixture. An ice cold solution of sodium nitrite (0.5 g in 4.0 ml of water) was added dropwise with vigorous stirring. Stirring was continued

for 1 hour at -5°, 20 ml of water was added and again stirred 2 hours at room temperature, cooled, filtered, washed with a little of ice cold water and recrystallised from hot water to give 100 mg (76%) of 3-hydroxy-1-phenylflavazole (45) mp 259° (decomp), identical in all respects with a sample prepared previously.

c) From 3-chloro-1-phenylflavazole (52)

A mixture of 60 mg (0.00019 mole) of 3-chloro-1-phenylflavazole (52) and 2.0 ml of 10% ethanolic potassium hydroxide was heated for 65 hours at 150° in a sealed corning glass tube inside a muffled furnace. Ethanol was removed under reduced pressure from the reaction mixture and the residue was diluted to 25 ml with water. The aqueous solution after extraction with chloroform to remove the traces of unreacted 3-chloro-1-phenylflavazole (52) was acidified with conc. hydrochloric acid and cooled. The crystalline material was filtered, washed with water, dried and recrystallised from 9:1 chloroform-hexane to give 35 mg (70%) of 3-hydroxy-1-phenyl-flavazole (45) mp 259°, identical in all respects with the sample prepared previously.

4.51 3-Acetyloxy-1-phenylflavazole (51)

A mixture of 130 mg (0.0005 mole) of 3-hydroxy-1-phenylflavazole (45) in 5.0 ml of dry pyridine and 2.0 ml of acetic anhydride was stirred for 24 hours at 50° under a $CaCl_2$ guard tube. The reaction mixture was cooled and poured into 100 ml of ice cold water with stirring and kept overnight. The crystals were filtered, washed with water, dried and recrystallised from hexane-chloroform (1:1) to give 120 mg (79%) of 3-acetyloxy-1-phenylflavazole (51) mp 219° (decomp).

Anal. Calcd. for $C_{17}H_{12}N_4O_2$: C, 67.09; H, 3.97; N, 18.42.
Found: C, 67.40; H, 3.90; N, 18.57.

4.52 Attempted conversion of 3-hydroxy-1-phenylflavazole (45) into 3-chloro-1-phenylflavazole (52)

a) Using phosphorous oxychloride

A mixture of 130 mg (0.0005 mole) of 3-hydroxy-1-phenylflavazole (45) and 10 ml of phosphorous oxychloride was heated under reflux for 30 hours at 130° under a $CaCl_2$ guard tube. An examination of the reaction

mixture by tlc indicated that no new product was formed. The reaction mixture was poured into about 50 ml of ice cold water with stirring, neutralised with saturated solution of sodium bicarbonate, cooled, filtered, washed with a little of ice cold water, dried and recrystallised from 2:1 methanol-water to give 100 mg (77%) of starting material, mp 259°.

b) Using phosphorous pentachloride

A mixture of 130 mg (0.0005 mole) of 3-hydroxy-1-phenylflavazole (45) and 200 mg of phosphorous pentachloride was fused on a flame. An examination of the reaction mixture by tlc indicated that no 3-chloro-1-phenylflavazole (52) was formed.

c) Using a mixture of phosphorous oxychloride and phosphorous pentachloride

A mixture of 130 mg (0.0005 mole) of 3-hydroxy-1-phenylflavazole (45), 10 ml of phosphorous oxychloride and 200 mg of phosphorous pentachloride was heated under reflux for 30 hours at 130° under a $CaCl_2$ guard tube. An examination of the reaction mixture by tlc indicated that no 3-chloro-1-phenyl-flavazole (52) was formed.

4.53 3-Chloro-1-phenylflavazole (52)

A mixture of 1.12 g (0.004 mole) of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (50) and 20 ml of freshly distilled phosphorous oxychloride was heated 12 hours under a $CaCl_2$ guard tube on a steam bath. The reaction mixture was cooled and poured into 200 ml of ice cold water with stirring. The crystalline material was filtered, washed with water and dried. It was dissolved in 50 ml of carbon tetrachloride, purified by passing over a column of silica gel and recrystallised from hexane to give 800 mg (71%) of 3-chloro-1-phenylflavazole (52) mp 210°.

