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ACTIVATION AND TRANSFER OF OXYGEN-X

A NEW AUTOXIDATIVE REARRANGEMENT OF TETRAHYDROPTERIDINES

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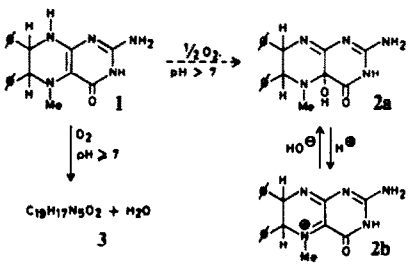
Abstract—The structure **2a** proposed by Viscontini and Okada [1,2] for the autoxidation product of 5-methyl-6,7-diphenyl-5,6,7,8-tetrahydropterin **1** was found to be incorrect. Alternative structures **3a**, **3b** were deduced from spectroscopic data. X-ray analysis of the acetyl derivative **8** proved the oxidation product to be 2-amino-8-methyl-4,9-dioxo-cis-6,7-diphenyl-6,7,8,9-tetrahydro-4H-pyrazino(1,2-a)-s-triazine **3a**. The mechanism of the rearrangement may involve an intermediate 4a-peroxy-pterin. A similar rearrangement on peroxide-level was observed for the corresponding lumazine **14**.

INTRODUCTION

Viscontini and Okada[1] described the autoxidation of 5-methyl-6,7-diphenyl-5,6,7,8-tetrahydropterin **1** at pH > 7. Structure **2a** was proposed for the oxidation product.[2]



As compound **2b** is of interest as the possible cationic species in pseudobase and pteridine-hydroperoxide equilibria,[3] we investigated this oxidation product. No evidence however, could be obtained in favor of the equilibrium **2a** ⇄ **2b**, which prompted us to question the proposed structure **2a**.

RESULTS

The preparation of 5 - methyl - 6,7-diphenyl - 5,6,7,8 - tetrahydropterin

Regarding the possible configurational isomers arising from chirality around C_6 and C_7, starting material was prepared in three ways: (a) by the reduction of 5 - methyl - 6,7 - diphenyl - 5,6 - dihydropterin with sodiumborohydride,[4] (b) by reducing the dihydropterin over palladium on charcoal in acetic acid, (c) by catalytic reduction of the formaldehyde adduct of 6,7 - diphenyl - 5,6,7,8 - tetrahydropterin as outlined by Matsuura and Sugimoto.[5] The products prepared by these routes proved to be identical. Apparently only one set of stereo-isomers is isolated in these cases.

Structure of the oxidation product.

From the autoxidation of **1** in methanolic phosphate buffer a single compound was isolated. Spectral data and elementary analysis were in accordance with the published figures (cf Table 1), but in our opinion they disagreed with the proposed structure **2a**. Our doubts were substantiated when the PMR spectrum (CF_3COOD) revealed the presence of only two exchangeable protons (apart from the proton contributed by the water of crystallization), while structure **2a** requires four exchangeable protons. The IR spectrum showed stronger absorptions in the C=O region, compared with **1**. Our attempts to quaternize the compound failed. To establish oxygen consumption by manometric measurement, methanolic phosphate buffer proved to be inconvenient, which is illustrated by the erroneous value of 0·5 mole of oxygen consumed per mole of tetrahydropterin, claimed in the literature.[2] When **1** was stirred in unbuffered water and an oxygen atmosphere for several weeks at room temp, a final uptake of 1·0 mole of oxygen per mole of tetrahydropterin was observed. Oxidation product, identical with the product isolated from autoxidations in methanolic phosphate, was obtained in quantitative yield, no hydroperoxide could be detected.

Summarising these results, we concluded that the structure proposed by Viscontini and Okada was incorrect.

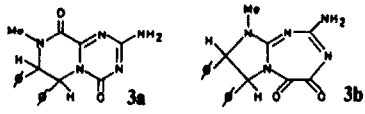
The PMR spectra (CF_3COOD) of **1** and **3** showed the presence of the diphenylethylene moiety in both compounds. Protonation at the 5 position is responsible for the relative low field resonance of the methyl protons and hence of the adjacent benzylic proton in the spectrum of **1**. In the spectrum of **3**, both benzylic protons resonate at nearly the same low field, while the methylgroup resonance occurs at a slightly higher field compared with **1**. We interpreted this in terms of a substitution at the original 8 position with an electron-withdrawing group, accompanied by distinct changes in the environment of

Table 1. Comparison of analytical data for the oxidation product of 5 - methyl - 6,7 - diphenyl - 5,6,7,8 - tetrahydropterin 1

Data from	Found lit[2]	Required 2a	Found†	Required 3
PMR	$-CH_3$;	additional	additional	additional
	$-CH-C_6H_5$ 2x	4 protons	2 protons	2 protons
IR		C–O stretch	C=O stretch	
Analysis		$C_{19}H_{19}N_5O_2$		$C_{19}H_{17}N_5O_2 \cdot \frac{1}{2}H_2O$
C,	65·6	65·31	64·2	64·03
H,	5·1	5·48	5·1	5·09
N,	19·9%	20·05	19·7	19·65
MS(M^+/e)	347	349	347	347
n-O_2	0·5	0·5	1·0	1·0

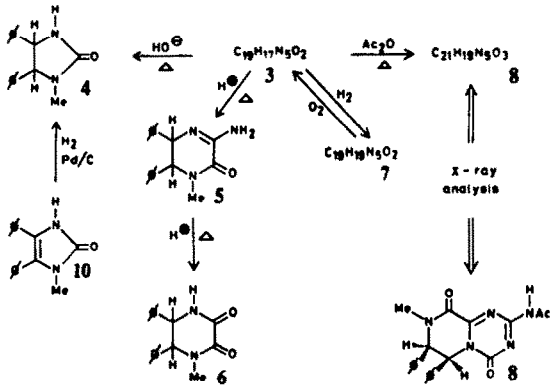
†Present authors.

the methylgroup. Structures **3a** and **3b** were considered:



Expecting an accumulation of amide linkages, controlled hydrolysis was carried out. Alkaline degradation gave the imidazolidinone **4**. Dissolving **3** in boiling N ethanolic hydrogen chloride yielded a compound assigned structure **5** among other products (cf Scheme 1).

