An Investigation into the Mechanism of Formation of Oxadiazoles and Arylidenehydrazides from the Action of Methanolic Potassium Hydroxide on 1,4-Dihydro-*s*-tetrazines.

Daniel Hunter and Douglas G. Neilson*
Department of Chemistry, The University of Dundee, Dundee, Scotland, DD1 4HN

3,6-Diaryl- and 3,6-dibenzyl-1,4-dihydro-*s*-tetrazines have been shown to be inert to methanolic alkali under nitrogen. Oxadiazoles derived from these substances by the action of alkali in methanol in air result from alkali attack on the tetrazines themselves and not on the reduced derivatives as previously believed. 1,4-Dihydro-3,6-bis(α-hydroxybenzyl)-*s*-tetrazine is likewise stable to alkali under nitrogen but on exposure to air yields the benzylidene derivative of mandelohydrazide. Mechanisms are here proposed for the formation of the oxadiazoles and hydrazides.

Our own work [1-4] and that of other groups [5-9] has clearly shown that the base-catalysed rearrangements of symmetrically 3,6-disubstituted *s*-tetrazines (**1**) and their 1,4-dihydro-derivatives† (**2**) are highly sensitive to the nature of the 3,6-substituents (Scheme 1). One unresolved problem in this work relates to the fact that the 3,6-dibenzyl-1,4-dihydrotetrazines (**2d, e**) afford the corresponding 2,5-dibenzyl-1,3,4-oxadiazoles (**5b,d**) on treatment with methanolic potassium hydroxide in air,[2,6] but are recovered unchanged when the reaction is carried out under nitrogen.[2] This suggests that the idea of a simple hydrolysis mechanism for the formation of an oxadiazole from a dihydrotetrazine (Scheme 2) does not hold and that an oxidative step is involved.[2]

Further evidence for the stability of 1,4-dihydrotetrazines towards alkali under nitrogen comes from the observation that the tetrazine (**1a**) yielded the pyrazolinone (**3a**) by a route involving a hydride transfer[3] to a further molecule of the tetrazine (**1a**) thus causing concomitant formation of the corresponding dihydrotetrazine (**2a**) (Scheme 3). When this reaction was carried out under nitrogen, the dihydrotetrazine (**2a**) was isolated itself or in the form of a 1:2 complex with the pyrazolinone (**3a**); when air was present however, the readily oxidisable dihydrotetrazine (**2a**) reverted to the tetrazine (**1a**) which was recycled. Thus the dihydrotetrazine formed in this reaction appears also to be stable to base under nitrogen.

This paper reports our attempts to explain this apparent difference in the reactivity of dihydrotetrazines towards alkali when under nitrogen as against when exposed to air. Our experiments show that the dihydrotetrazines are indeed unchanged by the action of alkali under nitrogen and that the identified products, *e.g.* oxadiazoles (**5b**) or (**5d**) from substrates (**2d**) or (**2e**) arise from the corresponding tetrazines (**1d,e**) formed by aerial oxidation of their dihydro derivatives. Furthermore, related experiments on 1,4-dihydro-3,6-bis(α-hydroxybenzyl)-*s*-tetrazines (**2a,b**) point to the isolated products, *viz.* the hydrazides (**6a,b**), also being derived from the corresponding tetrazines (**1a,b**) rather than directly from the dihydro compounds.