MS : m/e 280(M^+), 282($M^+ + 2$), 245($M^+ - Cl$), 219($M^+ - ClCN$), 77($C_6H_5^+$) etc.

UV : λ_{max}^{MeOH} 267.8 nm(ε 7.8 x 10^4), 334.2 nm(ε 1.9 x 10^4).

Anal. Calcd. for $C_{15}H_9N_4Cl$: C, 64.18; H, 3.23; N, 19.96.
Found: C, 64.10; H, 3.49; N, 19.82.

The aqueous filtrate after separating 52 gave an yellow crystalline substance on standing. It was cooled, filtered and recrystallised from methanol to

give 170 mg (16%) of 3-hydroxy-1-phenylflavazole (45) mp 259°, identical with a sample of the compound prepared earlier.

4.54 Conversion of 3-chloro-1-phenylflavazole (52) into 1-phenylflavazole (4)

A mixture of 140 mg (0.005 mole) of 3-chloro-1-phenylflavazole (52), 50 mg of red phosphorous and 3.0 ml of hydroiodic acid (sp gr 1.7) was heated under reflux for 3 hours with occasional shaking at 130-140° in an oil bath[100]. The mixture was cooled and diluted to 100 ml with water. The hydroiodic acid was neutralised with a dilute solution of potassium hydroxide and cooled overnight in a refrigerator. The yellow crystals of 1-phenylflavazole (4) was filtered, washed with water, dried and recrystallised from ethanol to give 90 mg (73%) of 1-phenylflavazole (4) mp 152°. A mixed melting point determination with an authentic sample of 4 was undepressed.

4.55 1-Phenylflavazole-3-carbonylchloride (56)

A mixture of 580 mg (0.002 mole) of 1-phenylflavazole-3-carboxylic acid (40) and 5.0 ml of thionyl chloride was heated under reflux for 2 hours

under a calcium chloride guard tube on a boiling water bath. It was cooled and excess thionyl chloride was removed under vacuum. The residue was recrystallised from petroleum ether to give 540 mg (88%) of 1-phenylflavazole-3-carbonylchloride (56) mp 203° (decomp).

Anal. Calcd. for $C_{16}H_9N_4OCl$: C, 62.24; H, 2.94; N, 18.15.

Found: C, 62.56; H, 2.98; N, 18.44.

4.56 1-Phenylflavazole-3-carboxamide (57)

A solution of 310 mg (0.001 mole) of 1-phenylflavazole-3-carbonylchloride (56) in 250 ml of dry ether was added dropwise to a cold ammonia solution (20 ml) with vigorous stirring. After the addition was complete stirring was continued for 1 hour more. From the mixture, the ether was removed under reduced pressure. The precipitated material was collected by filtration, washed with water and recrystallised from ethanol to give 270 mg (93%) of 1-phenylflavazole-3-carboxamide (57) mp 255° (decomp).

IR(KBr): 3390 and 3295 $cm^{-1}(NH_2)$, 1660 $cm^{-1}(C=O)$.

UV : λ_{max}^{MeOH} 249.8 nm(ϵ 4.86 x 10^5), 338.4 nm(ϵ 1.16 x 10^5).

Anal. Calcd. for $C_{16}H_{11}N_5O$: C, 66.42; H, 3.83; N, 24.21.

Found: C, 66.23; H, 3.98; N, 24.28.

4.57 3-Amino-1-phenylflavazole (58)

a) By Hofmann reaction of 1-phenylflavazole-3-carboxamide (57)

A solution of 300 mg (0.001 mole) of 1-phenylflavazole-3-carboxamide (57) in 10 ml of methanol was mixed with 8.0 ml of 0.5 N sodium hypochlorite and the mixture was kept at 60° for 30 hours with constant stirring. The reaction mixture was diluted to 100 ml with water, cooled, filtered, washed with water and dried. The red crystalline substance recrystallised from hexane to give 230 mg (88%) of 3-amino-1-phenylflavazole (58) mp 205° (decomp).

MS : m/e 261(M^+), 245($M^+ - NH_2$), 219($M^+ - H_2NCN$).

IR(CCl_4): 3460 and 3380 cm^{-1}(NH_2).

UV : λ_{max}^{MeOH} 290.6 nm(ϵ 1.35 x 10^5), 335.0 nm(sh).