As we were not able to detect oxalate in the alkaline hydrolysate, the imidazolidinone **4** was the more likely artifact. Comparison of the PMR spectra of **3** with **4** and **5** gave the same impression: the benzylic protons of **3** and **5** showed a coupling constant of about 5 Hz and a broad and very similar multiplet was observed for the phenyl protons. In the spectrum of **4** a coupling constant of 10 Hz and a smaller phenyl multiplet is observed. Reduction of the amide functions with lithium aluminium hydride could not be effected, presumably because of the extreme insolubility of **3**. Catalytic reduction over palladium on charcoal resulted in the uptake of 1 mole of hydrogen per mole of **3**. The hydrogenated product **7** analysed for $C_{19}H_{19}N_5O_2$. The PMR spectrum of **7** showed an extra one-proton singlet at 6·1 ppm (CF_3COOD) and an additional exchangeable proton, compared with **3**. Compound **7** was subjected to methylation with dimethylsulphate and potassiumcarbonate in DMF. Prolonged treatment at room temp did not result in detectable conversion, when heated, **7** was found to be spontaneously reoxidized to give **3**. Acylation with mixed anhydride or acetic anhydride of **3** gave the formylated and acetylated derivatives. The acetyl derivative **8**, crystallized from water-ethanol, gave crystals of sufficient size and quality for analysis by X-ray diffraction. The results, presented in an accompanying paper,[6] show structure **3a** to be correct.



Scheme 1. Degradation and derivatization of 3.

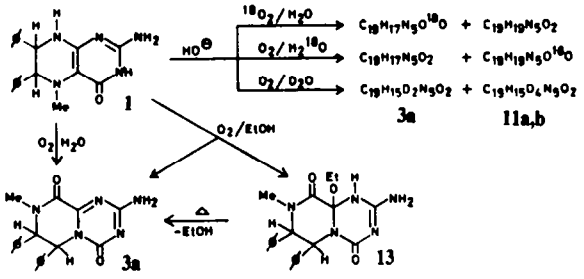
Autoxidations in different media

Autoxidation in water proceeded at an inconveniently slow rate, but was faster in alkali. Oxidations in 0·1 N sodium hydroxide were complete within 4 h; however, a different stoichiometry was observed. (cf Scheme 2). Oxygen uptake was about 0·8 mole per mole of tetrahydropterin and in addition to 0·6–0·7 mole of **3a**, 0·1–0·2 mole of a new compound **11** analysing for $C_{19}H_{19}N_5O_2$, was isolated.

Methylation of **11** with dimethylsulphate-potassiumcarbonate in DMF at elevated temp, gave a mixture from which compound **12** (cf scheme 5) could be separated in moderate yield by chromatography. Compound **12** analysed for $C_{21}H_{22}N_4O_3$, suggesting two extra methyl groups and hydrolysis of the amine function compared with **11**. This was confirmed by the PMR spectrum ($CDCl_3$), showing three singlet three-proton signals, the ten-proton multiplet and two one-proton doublets for the benzyl groups, and a broad one-proton absorption at 2·3 ppm (exchanged in CF_3COOD). The IR spectrum showed several absorptions in the C=O region, forming a pattern similar to that observed for methylated spiro-hydantoins.[7]

When oxidizing **1** with $^{18}O_2$ in $H_2{}^{16}O$ (0·1 N NaOH), incorporation of one atom of heavy oxygen was detected by MS of **3a**. Comparison of the fragmentation patterns of labelled and unlabelled **3a** showed the incorporation to be at the 9 position (position **4a** in the parent pterin). In $H_2{}^{18}O$ under $^{16}O_2$ no incorporation of label was detected in compound **3a**. For compound **11** the reverse situation was observed; the extra oxygen atom originated from water.

Upon autoxidation of **1** in absolute ethanol, a final



Scheme 2. Autoxidations of 1 in different media.

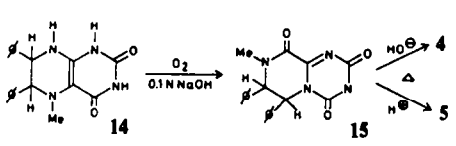
uptake of 1·0 mole of oxygen per mole of tetrahydropterin was observed. Varying ratios of **3a** and an ethanol-soluble compound **13** were isolated in a total amount accounting for the tetrahydropterin oxidized.

Compound **13** analysed for $C_{21}H_{23}N_5O_3$ and was irreversibly converted to **3a** when heated for several minutes at 150°. The PMR spectrum showed the presence of an ethylgroup and an extra exchangeable proton (compared with **3a**). Mass spectrometric fragmentations were in accordance with an ethanol adduct of **3a**, carrying the ethoxy group on the bridgehead carbon (C_{9a}).