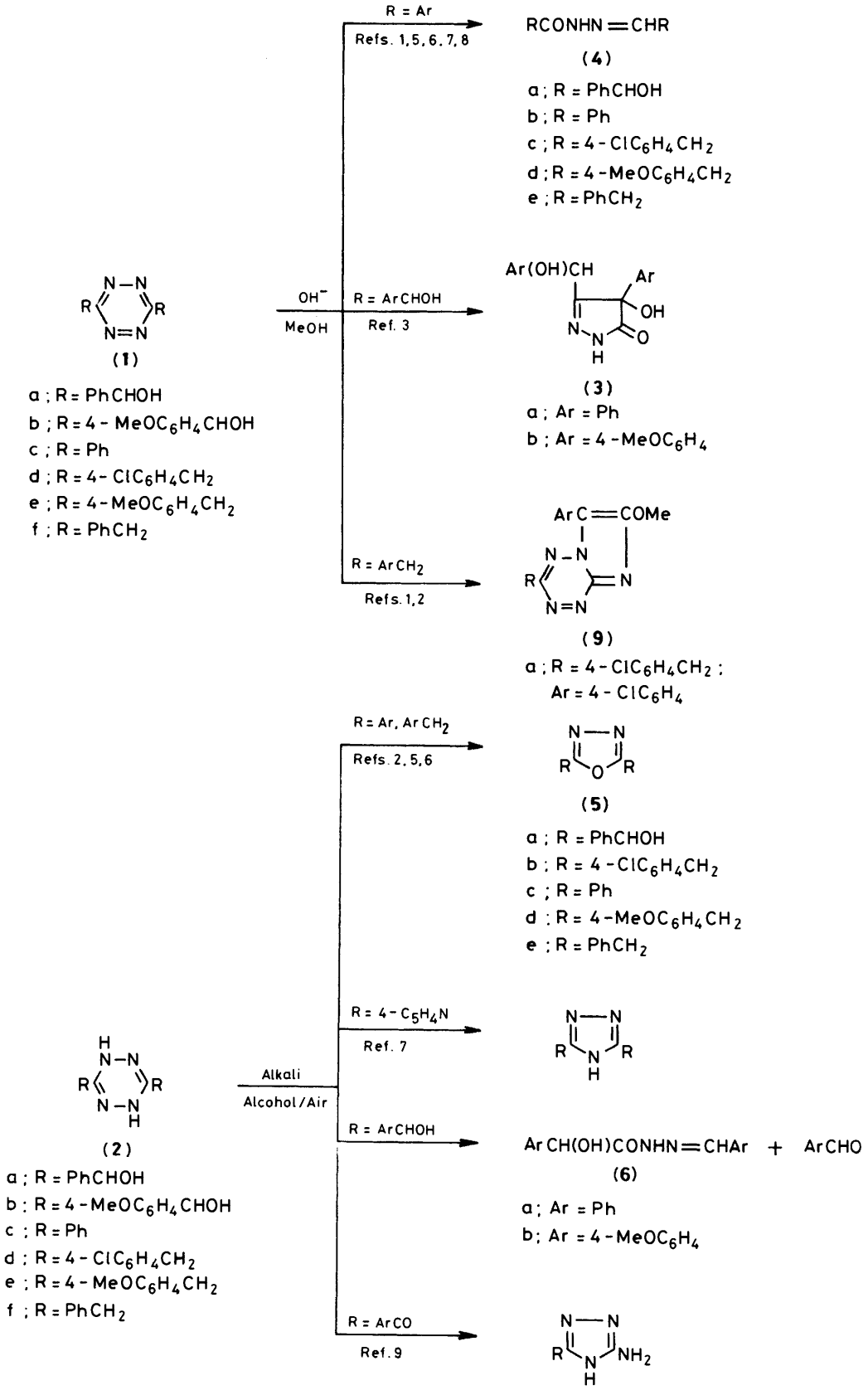
(a) *Action of Base on* 3,6-*Bis*(α-*hydroxybenzyl*)-s-*tetrazines* (**1a,b**) *and their* 1,4-*Dihydro Derivatives* (**2a,b**).—As stated above, 3,6-bis-(α-hydroxybenzyl)-*s*-tetrazine[3] (**1a**) yields the pyrazolinone (**3a**) on treatment with methanolic potassium hydroxide in air. When this reaction was carried out on the 4-methoxy-substituted analogue (**1b**), 4-methoxybenzaldehyde and the hydrazide (**6b**) were isolated in addition to the expected pyrazolinone (**3b**). Although more symmetrically substituted hydrazides of type (**4**) have commonly been reported as hydrolysis products of 3,6-diaryl-*s*-tetrazines (**1**; R = Ar), hydrazides of type (**6**) have not previously been reported as arising from tetrazines. As the formation of pyrazolinone (**3b**) from tetrazine (**1b**) gives concomitant formation of dihydrotetrazine (**2b**) as a by-product (Scheme 3), dihydrotetrazine was investigated as one potential source of the aldehyde and hydrazide (**6b**) in the above reaction sequence.

When the dihydrotetrazine (**2a**) was treated with methanolic potassium hydroxide in the presence of air, the hydrazide (**6a**) was isolated in good yield along with benzaldehyde. We could not isolate the free hydrazide, $PhCH(OH)CONHNH_2$, corresponding to the hydrazide (**6a**), despite attempts to do so. When the experiment was repeated in an atmosphere of nitrogen (under otherwise analogous conditions) the dihydrotetrazine (**2a**) was for the most part recovered unchanged, and no hydrazide (**6a**) or benzaldehyde was isolated. Hence an oxidative step appears to be required for the breakdown of the dihydrotetrazine (**2a**) in alkaline media. This oxidation may involve either tetrazine formation (**2a**)→(**1a**) or the oxidation of some other intermediate on the pathway (**2a**)→(**6a**).

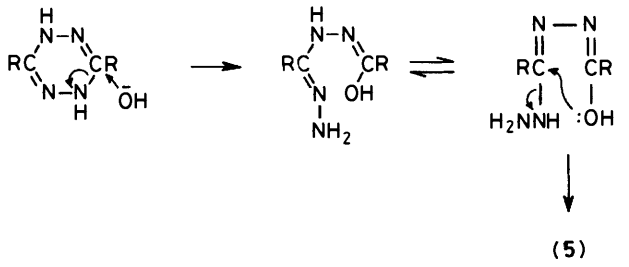
Regarding tetrazine as a possible source of the hydrazides (**6**), it was considered that if the reaction illustrated in Scheme 3 was carried out at much greater dilution (e.g. *ca.* 100-fold), some anion intermediate (**7**) might be unable to cyclise by transfer of a hydride ion to a further molecule of tetrazine, [*i.e.* (**1a**)→(**2a**)] but would in fact undergo protonation to give the hydrazide (**4a**). Although the pyrazolinone (**3a**) remained the main product of this reaction at high dilution, some hydrazide (**6a**), but no hydrazide of the type (**4a**) was isolated. The hydrazide (**4a**), prepared by the reduction of the condensation product of phenylglyoxal and mandelohydrazide, was then tested for stability under the same conditions as the foregoing reactions. The hydrazide (**4a**) was found to give low yields of the hydrazide (**6a**) on treatment with alkali but the quantities were too small (*i.e.* the reaction was too slow) to account for all the hydrazide (**6a**) formed *via* the dihydrotetrazine route. However, when benzaldehyde was introduced into the hydrazide (**4a**) reaction, facile exchange of aldehyde for mandelaldehyde took place and the hydrazide (**6a**) was formed in very high yield (Scheme 4).