Anal. Calcd. for $C_{15}H_{11}N_5$: C, 68.95; H, 4.24; N, 26.81.

Found: C, 69.12; H, 4.39; N, 26.98.

b) From 3-chloro-1-phenylflavazole (52)

A mixture of 50 mg (0.00017 mole) of 3-chloro-1-phenylflavazole (52), 2.0 ml of ethanol and 2.0 ml of liquor ammonia was heated for 70 hours at 200° in a sealed glass tube inside an oven. The reaction mixture was cooled, diluted to 100 ml with water, cooled in ice, filtered, washed with water, dried and re-crystallised from hexane to give 40 mg (91%) of 3-amino-1-phenylflavazole (58) mp 205°. A mixed melting point determination with the sample prepared previously was undepressed.

4.58 3-Acetamido-1-phenylflavazole (59)

A mixture of 130 mg (0.0005 mole) of 3-amino-1-phenylflavazole (58) in 5.0 ml of dry pyridine and 2.0 ml of acetic anhydride was stirred 24 hours at room temperature under a $CaCl_2$ guard tube. The reaction mixture was poured into 100 ml of ice cold water with vigorous stirring and kept overnight. The precipitated material was filtered, washed with water and dried. The crystals were recrystallised from hexane-chloroform (5:1) to give 125 mg (83%) of 3-acetamido-1-phenylflavazole (59) mp 214°.

IR(KBr): 3410 cm^{-1}(NH), 1720 cm^{-1}(CO).

UV : λ_{max}^{MeOH} 264.2 nm(ε 5.15 x 10^4), 336 nm(ε 1.46 x 10^4).

Anal. Calcd.for $C_{17}H_{13}N_5O$: C, 67.31; H, 4.32; N, 23.09.

Found: C, 67.48; H, 4.63; N, 22.89.

4.59 3-(N-Pyrrolidyl)-1-phenylflavazole (60)

A mixture of 280 mg (0.001 mole) of 3-chloro-1-phenylflavazole (52) and 10 ml of pyrrolidine was heated for 30 minutes on a boiling water bath. The mixture was cooled and poured into about 200 ml of ice cold water. The crystals were filtered, washed with water, dried and recrystallised from hexane to give 275 mg (87%) of 3-(N-pyrrolidyl)-1-phenylflavazole (60) as blood red shining crystals, mp 230° (decomp).

NMR($CDCl_3$): δ 2.1(4,t,CH_2-CH_2), 4.0(4,t,CH_2NCH_2), 7.0-8.7(9,Complex m, aromatic).

UV : λ_{max}^{MeOH} 300.0 nm(ε 2.0 x 10^5), 340.0 nm(sh).

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.35; H, 5.43; N, 22.21.

Found: C, 72.36; H, 5.68; N, 22.47.

4.60 3-(1-Phenylhydrazono-L-*threo*-2,3-4-trihydroxybutyl)-2-quinoxalinone[46] (*61*)

A mixture of 17.6 g (0.1 mole) of L-ascorbic acid, 400 ml of water, 250 ml of ethanol, 10.8 g (0.1 mole) of o-phenylene diamine, 21.6 g (0.2 mole) of phenylhydrazine and 15 ml of glacial acetic acid was heated under reflux for 1 hour. On cooling, a red crystalline product separated out which was recrystallised from ethanol to give 26.5 g (75%) of 3-(1-phenylhydrazono-L-*threo*-2,3,4-trihydroxybutyl)-2-quinoxalinone (*61*) mp 216° (lit.[46] mp 216°).

4.61 3-[1-(Phenylhydrazono)glyoxal-1-yl]-2-quinoxalinone[46] (*62*)

A solution of 3.5 g (0.01 mole) of 3-(1-phenylhydrazono-L-*threo*-2,3,4-trihydroxybutyl)-2-quinoxalinone (*61*) in 150 ml of water was mixed with a solution of 6.4 g (0.03 mole) of sodium metaperiodate in 50 ml of water and the mixture was stirred for 4 hours at room temperature and then kept overnight in the dark. The crystals were filtered off, washed with water and recrystallised from butanol to give 2.4 g (82%) of 3-[1-(phenylhydrazono)glyoxal-1-yl]-2-quinoxalinone (*62*) 235° (lit.[46] mp 235°).