From autoxidations in D_2O (0·1 NaOD) both **3a** and **11** were isolated without detectable deuterium incorporation at C_6, C_7 or in the methyl group, as judged from PMR spectra in CF_3COOD.

Autoxidation of 5 - *methyl* - 6,7 - *diphenyl* - 5,6,7,8 - *tetrahydrolumazine*

5 - Methyl - 6,7 - diphenyl - 5,6,7,8 - tetrahydrolumazine **14** was prepared by reductive methylation. When stirred under oxygen in 0·1N NaOH, a final uptake of 1·0 mole of oxygen per mole of tetrahydrolumazine was noted. No peroxide could be detected in the resulting solution, from which compound **15** ($C_{19}H_{16}N_4O_3$) was isolated in near quantitative yield. The PMR spectrum of **15** was almost identical with that of **3a**; only one exchangeable proton is present. Chemical degradation gave the imidazolidinone **4** and the amino-oxo-pyrazine **5**.



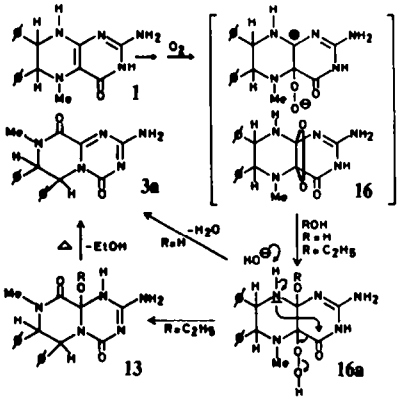
DISCUSSION

In studying the autoxidation of 5-methyl-6,7-diphenyl-5,6,7,8-tetrahydropterin **1** and related compounds (e.g. **14**), two objectives are met: (a) compound **1** may be studied as a model in connection with the questions on the possible peroxide formation at the C_{4a} or C_{8a} bridge atoms, and (b) it may be studied as a model for the natural co-factor 5 - methyl - tetrahydro - folic acid (5 - methyl - THF). Concerning the latter aspect, Gapski *et al.*[8] extended the proposal of structure **2a** to an oxidation product of 5 - methyl - THF, prepared either by peroxide oxidation of 5 - methyl - THF, or by peroxide oxidation of 5 - methyl - 5,6 - dihydro - folic acid (5 - methyl - DHF). From labeling experiments they concluded that one oxygen atom was incorporated, originating from the hydrogenperoxide, while the benzylic proton at C_6 was still present. The intermediacy of 5-methyl-DHF was proposed.

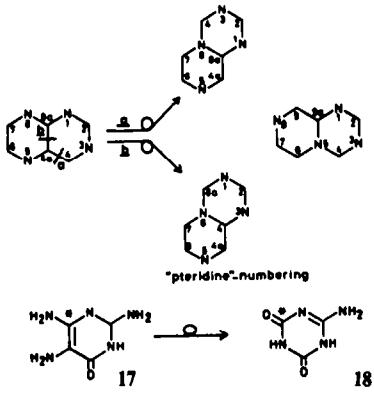
Replacing structure **2a** by the correct structure **3a** should not affect this proposal; however, we could not obtain **3a** by oxidizing 5 - methyl - 6,7 - diphenyl - 5,6 - dihydropterin, nor is the 5,6 - dihydro - compound found to be an intermediate in the air oxidation of **1** (as deuterium is not incorporated during autoxidations in D_2O), suggesting **1** to be a poor model for 5 - methyl - THF in oxidation experiments.

Regarding the first aspect, the consumption of one mole of oxygen, incorporation of one atom of oxygen and breaking of a carbon–carbon bond, is a strong indication for oxygen insertion resulting from a rearrangeing peroxy-pterin (Scheme 3).

The isolated ethanol adduct **13** may arise from the addition of ethanol (or an ethoxy-anion) to the (partial) positive bridgehead carbon of intermediate **16**. In a formal manner (cf Scheme 4), the rearrangement of **1** to **3a** can be brought about by breaking the C_4–C_{4a} bond (a) or the C_{4a}–C_{8a} bond (b), in both cases followed by subsequent linking of N_8 and C_4. The latter possibility was considered in connection with the rearrangement of the triamino-hydroxy-pyrimidine **17** to the s-triazine **18** upon treatment with hydrogen peroxide (Scheme 4), as studied by Pfleiderer *et al.*[9] In our case a similar mechanism



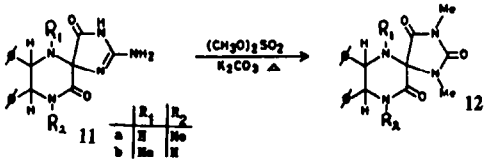
Scheme 3. Possible pathway for the autoxidative rearrangement of 5-alkyltetrahydropteridines.



Scheme 4. Two possible "one-break-one-fission" syntheses of the pyrazino-(1,2-a)-s-triazine nucleus from pteridine.

(pathway b) would lead to incorporation of oxygen from the medium at the original 8a position. This however, was not observed.

It should be emphasized that compound **11**, produced in alkaline media, represents a rearrangement on pseudo-base level, resulting from the transfer of one atom of oxygen. Analysis, spectral properties and oxygen uptake suggests a spiro - amino - hydantoin **11a**, **11b**. As the one-proton absorption in the PMR spectrum of the methylated and partly hydrolysed derivative **12** can be assigned to a secondary amine proton (as in **12a**), rather than to a mono substituted amide proton (as in **12b**), structure **11a**, 1 - methyl - 2 - oxo - 5,6 - diphenyl - piperazine - 3 - spiro - 5' - (2' - amino - 2' - imidazolin - 4' - one), is proposed for compound **11**. We are well aware that the identification of the relative configuration around the spiro centre represents an additional problem.