In addition, mandelohydrazide but not benzaldehyde was found to react with the dihydrotetrazine (**2a**) to give the hydrazide (**6a**) but no free benzaldehyde; 4-methoxymandelohydrazide with the dihydrotetrazine (**2a**) similarly yielded the

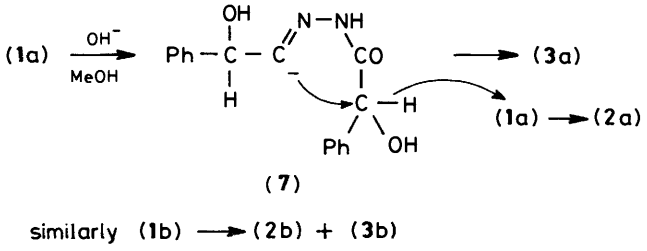
† There has been considerable confusion in the literature as to whether such species have 1,4- or 1,2-dihydro patterns. *X*-Ray evidence[2,10] now clearly points to the 1,4-dihydro structure (in the solid state at least) for compounds of type (**2**).


R = Ar
Refs. 1,5,6,7,8
RCONHN=CHR
(4)
a; R = PhCHOH
b; R = Ph
c; R = 4-ClC6H4CH2
d; R = 4-MeOC6H4CH2
e; R = PhCH2
OH⁻
MeOH
R = ArCHOH
Ref. 3
Ar(OH)CH
Ar
OH
(3)
a; Ar = Ph
b; Ar = 4-MeOC6H4
(1)
a; R = PhCHOH
b; R = 4-MeOC6H4CHOH
c; R = Ph
d; R = 4-ClC6H4CH2
e; R = 4-MeOC6H4CH2
f; R = PhCH2
R = ArCH2
Refs. 1,2
ArC=COMe
(9)
a; R = 4-ClC6H4CH2;
Ar = 4-ClC6H4
R = Ar, ArCH2
Refs. 2,5,6
(5)
a; R = PhCHOH
b; R = 4-ClC6H4CH2
c; R = Ph
d; R = 4-MeOC6H4CH2
e; R = PhCH2
R = 4-C5H4N
Ref. 7
Alkali
Alcohol/Air
(2)
a; R = PhCHOH
b; R = 4-MeOC6H4CHOH
c; R = Ph
d; R = 4-ClC6H4CH2
e; R = 4-MeOC6H4CH2
f; R = PhCH2
R = ArCHOH
ArCH(OH)CONHN=CHAr + ArCHO
(6)
a; Ar = Ph
b; Ar = 4-MeOC6H4
R = ArCO
Ref. 9
NH2

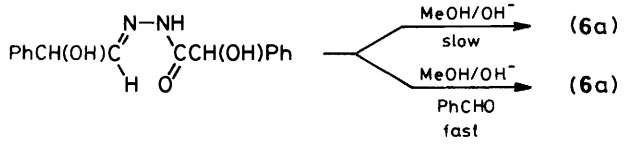
Scheme 1.



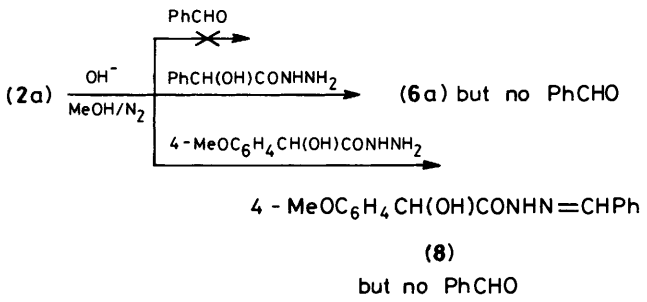
Scheme 2.



Scheme 3.



Scheme 4.

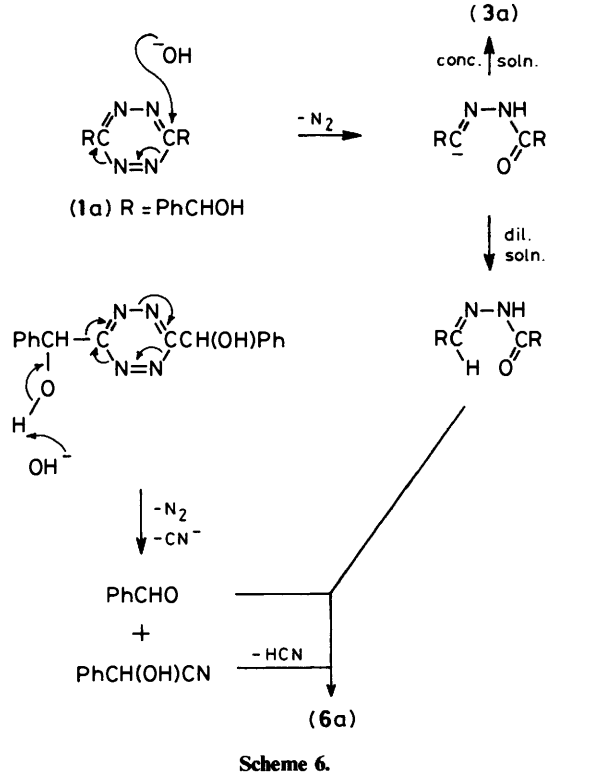


Scheme 5.

hydrazide (**8**) but no free benzaldehyde (Scheme 5). Although this shows that the dihydrotetrazine (**2a**) is susceptible to attack by an acid hydrazide, this is not envisaged as the major route to the hydrazides (**6**) as free mandelohydrazide has never been found in any of our reactions. Furthermore, in alkaline media any free mandelohydrazide would be very susceptible to attack by aldehyde (which is an identified product).