4.62 3-[2-Hydroxy-1-(phenylhydrazono)ethyl]-2-quinoxalinone[45] (63)

A mixture of 0.58 g (0.02 mole) of 3-[1-(phenylhydrazono)glyoxal-1-yl]-2-quinoxalinone (62) in 15 ml of N,N-dimethylformamide and 10 ml of methanol and 0.7 g of sodium borohydride was stirred at room temperature for 1 hour and then kept for a further 4 hours. The solution was diluted with water and the product formed was filtered and recrystallised from ethanol, to give 0.4 g (68%) of 3-[2-hydroxy-1-(phenylhydrazono)ethyl]-2-quinoxalinone (63) mp 230° (lit.[45] mp 230°).

4.63 3-(Hydroxymethyl)-1-phenylflavazole (64)

a) From 3-[2-hydroxy-1-(phenylhydrazono)ethyl)-2-quinoxalinone[45] (63)

A solution of 0.3 g (0.001 mole) of 3-[2-hydroxy-1-(phenylhydrazono)ethyl]-2-quinoxalinone (63) in 25 ml of 0.01 M sodium hydroxide and few drops of 1-butanol was boiled under reflux for 1 hour in an oil bath. The mixture was cooled, the crystalline product was filtered off, washed with water and recrystallised from ethanol to give 0.24 g (87%) of 3-(hydroxymethyl)-1-phenylflavazole (64) mp 195° (lit.[45] mp 205°).

b) From 1-phenylflavazole-3-carboxaldehyde (55)

A solution of 270 mg (0.001 mole) of 1-phenylflavazole-3-carboxaldehyde (55) in 50 ml of ethanol was mixed with 40 mg (0.001 mole) of sodium borohydride was stirred for 1 hour at room temperature. The reaction was shown to be completed by tlc. The solution was concentrated to 10 ml under reduced pressure, the residue was diluted to 50 ml with water, cooled in ice, filtered and recrystallised from ethanol to give 250 mg (91%) of 3-(hydroxymethyl)-1-phenyl-flavazole (64) mp 195° (lit.[45] mp 205°). A mixed mp determination of the two samples was undepressed.

4.64 3-(Acetyloxymethyl)-1-phenylflavazole[45] (65)

A mixture of 280 mg (0.001 mole) of 3-(hydroxymethyl)-1-phenylflavazole (64) in 10 ml of dry pyridine and 5 ml of acetic anhydride was stirred for 24 hours at room temperature. The reaction mixture was poured into 50 ml of ice-cold water and stirred well. The product was filtered, washed with water, dried and recrystallised from ethanol to give 290 mg (91%) of 3-(acetyloxymethyl)-1-phenylflavazole (65) mp 158° (lit.[45] mp 163-164°).

4.65 3-(Benzoyloxymethyl)-1-phenylflavazole (66)

A mixture of 280 mg (0.001 mole) of 3-(hydroxymethyl)-1-phenylflavazole (64) in 10 ml of pyridine and 1.0 ml of benzoyl chloride was stirred for 24 hours at room temperature. The reaction mixture was poured into 50 ml of ice cold water and stirred well. The product was filtered, washed with water, dried and recrystallised from ethanol to give 310 mg (85%) of 3-(benzoyloxymethyl)-1-phenylflavazole (66) mp 144° (lit.[45] mp 192°).

4.66 3-(Chloromethyl)-1-phenylflavazole (67)

A mixture of 280 mg (0.001 mole) of 3-(hydroxymethyl)-1-phenylflavazole (64) and 10 ml of thionyl chloride was heated under reflux for 2 hours under a calcium chloride guard tube. The excess thionyl chloride was removed under vacuum and the residue was recrystallised from ethanol to give 250 mg (85%) of 3-(chloromethyl)-1-phenylflavazole (67) mp 215° (decomp).

NMR($CDCl_3$): δ 5.25(2,s,CH_2), 7.3-8.8(9,Complex m, aromatic).

UV : λ_{max}^{MeOH} 267 nm(ϵ 1.09 x 10^5), 333.8 nm(ϵ 3.13 x 10^4).

Anal. Calcd. for $C_{16}H_{11}N_4Cl$: C,65.20; H, 3.76; N, 19.01.
Found: C, 65.48; H, 3.55; N, 19.14.