Scheme 5.

Apart from the implications for the mechanism of oxygen activation, the structure elucidation of **3a** allows a deduction concerning the relative positions of the phenyl groups in the tetrahydropterin **1**. As there is no incorporation of deuterium during autoxidations in 0·1 N NaOD, the erythroconfiguration around C_6 and C_7 in **3a** must be present in **1** (and **11a**). Apparently, both in the reductions of the dihydropterin by sodiumborohydride or catalytically, and in the catalytic reduction of 6,7-diphenyl-pterin, the more crowded isomers predominate.

EXPERIMENTAL

Analytical oxidations and hydrogenations were performed at room temp and atm pressure in an all-glass apparatus allowing continuous corrections for temp and pressure changes. Volume changes in the order of 50 ml could be measured with an accuracy better than 1%. PMR spectra were recorded on a Varian A-60 spectrometer and chemical shifts are represented in ppm from TMS as an internal standard. Incorporation of ^2H and ^{18}O was measured by MS and corrected for the natural isotope abundancy. M.ps were taken in evacuated capillary tubes and uncorrected.

Preparation of 5 - *methyl* - 6,7 - *diphenyl* - 5,6,7,8 - *tetrahydropterin*, **1**

(a) By reduction of 5 - methyl - 6,7 - diphenyl - 5,6 - dihydropterin[4] (4·00 g, 12·1 mmol) with $NaBH_4$ (60 g, 1·6 M) according to the literature,[2] crude **1** (3·3 g) was obtained as a yellow-orange powder. Crystallization from water-MeOH (1:1, 2 l) yielded **1** (1·88 g, 45·5%) as colorless crystals.

(b) By hydrogenation of 5 - methyl - 6,7 - diphenyl - 5,6 - dihydropterin (4·00 g, 12·1 mmol) over 10%-Pd/C (0·5 g) in glacial AcOH (250 ml). After the calculated amount of H_2 was consumed (15 h) the catalyst was filtered off under N_2 and the filtrate was concentrated to dryness under reduced pressure. Triturating the yellow oil with cold MeOH and filtering gave **1** (2·50 g) as a yellow powder, darkening upon exposure to air. Recrystallization from water-MeOH as above afforded pure **1** (2·10 g, 50·5%).

(c) 6,7-Diphenyl-pterin[10] (3·00 g, 9·51 mmol) was hydrogenated over pre-reduced PtO_2 (0·5 g) in a mixture of conc HCl and 96% EtOH (1:8, v/v, 200 ml). After the calculated amount of H_2 was absorbed (3 h), 40% aq CH_2O (10 ml, 145 mmol) was added and hydrogenation continued until a third molar eq of H_2 was consumed (15 h). Filtering off the catalyst under N_2 and evaporating the solvent under reduced pressure gave the hydrochloride of **1** as a yellow fluffy powder. Cold de-aerated water (250 ml) was added and the soln neutralized with 2N NaOH. The white ppt was filtered off, washed with water and crystallized from water-MeOH as above, yielding **1** (2·25 g, 69%).

Preparations by procedures (a), (b) and (c) were found to be identical according to PMR, IR, MS and mmp measurements. Mp 242–243·5°. ($C_{19}H_{19}N_5O\cdot\frac{1}{2}H_2O$ (342·39) Calcd: C, 66·65; H, 5·89; N, 20·45; Found: C, 66·7; H, 6·0; N, 20·6%). PMR (CF_3COOD): δ = 3·82 (s, 3); 4·98 and 5·68 (2d, J = 4Hz, 2); 6·8–7·5 (m, 10); 11·6 (s, 5). Mass spectrum, *m/e* (%): 333(M^+, 100); 318(19); 256(40); 242(46); 180(64). IR(KBr), cm^{-1}: 3485, 3440, 3370(NH); 1670, 1644(C=O); 1590(C=N).

Autoxidation of **1** *in different media.*

(*a*) *Methanolic phosphate buffer (as described*[2]). A soln of **1** (690 mg, 2·0 mmol) in a mixture of MeOH (250 ml) and aq phosphate buffer (250 ml, 0·01 M, pH 11·6) was stirred for 5 h under O_2. The resulting suspension was filtered and the white ppt washed with water and MeOH. After drying in a vacuum desiccator over P_2O_5, the oxidation product **3a** (625 mg, 87%) was obtained in a hygroscopic form absorbing less than half an equimolar amount of water when exposed to a moist environment. An analytical sample was prepared by keeping the washed ppt for several h at 60°. ($C_{19}H_{17}N_5O_2\cdot\frac{1}{2}H_2O$ (356·39) Calcd: C, 64·03; H, 5·09; N, 19·65; Found: C, 64·2; H, 5·1; N, 19·7%). PMR (CF_3COOD): δ = 3·09 (s, 3); 5·73 and 5·91 (2d, J = 5Hz, 2); 6·7–7·6 (m, 10); 11·6 (s, 3). Mass spectrum, *m/e* (%): 347(M^+, 47); 304(3); 200(34); 131(49); 120(93); 104(12); 91(8). IR(KBr), cm^{-1}: 3370, 3215(NH); 1687, 1647 (C=O); 1610(C=N).

