Pyrazine Chemistry. Part 13.[1] Preparation and Reactions of Pyrazine *N*-Oxides Related to Mycelianamide

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The synthesis is described of 2,5-dihydroxy-3-(4-hydroxybenzyl)-6-methylpyrazine 1,4-dioxide (9), an isomer of degeranylmycelianamide (7), starting with 3-(4-hydroxybenzyl)-6-methylpiperidine-2,5-dione. The chemistry of the dioxide (9) has been explored; acetylation of this compound and of degeranylmycelianamide has been shown to afford the same product.

MYCELIANAMIDE (1) has attracted attention both because of its biological properties [2] and because of the peculiarities of its structure.[3] Recently a successful synthesis of this compound has been achieved in which the hydroxamic acid groups were introduced prior to formation of the heterocycle.[4] Although examples of the incorporation of simple cyclic dipeptides, of the dioxopiperazine class, into various metabolites have been realised,[5] attempts to incorporate labelled dioxopiperazine precursors of the type (2) into mycelianamide resulted in very low uptake.[6] Despite this result, *N*-hydroxylation of amides is a well known biological process,[7] and an approach to compounds related to mycelianamide and which commences with the cyclic dipeptide (2) is described in this work.

In previous papers in this series the tautomeric equilibria between 3-arylmethylenepiperazine-2,5-diones (3) and their aromatic counterparts, the substituted dihydroxypyrazines (4),† have been described.[8] It was observed that aryl substituents, such as *p*-hydroxy-groups, tended to stabilise the arylmethylene isomer (3). A precept of the present approach was to establish whether a related tautomerisation could be observed between the corresponding 1,4-dihydroxypiperazinedione unit (5), as found in mycelianamide, and its aromatic counterpart, the dihydroxypyrazine-1,4-dione (6). Preliminary work had established routes to pyrazine di-*N*-oxides.[9]

It is known that mycelianamide is sensitive to treatment with both mild acid and base and, in order to be able to investigate tautomeric equilibria of the type (5) $\rightleftharpoons$ (6), it was considered necessary to prepare a derivative less sensitive to such conditions. The degeranylmycelianamide (7) was selected as a suitable target, since it was argued that the phenolate group should reduce the sensitivity of the arylmethylene double bond to nucleophilic attack and rearrangements involving the geranyl side chain [3] would be avoided. Formation of the phenol (7) was achieved by treatment of mycelianamide with trifluoroacetic acid. The product was a very polar, water-soluble substance which gave a blue-violet precipitate with aqueous iron(III) chloride. Its ^{1}H n.m.r. spectrum (solvent trifluoroacetic acid) indicated the intact nature of the heterocyclic nucleus. Whereas mycelianamide is rapidly attacked by methanolic 0.5M-potassium hydroxide, forming compounds such as (8),[10] degeranylmycelianamide (7) was only slowly decomposed under these conditions. The initial target of the synthetic studies was, therefore, the isomer (9), in order to establish whether it could equilibrate with the degeranylmycelianamide tautomer (7).

Preparation of the parent skeleton (2) was achieved by standard methods. Methyl L-tyrosinate was coupled with *N*-benzoyloxycarbonyl-DL-alanine, using dicyclohexylcarbodi-imide; catalytic hydrogenation of the product (10) produced the dioxopiperazine (2), as a mixture of stereoisomers. The phenolic group was protected by acetylation [to give (11)] before chlorination.

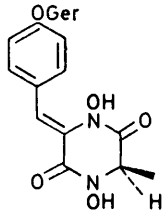
Chlorination was effected with phosphoryl chloride at room temperature. The principal product, the desired dichloropyrazine (12), was accompanied by the free phenol (13) and traces of the monochloropyrazines (14) and (15). Formation of the dichloropyrazine (12) involves concomitant oxidation of the starting material by the chlorinating reagent.[9]

The dichloropyrazine (12) was subjected to oxidation with trifluoroperacetic acid at 0—5 °C to produce the di-*N*-oxide (16). When short reaction times were used, the mono-*N*-oxides (17) and (18) were obtained as side products. Although these could be converted into the di-*N*-oxide on prolonged reaction, general decomposition of these materials then set in; optimal oxidation times were in the order of 24 h.

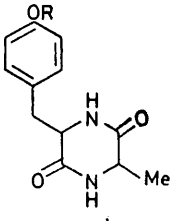
A method for the replacement of the chloro-groups by hydroxy-functions had now to be established. Direct hydrolysis proved unsatisfactory. Treatment of the dichloride (12) with an excess of aqueous 2N-sodium hydroxide at room temperature produced a new compound identified as the mono-*N*-oxide (19). That this was the 4-oxide, rather than the isomeric 1-oxide, was established by careful hydrolysis of the 1-oxide (18), to produce the phenol (20). Assignment of structures to the pairs of 1- and 4-mono-oxides was aided by ^{1}H n.m.r. spectroscopy; introduction of the *N*-oxide function produces a local deshielding (0.1—0.3 p.p.m.) of the methyl or methylene protons relative to the unoxidised precursors. Deoxygenation of aromatic *N*-oxides is

† Compounds such as the dihydroxypyrazines can also exist as tautomers, *e.g.* pyrazinones. For the purposes of the discussion, such possibilities will be assumed except where pertinent to the assignment of structures.