4.67 3-(N-Pyrrolidylmethyl)-1-phenylflavazole (68)

A mixture of 300 mg (0.001 mole) of 3-(chloromethyl)-1-phenylflavazole (67) and 10 ml of pyrrolidine was heated on a boiling water bath for 3 hours. The reaction mixture was cooled and poured into 100 ml of ice cold water with stirring. The yellow crystals formed were filtered, washed with water and recrystallised from 90% methanol to give 290 mg (88%) of 3-(N-pyrrolidylmethyl)-1-phenylflavazole (68) mp 162°.

NMR($CDCl_3$): δ 1.85(2,t,CH_2CH_2), 2.9(2,t,CH_2NCH_2), 4.4(2,s,CH_2), 7.3-8.7(9,m,aromatic).

UV : λ_{max}^{MeOH} 267.6 nm(ϵ 6.07 x 10^4), 336.6 nm(ϵ 1.4 x 10^4).

Anal. Calcd. for $C_{20}H_{19}N_5$: C, 72.92; H, 4.81; N, 21.26.
Found: C, 72.98; H, 5.06; N, 21.56.

4.68 1-Phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid (69)

A suspension of finely powdered 1-phenylflavazole (4) (550 mg, 0.002 mole) in a solution of

4% potassium permanganate in water was boiled under reflux for 5 hours with vigorous stirring. The excess permanganate was removed by adding few drops of methanol and heating for about 10 minutes. The mixture was filtered while hot through a sintered glass funnel and washed with 25 ml of water. The filtrate and washings were combined and concentrated to 50 ml under reduced pressure, cooled in ice and acidified with conc. hydrochloric acid. The solution was cooled overnight in a refrigerator, the crystals were filtered, washed with a little of ice cold water and recrystallised from hot water to give 350 mg (62%) of 1-phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid (69) mp 236° (decomp).

MS : m/e 266($M^+ - H_2O$), 240($M^+ - CO_2$), 222($M^+ - H_2O,CO_2$), 194($M^+ - H_2O,CO,CO_2$), 77($C_6H_5^+$)etc.

Anal. Calcd. for $C_{13}H_8N_4O_4$: C, 54.93; H, 2.84; N, 19.72.
Found: C, 55.28; H, 2.99; N, 19.98.

4.69 Dimethyl 1-phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylate (70)

A mixture of 18 ml of 50% aqueous potassium hydroxide and 60 ml of ether was cooled to 5° and 6.2 g of nitrosomethylurea was added with stirring. The

ether layer containing diazomethane was immediately separated and added to a cold solution of 280 mg (0.001 mole) of 1-phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid (69) in 150 ml of methanol. After the completion of addition the mixture was stirred 1 hour and the solvent was evaporated to dryness under reduced pressure. A 5% sodium bicarbonate solution (50 ml) was added to the residue and stirred for 1 hour at room temperature. The mixture was extracted with ether, the ether layer was dried (Na_2SO_4) and evaporated to dryness and the residue recrystallised from methanol to give 270 mg (87%) of dimethyl 1-phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylate (70) mp 140°.

MS : m/e 312(M^+), 281($M^+ - OCH_3$), 253($M^+ - COOCH_3$), 222($M^+ - CH_3O$, $COOCH_3$), 194($M^+ - 2COOCH_3$), 77($C_6H_5^+$) etc.

NMR($CDCl_3$): δ 4.1(6,s,$2CH_3$), 7.6 and 8.3(5,m,phenyl), 8.65(1,s,3H).

UV : λ_{max}^{MeOH} 268.6 nm(ϵ 5.3 x 10^5).

Anal. Calcd. for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.95.

Found: C, 57.60; H, 4.18; N, 17.80.

4.70 Attempted reduction of 1-phenylflavazole (4) with lithium aluminium hydride

A solution of 125 mg (0.0005 mole) of 1-phenylflavazole (4) in 200 ml of dry ether was mixed with 500 mg of lithium aluminium hydride and the mixture was stirred for 70 hours under a calcium chloride guard tube. An examination of the reaction mixture by tlc indicated that no new product was formed. The ether layer was separated, concentrated to dryness and recrystallised from ethanol to give 110 mg (88%) of 4, mp 152°.