The presence of benzaldehyde can be accounted for by alkali attack on the tetrazine (**1a**) (Scheme 6) [this mechanism is preferred to a retro Diels-Alder process leading to mandelonitrile as a precursor of the benzaldehyde, as tetrazines of type (**1a,b**) are relatively stable in neutral or weakly acid media].

On the basis of the foregoing experiments, it is our contention that the dihydrotetrazines [*e.g.* (**2a**) and (**2b**)] are stable to base in methanol under nitrogen but when exposed to the air *slowly* oxidise to the corresponding tetrazine which is then attacked by the alkali. This tetrazine [*e.g.* (**1a**)] decomposes as shown in Scheme 6 (dilute solution pathway) giving the hydrazide [*e.g.* (**4a**)]; other tetrazine molecules yield benzaldehyde which



Scheme 6.

exchanges rapidly for mandelaldehyde in the hydrazide (**4a**), yielding the hydrazide (**6a**) and an excess of benzaldehyde as the final identified products.

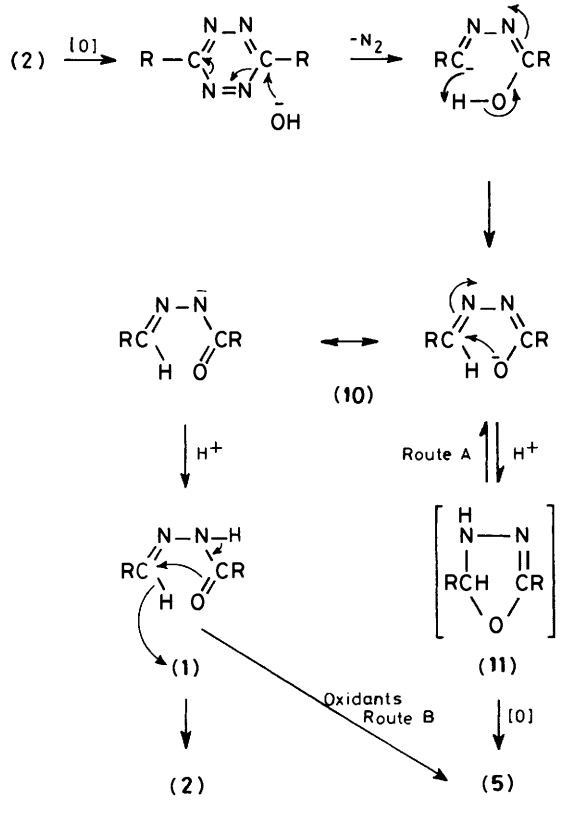
(b) *Formation of Oxadiazoles* (**5**).—This work has clearly established that the 1,4-dihydro-*s*-tetrazines (**2a—e**) are stable to methanolic solutions of alkali under nitrogen. The formation of oxadiazoles (**5c,b,d,e**) from dihydrotetrazines (**2c—f**) under the same conditions in air[2,5,6] must, therefore, of necessity involve some oxidation step and cannot be represented by a simple hydrolysis reaction as in Scheme 2. The following evidence is presented to show that the oxadiazoles (**5**) arise from the tetrazines (**1**) and not from their 1,4-dihydro-derivatives. To date oxadiazoles have only been isolated from tetrazines in the presence of peracids.[11-13]

Although Pinner[5,6] isolated only the hydrazides (**4**; R = Ar) from the action of hot ethanolic alkali on 3,6-diaryl-*s*-tetrazines (**1**; R = Ar), a result confirmed by us, other experiments conducted by us have shown that in cold methanolic alkali, the oxadiazole (**5c**) can be obtained in addition to the hydrazide (**4b**). The ratio of these two products appears to be somewhat sensitive to concentration, temperature, and solvent. When a similar experiment was carried out under nitrogen on the tetrazine (**1c**) [under conditions known to give the oxadiazole (**5c**) in air], no oxadiazole was observed and the hydrazide (**4b**) was the main product. This experiment further points to oxidative step(s) in the formation of oxadiazoles from tetrazines or their dihydro derivatives.

Attention was then turned to 3,6-bis(4-chlorobenzyl)-*s*-tetrazine (**1d**) which in concentrated solution yields the imidazotetrazine (**9a**) and 3-(4-chlorobenzyl)-5-(4-chlorophenyl)-1,2,4-triazole from the action of cold methanolic alkali, but which in dilute solutions gives the oxadiazole (**5b**) in addition to the above products. This result can be understood on the following basis: the formation of the imidazotetrazine (**9a**) and triazole involves two hydride shifts to the tetrazine (**1d**), which acts as a receptor (see ref. 2 for a full mechanism); hence the more dilute

the solution, the less tetrazine available to act as acceptor and the more likelihood there is of an alternative pathway.