(*b*) *Water.* A suspension of **1** (342·4 mg, 1·00 mmol) in water was stirred under O_2. In 60–70 days a final uptake of 0·96 mmol of O_2 was noted. The solid was filtered off, washed and dried as above. Yield 327·5 mg (94·3%) of **3a**, identical (PMR, IR and MS) with the product obtained under (a).

(*c*) 0·1N *aq* NaOH. A suspension of **1** (2·79 g, 8·15 mmol) in 0·1N NaOH (200 ml) was stirred under O_2. In 10 h a limiting amount of 6·30 mmol (77·3%) of O_2 was consumed. The white solid was filtered off, washed twice with 0·1N NaOH (25 ml),

water, MeOH and dried as above, yielding **3a** (1·96 g, 69·2%) identical with the products from (a) and (b). A peroxide test (KI-starch) performed with a sample of the filtrate was negative. The combined filtrate and alkali washings were neutralized (2N H_2SO_4) and the resulting suspension placed in the refrigirator overnight. Filtering and washing the residue with water and MeOH (25 ml) gave **11** (0·56 g, 15·2%). ($C_{19}H_{19}N_5O_2 \cdot H_2O$ (367·41) Calcd: C, 62·11; H, 5·76; N, 19·06; Found: C, 62·4; H, 5·8; N, 18·9%). PMR (CF_3COOD): δ = 3·15 (s, 3); 4·73 and 5·84 (2d, J = 4Hz, 2); 6·7–7·4 (m, 10); 11·6 (s, 6). Mass spectrum, *m/e*: 349(M^+, 30); 347(15); 332(42); 321(3); 305(28); 290(12); 276(8), 264(44); 262(40); 244(28); 180(38); 178(48); 165(20); 132(22); 120(100); 118(93); 104(26). IR(KBr), cm^{-1}; 3420, 3310(NH); 1721, 1677, 1632(C=O); 1575(C=N).

Concentrating the MeOH washing gave a yellow oil (0·11 g) showing several spots on TLC (polar eluents). PMR of the mixture showed absorptions in the δ = 2·5–3·5 region (N–CH_3) accounting for 1 to 1·5 protons (as compared to the 10 proton multiplet for the phenylprotons), suggesting partial demethylation.

(*d*) 0·1N NaOD. In an exp similar as described under (*c*), **1** (342·4 mg, 1·00 mmol) was suspended in 0·1 N NaOD (99·75% D, 20 ml) and stirred under O_2. After 5 h the white solid was filtered off and washed with D_2O (2 ml), yielding **3a** (233·2 mg, air-dry). The filtrate was neutralized (2N D_2SO_4, 99·5% D), cooled and the yellow suspension filtered, yielding **11** (56·2 mg) after washing with D_2O (2 ml), cold MeOH (5 ml) and drying at 60°. PMR (CF_3COOD) showed the ratio of benzylic to phenylic protons to be essentially 2:10 in both **3a** and **11**.

(*e*) 0·5N $Na^{18}OH$ *under* $^{16}O_2$. A suspension of **1** (50 mg) was stirred overnight in 1 ml of 0·5N $Na^{18}OH$ (83% ^{18}O) under O_2. The ppt was filtered off, washed with MeOH (2 ml) and dried, yielding 27 mg of **3a**. Mass spectrum, no enrichment detected. The filtrate was neutralized (2N H_2SO_4), cooled and filtered again, yielding 9 mg yellow solid. Shaking for several minutes with cold MeOH, filtering the residual white solid and drying at 60° afforded **11**–^{18}O (5 mg). Mass spectrum, *m/e* of peaks displaced by 2 mass units (% enrichment): 351, 349 (80); 334(79); 323(81); 292(80); 244(81). No double labelling detected.

(*f*) 0·1N $Na^{16}OH$ *under* $^{18}O_2$. A suspension of **1** (100 mg) was stirred overnight in 10 ml of 0·1N NaOH under $^{18}O_2$ (93% ^{18}O). The ppt was filtered off, washed with MeOH (5 ml) and dried, yielding 62 mg of **3a**-^{18}O. Mass spectrum, *m/e* of peaks displaced by 2 mass units (% enrichment): 349(91·7); 320(91); 306(91·5); 244(90); 230(90·5). M^+–57 (H_3CNO) is supported by a metastable peak at *m/e* 242·5 in the spectrum of unlabeled **3a**; M^+-59 ($H_3CN^{18}O$) is supported by a metastable at 241·2 (*m/e*). The filtrate was neutralized (2N H_2SO_4), cooled and filtered again, yielding 12 mg of **11**. In this compound no incorporation of label could be detected.

(*g*) EtOH. A suspension of **1** (1·89 g, 5·48 mmol) was stirred in EtOH (150 ml, abs) under O_2. In 10 days a final consumption of 5·40 mmol (98·5%) of O_2 was measured. The resulting suspension was filtered, the residue washed with EtOH (50 ml) and dried, yielding 1·29 g (65·9%) **3a**. Filtrate and EtOH washing were concentrated (20 ml) and cooled overnight. The crystalline ppt was collected by filtration and dried over P_2O_5 *in vacuo*, yielding 0·52 g (24·1%) of compound **13**. ($C_{21}H_{23}N_5O_3$ (393·45) Calcd: C, 64·11; H, 5·89; N, 17·80; Found: C, 64·4; H, 6·0; N, 18·0%). PMR (CF_3COOD): δ = 1·39 (t, J = 7 Hz, 3); 3·21 (s, 3); 4·46 (q, J = 7 Hz, 2); 4·95 and 5·72 (2d, J = 6·5 Hz, 2); 6·5–7·4 (m, 10); 11·4 (s, 3). Added EtOH (δ = 1·36(t) and 3·92(q)) is gradually converted to CF_3COOEt (δ = 1·43(t) and 4·51(q)), absorptions of the latter compounds can be clearly distinguished from the ethyl absorptions in the spectrum of **13**. Mass spectrum, *m/e* (%): 393(M^+, 100); 348(32); 347(53); 335(10); 320(5); 304(6); 274(14); 246(26); 200(31). IR(KBr), cm^{-1}: 3210(NH); 1675. 1645 (C=O); 1570 (C=N).