4.71 2-Anilinoquinoxaline-3-carboxamide (71)

A solution of 370 mg (0.0015 mole) of 1-phenylflavazole (4) in 200 ml of isopropanol was mixed in 200 mg portions with 1.0 g of powdered sodium borohydride and the mixture was heated under reflux for 80 hours on a boiling water bath. The unreacted sodium borohydride was decomposed by the addition of a few ml of water, the isopropanol was removed under reduced pressure and 50 ml of water was added to the residue. The precipitate was filtered, washed with water and dried. A tlc examination showed that

the product contained two compounds which were separated on a column of silica gel using benzene as solvent to give 50 mg (13.5%) of 1-phenylflavazole (4) mp 152° and 230 mg (58%) of 2-anilinoquinoxaline-3-carboxamide (71) as orange needles mp 220° after recrystallisation from chloroform-hexane.

MS : m/e 264($M^{+\cdot}$), 220($M^+ - CONH_2$), 91($C_6H_5N^+$), 77($C_6H_5^+$) etc.

IR(KBr): 3410 and 3320 cm^{-1}(NH_2), 1680 cm^{-1}(C=O).

UV : λ_{max}^{MeOH} 220.4 nm(ε 1.01 x 10^5), 288.2 nm(ε 1.2 x 10^5).

Anal. Calcd. for $C_{15}H_{12}N_4O$: C, 68.16; H, 4.58; N, 21.24.

Found: C, 68.35; H, 4.83; N, 21.39.

4.72 2-Anilinoquinoxaline-3-carboxylic acid (73)

a) From the mother liquor of 71

The mother liquor after separating 71 was cooled, acidified with hydrochloric acid and extracted with chloroform. The extract was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The yellow residue was recrystallised from 1:5 chloroform-pentane

to give 40 mg (10%) of 2-anilinoquinoxaline-3-carboxylic acid (73) mp 165° (decomp).

IR(CCl_4): 3300-2800 cm^{-1}(broad) and 1730 cm^{-1}(COOH).

UV of sodium salt: λ_{max}^{MeOH} 283.6 nm(ε 9.7×10^4),398 nm(ε 1.8×10^4)
[lit.[101] λ_{max}^{EtOH} 285 nm(ε 29300), 395 nm(ε 6250)].

b) From 2-anilinoquinoxaline-3-carboxamide (71)

A mixture of 100 mg (0.000375 mole) of 2-anilinoquinoxaline-3-carboxamide (71), 25 ml of 10% sodium hydroxide solution and 20 ml of n-propanol was heated under reflux for 4 hours. The reaction mixture was evaporated to 25 ml, cooled in an ice bath, neutralised with hydrochloric acid and extracted with chloroform. The extract was dried with anhydrous sodium sulphate, evaporated to dryness under reduced pressure and the residue recrystallised from 1:5 chloroform-pentane to give 85 mg (85%) of 2-anilino-quinoxaline-3-carboxylic acid (73) mp 165°. A mixed melting point determination with the sample from (a) was undepressed.

c) From 1-phenylflavazole (4)

A mixture of 200 mg (0.000815 mole) of 1-phenylflavazole (4), 25 ml of 10% sodium hydroxide

solution and 20 ml of n-propanol was heated under reflux for 25 hours. The reaction mixture was concentrated to 25 ml, cooled in an ice bath, neutralised with hydrochloric acid and extracted with chloroform. The extract was dried with anhydrous sodium sulphate, evaporated to dryness under reduced pressure and recrystallised from 1:5 chloroform-pentane to give 180 mg (83%) of 2-anilinoquinoxaline-3-carboxylic acid (73) mp 165° identical with the samples prepared previously.

4.73 2-Anilinoquinoxaline (74)

a) By the reaction of 2-anilinoquinoxaline-3-carboxamide (71) with conc. hydrochloric acid

A mixture of 100 mg (0.000375 mole) of 2-anilinoquinoxaline-3-carboxamide (71) and 15 ml of conc. hydrochloric acid was heated under reflux for 4 hours. The reaction mixture was concentrated to 5 ml under reduced pressure, cooled in an ice bath and neutralised with 10% sodium hydroxide solution. The mixture was cooled in a refrigerator overnight, filtered, washed with cold water, dried

and recrystallised from 1:9 chloroform-hexane to give 70 mg (84%) of 2-anilinoquinoxaline (74) mp 137° (lit.[102] mp 137°).

b) By heating of 2-anilinoquinoxaline-3-carboxylic acid (73) at 200°

2-Anilinoquinoxaline-3-carboxylic acid (73) (100 mg, 0.000375 mole) was heated in a test tube at 200° for 30 minutes in an oil bath. The mixture was purified by chromatographing over a column of silica gel using chloroform as solvent and the product was recrystallised from 1:9 chloroform-hexane to give 60 mg (72%) of 2-anilinoquinoxaline (74) mp 137°. A mixed melting point determination of the two samples obtained by (a) and (b) above was undepressed.