A detailed mechanistic route to the oxadiazoles (**5**) from the dihydrotetrazines (**2**) is proposed in Scheme 7. Our premise is that the dihydrotetrazine (**2**) slowly oxidises in air to the tetrazine (**1**) which is attacked by alkali to give the resonance-stabilised anion (**10**). This ion has two potential routes to the oxadiazoles (**5**). In the first (Route A), the ion (**10**) ring closes to give a highly unstable 2,3-dihydro-oxadiazole (**11**) which oxidises in air to the aromatic structure (**5**). Such dihydro-oxadiazoles (**11**) are unknown in the literature although attempts have been made to synthesize them.[14] The alternative route (Route B) is also feasible because the hydrazides (**4**) can be oxidised[15,16] to the oxadiazoles (**5**) by *e.g.* lead tetra-acetate or potassium ferricyanide in alkaline solution. In the case of our media this would require a tetrazine molecule (**1**) to act as a hydride acceptor and become reduced to the dihydrotetrazine (**2**). Route B has been rejected on the grounds that the 3,5-dibenzyl-1,4-dihydrotetrazines (**2d—f**) yield the corresponding oxadiazoles (**5b,d,e**) quite readily under treatment with alkali in air[2], but neither they (**2d—f**) nor the related tetrazines (**1d—f**) have ever been found to give even a trace of hydrazide (**4c—e**). In addition, when the hydrazide (**4e**) was treated with potassium hydroxide in methanol in the presence of 3,6-diphenyl-*s*-tetrazine (**1c**) (tetrazines being known hydride acceptors), no 3,5-dibenzyloxadiazole (**5e**) was isolated [although some 3,5-diphenyloxadiazole (**5c**) formed from decomposition of the tetrazine (**1c**) was found]. On this evidence, Route B appears non-viable and Route A, which involves an oxidative step but not hydride transfer, is the more likely. The fact that the tetrazine (**1c**) in alkali under nitrogen does not yield the oxadiazole (**5c**) further supports the concept of an equilibrium between the resonance-stabilised anion (**10**) and the dihydro-oxadiazole (**11**) (Scheme 7, Route A) since in air the dihydro-oxadiazole (**11**) would be irreversibly oxidised to the oxadiazole (**5**), thus upsetting the equilibrium which appears to be very much more favourable towards the anion (**10**); under nitrogen, this route is blocked and hence the hydrazide (**4b**) is isolated instead. Route B is again rejected as this pathway would be expected to be viable even in the absence of air because it depends for oxadiazole formation on a hydride transfer step and tetrazines have clearly been shown to be hydride acceptors.[1-3]



Scheme 7.

Experimental

M.p.s are uncorrected. ^{1}H n.m.r. spectra were run on a Varian EM 360 (60 MHz) instrument. Ether refers to diethyl ether.

Preparation of s-*Tetrazines.*—The tetrazines (**1a**[3],**1b**[3],**1c**[17]**1d**[1]) were prepared according to literature methods and had m.p.s as follows: compound (**1a**) 158—168 °C (lit.,[9] 174—175 °C: the diastereoisomers could not be separated; the literature m.p. refers to a hand-picked crystal sample[18]); compound (**1b**) 199—203 °C (lit.,[9] 206—207 °C); compound (**1c**) 196—198 °C (lit.,[19] 198 °C); compound (**1d**) 135—136 °C (lit.,[1] 135—137 °C).

Preparation of Dihydro-s-*tetrazines.*—The dihydro-*s*-tetrazines (**2a,2c**) were prepared according to the literature,[2] and had m.p.s as follows: compound (**2a**) 181—186 °C (lit.,[20] 193 °C); compound (**2c**) 192 °C (lit.,[21] 192—193 °C).

Action of Methanolic Potassium Hydroxide on 3,6-Bis(α-hydroxy-4-methoxybenzyl)-s-*tetrazine* (**1b**).—(a) *In air.* A solution of potassium hydroxide (0.4 g) in methanol (5 ml) was added dropwise with stirring at room temperature in air to a mixture of the tetrazine (**1b**) (1.2 g) and methanol (10 ml). Stirring was continued for 3 h and the reaction mixture left sealed overnight. Dilute hydrochloric acid (0.15M; 25 ml) was then added and the mixture extracted with ethyl acetate (3 × 25 ml). The remaining undissolved solid, when filtered off, yielded the hydrazide (**6b**) (10 mg) identical by t.l.c. and by i.r. and n.m.r. spectrometry with a sample prepared by an independent route (see below). The ethyl acetate extract yielded, after drying ($MgSO_4$) and evaporation of solvent, a brown oil which on trituration with chloroform gave crystals of the *pyrazolinone* (**3b**) (20 mg) which had m.p. 152—155 °C (from chloroform–ethanol) (Found: C, 62.4; H, 5.3; N, 8.1; M^+, 342.120 73. $C_{18}H_{18}N_2O_5 \cdot H_2O$ requires C, 62.3; H, 5.3; N, 8.1%; M^+ for $C_{18}H_{18}N_2O_5$ requires 342.121 54); δ[$(CD_3)_2CO$] 3.68 (3 H, s, OMe), 3.7 (3 H, s, OMe), 4.75 (1 H, d, CHO*H*), 5.35 (1 H, d, C*H*OH), 5.6 [1 H, s, C(4-$MeOC_6H_4$)O*H*], 6.5—7.2 (8 H, m, ArH), 10.2 (1 H, br s, NH). The chloroform used for tritutration yielded 4-methoxybenzaldehyde.