Prepraration of 1 - *methyl* - 4,5 - *imidazolidin* - 2 - *one*, **4**

(*a*) *By alkaline hydrolysis of* **3a**. A suspension of **3a** (250 mg, 0·70 mmol) in 2N NaOH (10 ml) was refluxed for 15 h. The resulting suspension was cooled and extracted with $CHCl_3$ (3 × 25 ml). The combined $CHCl_3$ layers were washed with water (2 × 50 ml), dried (Na_2SO_4) and evaporated to leave 93·6 mg of crude **4**. Crystallization from MeOH (10 ml) gave 85·5 mg (48·4%) of pure **4**. Mp 214·5–215·5° (Lit[11] mp 214–215° for trans-**4**). ($C_{16}H_{16}N_2O$ (252·32) Calcd: C, 76·16; H, 6·39; N, 11·10; Found: C, 76·1; H, 6·5; N, 11·1%). PMR ($CDCl_3$): δ = 2·72 (s, 3); 4·86 and 5·07 (2d, J = 9 Hz, 2); 5·68 (s, broad, 1); 6·7–7·3 (m, 10), (CF_3COOD): δ = 2·87 (s, 3); 5·22 and 5·45 (2d, J = 10 Hz, 2); 6·65–7·25 (m, 10); 11·6 (s, 1). Mass spectrum, *m/e* (%): 252(M^+, 100); 195(22), 185(13); 165(5); 120(97); 118(76); 106(22). IR(KBr), cm^{-1}: 3225(NH); 1693(C=O).

(*b*) *By alkaline hydrolysis of* **15**. As described above, 250 mg (0·72 mmol) 15 was hydrolysed (5 h) and the product crystallized (MeOH) to yield 123·3 mg (68%) of compound **4**. This product was identical (PMR, IR, MS and mmp) with the product from **3a**. As compound **15** was readily soluble in hot 2N NaOH, hydrolysis proceeded at a faster rate. An intermediate (which may be the initially formed cis-**4**) accumulated and could be detected by PMR spectroscopy of the products isolated after refluxing for 1 h. PMR (CF_3COOD): =3·05 (s, 3); 5·42 and 5·65 (2d, J = 10 Hz, 2); 6·65–7·3 (m, 10).

(*c*) *By catalytic hydrogenation of* 1 - *methyl* - 4,5 - *diphenyl* - 4 - *imidazolin* - 2 - *one*, **10** Imidazolinone **10** was prepared by heating equimolar amounts of benzoin and monomethyl urea at 160–170° for 20 min. After removing a yellow impurity by shaking the resulting solid with small quantitites of DMF, the residue was dried (80 Torr, 150°) and purified by continuous extraction with MeOH. Mp 285–287°. ($C_{16}H_{14}N_2O$ (250°30) Calcd: C, 76·78; H, 5·63; N, 11·19; Found: C, 76·7; H, 5·6; N, 11·0%). PMR (CF_3COOD) δ = 3·40 (s, 3); 7·15–7·7 (m, 10); 11·6 (s, 1). Mass spectrum, *m/e* (%): 250(M^+, 100); 193(15); 165(9); 118(29); 104(13). Compound **10** (6·50 g, 26·0 mmol) was hydrogenated over 10%-Pd/C (1·0 g) in glacial AcOH (150 ml). After the calculated amount of H_2 had been consumed (5 h), the catalyst was filtered off and the colorless filtrate taken to dryness under reduced pressure. The resulting yellow oil was dissolved in EtOH (100 ml) and concentrated again to remove excess AcOH. This procedure was repeated several times until a crystalline product was obtained. The white product was collected, dried and recrystallized from MeOH (75 ml), affording 4·95 g (75·4%) of **4**. This product proved identical (PMR, IR, MS and mmp) with the products isolated under (a) and (b).

3 - *Amino* - 1 - *methyl* - 2 - *oxo* - 5,6 - *diphenyl* - 1,2,5,6 - *tetrahydropyrazine*, **5**

(*a*) *By acid hydrolysis of* **3a**. Compound **3a** (1·60 g, 4·49 mmol) was refluxed for 5 h in a mixture of 96% EtOH and conc HCl (20:1, v/v, 100 ml). The yellow soln was cooled (0°) overnight and the colorless crystals were filtered off and dried at 80°, yielding 510 mg (36%) of compound **5** as the hydrochloride. Mp 275–281 (dec). ($C_{17}H_{17}N_3O \cdot HCl$ (315·80), Calcd: C, 64·66; H, 5·75; N, 13·31; Found: C, 64·4; H, 5·7; N, 13·2%). PMR (DMSO-d_6): δ = 3·00 (s, 3); 5·27 and 5·78 (2d, J = 5 Hz, 2); 6·5–7·4 (m, 10); 10·2 (s, 3). Mass spectrum, *m/e* (%): 279 (M^+, 56); 132(11); 131(8); 120(100); 118(5); 104(8). IR(KBr), cm^{-1}: 3150(NH, Broad); 1712(w), 1660(C=O).