CHAPTER V

SUMMARY AND CONCLUSIONS

1-Phenylflavazole (1-phenyl-1-H-pyrazolo [3,4-b]quinoxaline) is known to be prepared by the treatment of quinoxaline-2-carboxaldehyde phenylhydrazone with phenylhydrazine. However, when stored or oxidised phenylhydrazine was used for this cyclisation, an unusual phenylation reaction was found to take place producing significant quantities of 1,3-diphenylflavazole. This phenylation reaction was established as taking place by a free radical mechanism involving phenyl radicals formed from oxidised phenylhydrazine. Benzoyl peroxide which also produces phenyl radicals gave 1,3-diphenylflavazole under the same reaction conditions, thus providing additional evidence for the free radical mechanism. By using oxidised substituted phenylhydrazines, a number of new flavazoles such as 1-p-tolyl-3-phenyl, 1-(p-chlorophenyl)-3-phenyl, 1-(p-bromophenyl)-3-phenyl and 1-phenyl-3-p-tolylflavazoles were also prepared and characterised. The structures of these compounds were confirmed by their spectral data.

1-Phenylflavazoles with different substituents at position 3 such as the amino, chloro, hydroxy, chloromethyl, trichloromethyl, carboxamido, N-pyrrolidyl

and N-pyrrolidylmethyl groups were also prepared for the first time. 3-Amino-1-phenylflavazole was prepared by two methods, ie, by the reaction of 3-chloro-1-phenylflavazole with ammonia and also by a Hofmann reaction of 1-phenylflavazole-3-carboxamide. Other interconversions of the 3-amino, 3-hydroxy and 3-chloro-1-phenylflavazoles were also investigated.

On the new reactions of flavazoles studied, the oxidation, reduction, bromination and hydrolysis reactions are worth mentioning. Thus the oxidation of 1-phenylflavazole produced 1-phenylpyrazolo [3,4-b]pyrazine-5,6-dicarboxylic acid which was also characterised as its dimethyl ester. The flavazole ring was not easily reduced either with lithium aluminium hydride or with sodium borohydride. When 1-phenyl-flavazole was heated under reflux with sodium boro-hydride in isopropyl alcohol, the heterocyclic ring was broken and 2-anilinoquinoxaline-3-carboxamide was produced showing that the sodium borohydride acted only as a base rather than as a reducing agent under the reaction conditions. Also treatment of 1-phenyl-flavazole with hot aqueous sodium hydroxide again ruptured the heterocyclic ring system and produced

2-anilinoquinoxaline-3-carboxylic acid. This carboxylic acid underwent decarboxylation easily when heated giving the known 2-anilinoquinoxaline. The treatment of both 1-phenylflavazole and 1,3-diphenylflavazole with bromine in acetic acid led to clean bromination at the para position of the 1-phenyl group, thus producing 1-(p-bromophenyl)flavazole and 1-(p-bromophenyl)-3-phenylflavazole respectively in excellent yields.

The cyclisation reaction of several quinoxaline-2-carboxaldehyde phenylhydrazones was shown to take place by using a mild dehydrogenating agent such as azobenzene. Also the same cyclisation took place although in a lower yield when these compounds were heated at a temperature above their melting points in an atmosphere containing oxygen.

An analysis of the mass spectra of several flavazoles showed similar fragmentation patterns. Also the flavazoles have characteristic ultraviolet absorptions as seen from the correlation of the uv spectra of a number of flavazole derivatives.

As a large number of previously prepared flavazole derivatives have shown significant biological

activity as potential diuretic, anti-inflammatory, analgesic, anti-leukaemic,tuberculostatic and immuno-chemical agents, all the new flavazoles obtained will be submitted for screening their pharmacological properties.

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