(b) *Under nitrogen.* The experiment was repeated under nitrogen. On evaporation of solvent, the ethyl acetate extract yielded a brown oil which on trituration with chloroform gave a pinkish white solid which gave a positive test for dihydrotetrazine (using sodium nitrite and glacial acetic acid). This solid, on repeated chromatographic separation (silica eluted with ether), yielded the pyrazolinone (**3b**) (88 mg) and the tetrazine (**1b**) (93 mg) formed by oxidation of the dihydrotetrazine (**2b**) during work-up.

Action of Methanolic Potassium Hydroxide on 1,4-*Dihydro*-3,6-*bis(α-hydroxybenzyl)*-s-*tetrazine* (**2a**).—(a) *In air.* A solution of potassium hydroxide (0.7 g) in MeOH (10 ml) was added dropwise with stirring at room temperature in air to a mixture of the dihydrotetrazine (**2a**) (2 g) in MeOH (20 ml). After being stirred for 1 h and left sealed overnight, the reaction mixture was evaporated to small bulk under reduced pressure and poured into dilute hydrochloric acid (1M; 70 ml). The

precipitate, when filtered off, yielded the hydrazide (**6a**) (0.677 g); identical, by mixed m.p. and by n.m.r. spectroscopy, with a sample prepared by an independent route (see below). The filtrate was extracted with ethyl acetate, dried ($MgSO_4$) and evaporated to afford an oil which was separated chromatographically (silica eluted with ether), affording benzaldehyde (0.098 g) and a further quantity of the hydrazide (**6a**) (0.353 g), but no α-hydroxyphenylacetohydrazide. The aqueous layer was made basic with ammonia (*d* 0.880) and again extracted with ethyl acetate. After drying ($MgSO_4$) and evaporation of solvent, no α-hydroxyphenylacetohydrazide was found.

(b) *Under nitrogen.* The experiment was repeated under nitrogen. No hydrazide (**6a**) was detected, and the starting material (**2a**) (1.6 g) was recovered unchanged. Addition of benzaldehyde (1.5 g) gave a similar result.

(c) *Under nitrogen in the presence of α-hydroxyphenylacetohydrazide.* A solution of potassium hydroxide (0.3 g) in methanol (4 ml) was added dropwise with stirring under nitrogen at room temperature to a mixture of the dihydrotetrazine (**2a**) (0.25 g) and α-hydroxyphenylacetohydrazide (0.25 g) in methanol (10 ml). The mixture was stirred for 1 h and left overnight under nitrogen, after which dilute hydrochloric acid (0.1M; 50 ml) was added and the mixture extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated. Chromatographic separation (silica eluted with ether) yielded the dihydrotetrazine (**2a**) (97 mg), the hydrazide (**6a**) (98 mg), and α-hydroxyphenylacetohydrazide (20 mg).

(d) *Under nitrogen in the presence of α-hydroxy-4-methoxyphenylacetohydrazide.* A similar experiment to that described in (c) above, in which α-hydroxy-4-methoxyphenylacetohydrazide replaced the α-hydroxyphenylacetohydrazide, yielded only the dihydrotetrazine (**2a**) (127 mg) and the hydrazide (**8**) (40 mg) which had m.p., t.l.c., and i.r. spectra identical with a sample prepared by an independent route (see below) and α-hydroxy-4-methoxyphenylacetohydrazide (99 mg). No hydrazides (**6a**) or (**6b**) were observed.

Action of Methanolic Potassium Hydroxide on 3,6-*Bis*(α-*hydroxybenzyl*)-s-*tetrazine* (**1a**) *in Dilute Solution in Methanol.*—A solution of potassium hydroxide (0.7 g) in methanol (10 ml) was added dropwise to a solution of the tetrazine (**1a**) (0.2 g) in methanol (200 ml) with stirring at room temperature. After being stirred for 2 h and left sealed overnight, the volume of the mixture was reduced to *ca.* 20 ml under reduced pressure, and dilute hydrochloric acid (0.1M; 100 ml) was added. After extraction with ethyl acetate, drying ($MgSO_4$), and evaporation of solvent, a brown oil remained which was separated chromatographically [silica eluted with ether–light petroleum (b.p. 40—60 °C), 3:1] to yield the pyrazolinone (**3a**) (65 mg) and the hydrazide (**6a**) (12 mg).

Action of Methanolic Potassium Hydroxide on the Hydrazide (**4a**).—A solution of potassium hydroxide (0.02 g) in methanol (0.25 ml) was added dropwise with stirring to a mixture of the hydrazide (**4a**) (50 mg) in methanol (0.5 ml). The mixture was stirred for 1 h and left sealed overnight before being poured into dilute hydrochloric acid (0.1M; 50 ml) and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated. Separation of products by preparative t.l.c. [silica eluted with ether–light petroleum (b.p. 40—60 °C), 1:1] yielded the hydrazide (**6a**) (4 mg) which was identified by t.l.c. and i.r. spectroscopy.

Action of Benzaldehyde on the Hydrazide (**4a**) *in the Presence of Alkali.*—A solution of potassium hydroxide (0.02 g) in methanol (0.5 ml) was added dropwise with stirring to a mixture of the hydrazide (**4a**) (50 mg) and benzaldehyde (100 mg) in methanol (1.0 ml) at room temperature. After being stirred for 1 h and left sealed overnight, the mixture was poured into dilute hydrochloric acid (0.1M; 100 ml), and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and the solvent removed. The product was purified by preparative t.l.c. (silica eluted with ether), yielding the hydrazide (**6a**) (32 mg), which was identical with an authentic sample by t.l.c. and i.r. spectroscopy.