(*b*) *By acid hydrolysis of* **15**. Compound **15** (250 mg, 0·72 mmol) was hydrolysed as described under (a) in the appropriate amount of ethanolic HCl. After refluxing for 5 h and cooling overnight 5 mg of colorless crystals was filtered off. This product was found to be identical (TLC, IR, MS) with the product isolated under (a).

1 - *Methyl* - 5,6 - *diphenyl* - *piperazin* - 2,3 - *dione*, **6**

(*a*) *By acid hydrolysis of* **3a**. Compound **3a** (713 mg, 2·00 mmol) was dissolved in a mixture of water, 96% EtOH and conc HCl (1:1:2, v/v, 40 ml) by stirring for 5 h at 60–70°. The yellow soln was concentrated under reduced pressure and excess HCl was removed by evaporating 250 ml of gradually introduced EtOH. The suspension formed upon addition of water (250 ml) was filtered and the yellowish residue washed with MeOH (50 ml) and dried, yielding 140 mg (19·5%) of unreacted **3a**. Filtrate and MeOH washing were combined, the solvent evaporated and the residue dissolved in 150 ml of water. The yellow soln was neutralized (2N aq NH_3) and extracted twice with 100 ml portions of $CHCl_3$. The $CHCl_3$ layers were combined, dried (Na_2SO_4) and concentrated (2 ml). This soln, showing several spots on TLC (EtOAc), was chromatographed on silicagel (50 g silicagel). Elution of several yellow bands was effected with $CHCl_3$–EtOAc (1:1). A fluorescent band (TLC R_f 0·10, EtOAc) was then eluted with EtOAc. The eluate was evaporated and the residue crystallized from benzene (25 ml), yielding 120 mg (20%) of compound **6** as the colorless monohydrate. Mp 133–137° (dec). ($C_{17}H_{16}N_2O_2 \cdot H_2O$ (298·34) Calcd: C, 68·44; H, 6·08; N, 9·39; Found: C, 68·1; H, 6·2; N, 9·5%). PMR (CF_3COOD): δ = 3·24 (*s*, 3); 4·83 and 5·68 (2*d*, J = 5Hz, 2); 6·65–7·4 (m, 10); 11·6 (s, 1). Mass spectrum, *m/e* (%): 280(M^+, 100); 132(5); 120(26); 118(50). IR(KBr), cm^{-1}: 3205(NH); 1690, 1672(C=O).

(*b*) *By acid hydrolysis of* **5**. Compound **5** (100 mg, 0·32 mmol) was heated for 2 h in a mixture, EtOH and conc HCl (1:1:2, v/v, 5 ml) at 60°. Disappearance of **5** and formation of **6** was judged from TLC (EtOAc, spots treated with NH_3(g) before elution).

Catalytic hydrogenation of **3a**

2 - Amino - 8 - methyl - 4,9 - dioxo - 1,6,7,8,9,9a - hexahydro - 4*H* - pyrazino - (1,2 - a) - s - triazine **7** was prepared by hydrogenation of **3a** (1·50 g, 4·21 mmol) over 10%-Pd/C (0·30 g) in glacial AcOH (150 ml). After stirring for 50 h a limiting amount of H_2 (4·18 mmol) had been consumed. The catalyst was filtered off and the filtrate concentrated under red pressure. The resulting slightly yellow oil was triturated with cold MeOH yielding 1·34 g (91·1%) of an amorphous white solid. Crystallization from large amounts of water-MeOH (1:1) gave 1·10 g (74·8%) of **7**. ($C_{19}H_{19}N_5O_2$ (349·39), Calcd: C, 65·32; H, 5·48; N, 20·04; Found: C, 65·3; H, 5·6; N, 20·2%). PMR (CF_3COOD): δ = 2·93 (s, 3); 5·37 and 5·50 (2d, J = 3·5 Hz, 2); 6·12 (s, 1); 6·6–7·5 (m, 10); 11·5 (s, 3). Mass spectrum, *m/e* (%): 349(M^+, 28); 348(24); 347(100); 304(3); 265(15); 192(13); 202(8); 200(22). IR(KBr), cm^{-1}: 3420, 3300(NH); 1686, 1662(C=O).

Conversion of **7** *to* **3a**. A suspension of **7** (500 mg) in DMF (50 ml) was stirred at 80°. After 5 min a clear soln was obtained, prolonged heating (30 min) produced a suspension again, from which **3a** (483 mg) was isolated by filtration, washing with large amounts of water and MeOH and drying at 120°.