Action of Methanolic Potassium Hydroxide on 1,4-*Dihydro*-3,6-*diphenyl*-s-*tetrazine* (**2c**).—(a) A solution of potassium hydroxide (0.02 g) in methanol (0.5 ml) was added dropwise with stirring to a mixture of the dihydrotetrazine (**2c**) (100 mg) and methanol (1.0 ml). After being stirred for 1 h and left sealed overnight, the mixture was poured into dilute hydrochloric acid (0.1M; 5 ml) and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated. The products were separated by preparative t.l.c. [silica eluted with ether–light petroleum (b.p. 40—60 °C), 1:1] to yield the tetrazine (**1c**) (23 mg) and the oxadiazole (**5c**) (60 mg).

(b) When the above experiment was repeated under nitrogen with the exclusion of air up to the stage of acidification, only starting material and a trace of the tetrazine (**1c**) were obtained.

Action of Methanolic Potassium Hydroxide on 3,6-*Diphenyl*-s-*tetrazine* (**1c**).—(a) The tetrazine (**1c**) (50 mg) was added to a solution of potassium hydroxide (1.0 g) in methanol (100 ml). The solution was stirred at room temperature for 1.5 h and left sealed for 10 days. The volume was then reduced to 25 ml under reduced pressure and the solution was poured into dilute hydrochloric acid (1M; 100 ml), and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated. The products were separated by preparative t.l.c. [silica eluted with ether–light petroleum (b.p. 40—60 °C), 1:1], to yield the oxadiazole (**5c**) (16 mg) and the hydrazide (**4b**) (21 mg), which were identical with authentic samples by t.l.c. and by i.r. spectroscopy.

(b) A solution of potassium hydroxide (0.02 g) in methanol (0.5 ml) was added dropwise with stirring to a mixture of the tetrazine (**1c**) (100 mg) and methanol (1.0 ml) at room temperature. Stirring was continued for 1 h and the mixture left sealed for 10 days. Unchanged tetrazine (**1c**) (66 mg) was then filtered off, and the filtrate was poured into dilute hydrochloric acid (0.1M; 5 ml) and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated. The products were separated by preparative t.l.c. [silica eluted with ether–light petroleum (b.p. 40—60 °C), 1:1] to yield a further quantity of the tetrazine (**1c**) (14 mg) and the oxadiazole (**5c**) (15 mg), which had m.p. 139 °C [from light petroleum (b.p. 60—80 °C)] (lit.,[5] 140 °C).

(c) *Under nitrogen.* A solution of potassium hydroxide (0.02 g) in methanol (0.5 ml) was added dropwise with stirring to a mixture of the tetrazine (**1c**) (100 mg) and methanol (1.0 ml) at room temperature under an atmosphere of nitrogen. The mixture was stirred for 1 h and left sealed under nitrogen for 10 days. Hydrochloric acid (0.2M; 5 ml) was then added with stirring, and the mixture poured into water (100 ml) and extracted with ethyl acetate. The extract was dried ($MgSO_4$) and evaporated. Dry column chromatography [silica eluted with ether–light petroleum (b.p. 40—60 °C), 1:1] yielded unchanged tetrazine (**1c**) (67 mg) and the hydrazide (**4b**) (17 mg), but not the oxadiazole (**5c**).

Action of Methanolic Potassium Hydroxide on 3,6-*Bis*(4-*chlorobenzyl*)-s-*tetrazine* (**1d**) *in Dilute Solution.*—A solution of potassium hydroxide (3.5 g) in methanol (200 ml) was added dropwise to a stirred solution of the tetrazine (**1d**) (0.5 g) in methanol (350 ml) at room temperature. The mixture was stirred for 1.5 h and left sealed overnight before addition of concentrated hydrochloric acid (6 ml). The volume was then

reduced to *ca.* 40 ml under reduced pressure, water (150 ml) was added, and the solution was extracted with ethyl acetate. The extracts were dried ($MgSO_4$) and evaporated, and the products were separated by dry column chromatography [silica eluted with ether–light petroleum (b.p. 40—60 °C), 4:1], to yield the imidazotetrazine (**9a**) (109 mg), and a mixture of 3-(4-chlorobenzyl-5-(4-chlorophenyl)-1,2,4-triazole (93 mg) and the oxadiazole (**5b**) (67 mg): these could not be separated and the figures for yields are based on the ^1H n.m.r. spectrum of the mixture.

Action of Methanolic Potassium Hydroxide on the Hydrazide (**4e**) *in the Presence of the Tetrazine* (**1c**).—A solution of potassium hydroxide (0.03 g) in methanol (0.5 ml) was added dropwise with stirring to a mixture of the hydrazide (**4e**) (125 mg) and the tetrazine (**1c**) (35 mg) in methanol (2 ml) at room temperature. The mixture was stirred for 1 h, and left sealed overnight before being poured into dilute hydrochloric acid (0.1M; 50 ml). The solution was extracted with ethyl acetate, dried ($MgSO_4$), and evaporated. Column chromatography [silica eluted with ether–light petroleum (b.p. 40—60 °C), 1 : 1] yielded a fore-run of unchanged tetrazine (**1c**), a quantity of unchanged hydrazide (**4e**) (43 mg) and 2,5-diphenyl-1,3,4-oxadiazole (**5c**) (10 mg), but no 2,5-dibenzyl-1,3,4-oxadiazole (**5e**) according to t.l.c. and n.m.r. comparison with an authentic sample.[22]

General Method for Preparation of Hydrazides.—The appropriate aldehyde and hydrazide were heated in ethanol under reflux until they had completely reacted (by t.l.c.). The volume of the reaction mixture was then reduced under reduced pressure until solid product separated; this was then filtered off.

Hydrazide (**6a**). By the above method, benzaldehyde (1.1 g) and α-hydroxyphenylacetohydrazide[23] (1.7 g) yielded the hydrazide (**6a**) (1.78 g) which had m.p. 155 °C (from aqueous acetone) (lit.,[23] 149 °C); δ[$(CD_3)_2SO$] (mixture of 2 isomers) 5.1 and 5.9 (1 H, d, C*H*OH), 5.5 and 6.4 (1 H, d, CHO*H*), 7.0—7.8 (10 H, m, ArH), 8.4 (1 H, s, HC:N), 11.4 (1 H, s, NH).

Hydrazide (**6b**). By the above method 4-methoxybenzaldehyde (1.36 g) and α-hydroxy-4-methoxyphenylacetohydrazide (1.96 g) yielded the novel *hydrazide* (**6b**) (2.2 g), m.p. 165—166 °C (from ethanol) (Found: C, 65.0; H, 5.9; N, 8.7. $C_{17}H_{18}N_2O_4$ requires C, 65.0; H, 5.7; N, 8.9%); δ[$(CD_3)_2CO$] 3.7 (3 H, s, OMe), 3.8 (3 H, s, OMe), 5.5 (1 H, d, C*H*OH), 5.8 (1 H, d, CHO*H*), 6.7—7.9 (8 H, m, ArH), 8.3 (1 H, s, *H*C=N), and 10.5 [1 H, br s, NH].

Hydrazide (**8**). By the above method, benzaldehyde (0.106 g) and α-hydroxy-4-methoxyphenylacetohydrazide (0.196 g) yielded the *hydrazide* (**8**) (0.24 g), m.p. 105—106 °C (from ethanol) [Found: C, 63.8; H, 6.0; N, 9.2. $C_{16}H_{16}N_2O_3$ requires C, 63.6; H, 6.0; N, 9.3%); δ[$(CD_3)_2SO$] 5.1 (1 H, d, C*H*OH), 6.2 (1 H, d, CHO*H*), 6.9—8.0 (9 H, m, ArH), 8.5 (1 H, s, *H*C=N), and 10.6 (1 H, s, NH).

Hydrazide (**4a**). By the above method, phenylglyoxal monohydrate (1.38 g) and α-hydroxyphenylacetohydrazide (1.52 g) yielded α-*hydroxy*-N-(2-*oxo*-2-*phenylethylidene*)*phenylacetohydrazide* (1.6 g), m.p. 181—183 °C (from ethanol) (Found: C, 68.0; H, 5.2; N, 10.2. $C_{16}H_{14}N_2O_3$ requires C, 68.1; H, 5.0; N, 9.9%); δ[$(CD_3)_2SO$] 5.2 (1 H, d, C*H*OH), 6.5 (1 H, d, CHO*H*), 7.4 (10 H, m, ArH), 8.3 (1 H, s, HC:N), 12.0 (1 H, s, NH). Reduction of this hydrazide by a published method[24] yielded the *hydrazide* (**4a**) (0.32 g) which had m.p. 174—175 °C (from acetone) (Found: C, 67.7; H, 5.8; N, 10.0. $C_{16}H_{16}N_2O_3$ requires C, 67.6; H, 5.6; N, 9.9%); δ[$(CD_3)_2SO$] 5.0 (1 H, dd, C*H*OH·CH), 5.2 (1 H, d, C*H*OH·CO), 6.0 (1 H, d, CHO*H*), 6.3 (1 H, d, CHO*H*), 7.4 (10 H, m, ArH), 7.8 (1 H, d, *H*C:N), 11.0 (1 H, s, NH).

Hydrazide (**4e**). By the above method phenylacetaldehyde (0.4 g) and phenylacetohydrazide[25] (0.5 g) yielded the hydrazide (**4e**) (0.54 g), which had m.p. 178—179 °C (from ethanol); δ[$(CD_3)_2SO$] (mixture of 2 isomers) 3.37, 3.43, 3.53, and 3.73 (4 H, 4 s, CH_2), 7.2 (10 H, m, ArH), 7.5 [1 H, br s, *H*C:N], and 10.9 and 11.2 (1 H, 2 s, NH).

Preparation of α-Hydroxy-4-methoxyphenylacetohydrazide.—Ethyl α-hydroxy-4-methoxyphenylacetate (15 g) was dissolved in ethanol (5 ml) and the solution added dropwise to hydrazine hydrate (5.3 g) on a water-bath under a reflux condenser. When the reaction mixture began to reflux, 5 ml of ethanol were removed by distillation, and refluxing was continued for 2 h. After cooling, the novel compound *α-hydroxy-4-methoxyphenylacetohydrazide* (11.9 g) was filtered off and had m.p. 152 °C (from ethanol) (Found: C, 54.7; H, 6.1; N, 14.0. $C_9H_{12}N_2O_3$ requires C, 55.1; H, 6.1; N, 14.3%).

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