Acetylation of **3a**

2 - Acetamino - 8 - methyl - 4,9 - dioxo - 6,7 - diphenyl - 6,7,8,9 - tetrahydro - 4H - pyrazino - (1,2-a) - s - triazine **8** was prepared by refluxing **3a** (300 mg, 0·84 mmol) in Ac_2O (10 ml). After 15 min a clear yellow soln was obtained, which was cooled and concentrated under red pressure. The resulting oil was taken up in MeOH (10 ml) and cooled (0°). Colorless crystals were filtered off yielding 225 mg (56·5%) **8**, containing one molar eq of MeOH (PMR) which was gradually lost upon standing at room temp for longer periods. Recrystallization from EtOH-water (8:1) gave rather poor crystals of the monohydrate. Crystallization at room temp of 50 mg **8** in 50 ml EtOH-water (1:1) afforded crystals of the dihydrate of sufficient size and quality to perform an X-ray analysis. Mp 163–165°. ($C_{21}H_{19}N_5O_3 \cdot 2H_2O$ (425·45) Calcd: C, 59·29; H, 5·45; N, 16·46; Found: C, 59·2; H, 5·5; N, 16·6%). PMR (of **8** · H_2O, CF_3COOD): δ = 2·57 (*s*, 3); 3·08 (*s*, 3); 5·78 and 5·96 (2d, J = 5Hz, 2); 6·7–7·5 (m, 10); 11·4 (s, 3), (of **8**, anhydrous, $CDCl_3$): δ = 2·48 (s, 3); 2·88 (s, 3); 5·68 and 5·78 (2d, J = 5Hz, 2); 6·6–7·35 (m, 10); 9·3 (s, 1). Mass spectrum, *m/e* (%): 389(M^+, 29); 347(7); 346(6); 265(34); 239(50); 222(12); 209(100); 149(81); 132(21). IR(KBr), cm^{-1}: 3340(NH); 1740, 1718, 1690, 1675(C=O); 1615(C=N).

Preparation of 5 - methyl - 6,7 - diphenyl - 5,6,7,8 tetrahydrolumazine, **14**

6,7-Diphenyllumazine[10] (5·00 g, 15·8 mmol) was hydrogenated over prereduced PtO_2 (0·5 g) in a mixture of 96%-EtOH and conc HCl (7:1, v/v, 400 ml). After the calculated amount of H_2 had been consumed (24 h), 40% aq CH_2O (10 ml, 145 mmol) was added to the resulting soln. Hydrogenation was then continued until a third molar eq of H_2 was taken up (30 min). The suspension was filtered under N_2 and residual **14** dissolved in 5N HCl (100 ml). Catalyst was filtered off and the combined filtrates were taken to dryness under red pressure. The yellowish residue was suspended in deaerated water (150 ml) and neutralized (2N NaOH). The white ppt was filtered off, washed with water (3 × 25 ml) and recrystallized from water-MeOH (1:1). The white microcrystalline powder was dried for several h at 60°, yielding **14** (3·20 g, 60·6%). ($C_{19}H_{18}N_4O_2$ (334·38), Calcd: C, 68·25; H, 5·43; N, 16·76; Found: C, 68·4; H, 5·4; N, 16·7%). PMR (CF_3COOD): δ = 3·78 (s, 3); 4·90 and 5·62 (2d, J = 4Hz, 2); 6·9–7·6 (m, 10); 11·4 (s, 3). Mass spectrum, *m/e* (%): 334(M^+, 100); 304(15); 191(8); 180(65); 165(15).

Autoxidation of **14**

Compound **14** (3·34 g, 10·0 mmol) was stirred under O_2 in 0·1 N NaOH (200 ml). A limiting amount of O_2 (9·75 mmol) had been consumed in 8 h. The resulting soln was neutralized (2N H_2SO_4) and the ppt filtered off. The white solid (3·26 g) was washed with water (50 ml) and dried overnight at 100°. Crystallization from water-EtOH (1:1) gave 2·38 g (68·3%) of **15**. Mp 259–261°. ($C_{19}H_{16}N_4O_3$ (348·36), Calcd: C, 65·51; H, 4·63; N, 16·08; Found: C, 65·4; H, 4·9; N, 16·1%). PMR (CF_3COOD): δ = 3·07 (s, 3); 5·75 and 5·89 (2d, J = 5Hz, 2); 6·7–7·6 (m, 10); 11·4 (s, 1). Mass spectrum, *m/e* (%): 348(M^+, 17); 305(100); 277(7); 248(27); 219(8); 201(10); 158(23); 130(77); 103(38).

Methylation of **11**.

Compound **11** (400 mg, 1·14 mmol, anhydrous) was treated with a mixture of powdered K_2CO_3 (dried at 120°) (2·00 g) and $(CH_3O)_2SO_2$ (2·0 ml) in dry DMF (50 ml) for 12 h at 40°. The suspension was filtered, the residue washed with $CHCl_3$ (2 × 50 ml) and the combined filtrate and washings concentrated to dryness under red pressure. The resulting yellow oil was dried overnight (100 Torr, 100°) and introduced onto a silica gel column. Several yellow bands were eluted with $CHCl_3$. A major band with R_f 0·84 (TLC, EtOAc) was eluted with $CHCl_3$–EtOAc (1:1). This eluate was concentrated to dryness and the colorless residue was crystallized from $CHCl_3$ yielding 155 mg (36%) of compound **12**. Mp 274–276°. ($C_{21}H_{22}N_4O_3$ (378·43), Calcd: C, 66·65; H, 5·85; N, 14·80; Found: C, 66·4; H, 6·0; N, 14·9%). PMR ($CDCl_3$): δ = 2·08 (broad, 1); 2·93, 3·02 and 3·13 (3s, 9); 4·50 and 6·00 (2d, J = 4Hz, 2); 6·75–7·4 (m, 10); (CF_3COOD): δ = 3·15, 3·18 and 3·36 (3s, 9); 4·78 and 6·02 (2d, J = 4Hz, 2); 6·75–7·4 (m. 10); 11·4 (s, 1). Mass spectrum, *m/e* (%); 378(M^+, 35); 270(4); 273(66); 216(100); 180(87); 165(12); 154(14); 145(8); 131(15); 120(23); 118(29); 104(9); 69(25). IR(KBr), cm^{-1}; 3275(NH); 1771, 1712(C=O).

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