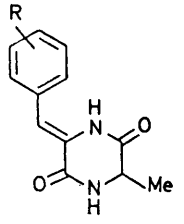
known to occur with a variety of reagents,[11] although the use of aqueous sodium hydroxide is unusual in causing this transformation. The oxygen atom is lost either as oxygen or as hydrogen peroxide and presumably involves



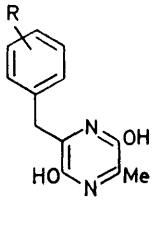
(1) Ger = geranyl throughout



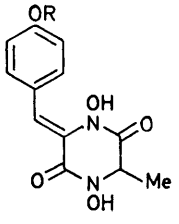
(2) R = H
(11) R = Ac



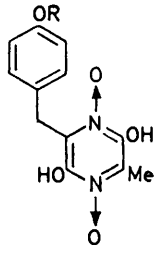
(3)



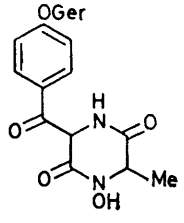
(4)



(5)
(7) R = H
(37) R = Ac



(6)
(9) R = H
(31) R = Ac

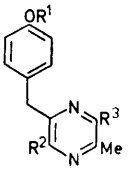


(8)

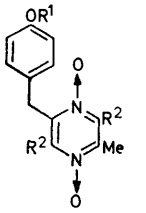
Z - Ala - Tyr - OMe

(10)

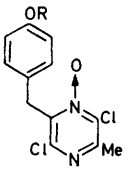
nucleophilic attack on the less hindered 1-*N*-oxide function of the starting 1,4-di-*N*-oxide (16). Pyrazine 1,4-dioxides have previously been observed to suffer deoxygenation rather readily.[9] When the hydrolysis of the 1,4-dioxide (16) was carried out with just 1 equiv. of sodium methoxide in methanol, deacetylation occurred and the free phenol (2) was isolated. Because of the failure to replace the chloro-groups by direct hyrolysis a two-step approach was adopted. Earlier work on the model di-*N*-oxide (22) had shown it was possible to displace the chlorine atoms with sodium benzyloxide, to give the ether (23); mild acid-catalysed cleavage of the benzylic groups then gave the dihydroxypyrazine di-*N*-oxide (24).[9]



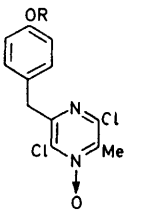
(12) R^1 = Ac, R^2 = R^3 = Cl
(13) R^1 = H, R^2 = R^3 = Cl
(14) R^1 = Ac, R^2 = H, R^3 = Cl
(15) R = Ac, R^2 = Cl, R^3 = H



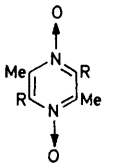
(16) R^1 = Ac, R^2 = Cl
(21) R^1 = H, R^2 = Cl
(25) R^1 = H, R^2 = $PhCH_2O$
(30) R^1 = Ac, R^2 = $PhCH_2O$



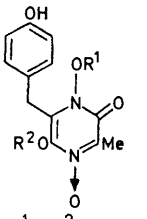
(17) R = Ac
(19) R = H



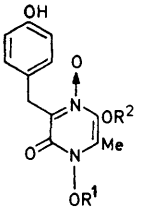
(18) R = Ac
(20) R = H



(22) R = Cl
(23) R = $PhCH_2O$
(24) R = OH



(26) R^1 = R^2 = CH_2Ph
(28) R^1 = H, R^2 = CH_2Ph
(28A) R^1 = CH_2Ph, R^2 = H



(27) R^1 = R^2 = CH_2Ph
(29) R^1 = H, R^2 = CH_2Ph
(29A) R^1 = CH_2Ph, R^2 = H

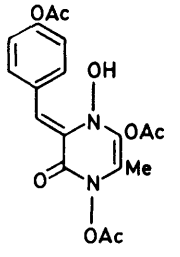
Treatment of the di-*N*-oxide (16) with sodium benzyloxide in tetrahydrofuran at room temperature proceeded smoothly to afford the crude phenolic bisbenzyloxypyrazine 1,4-dioxide (21) in good yield. Attempted recrystallisation of the oxide (25) from common organic solvents resulted in a chemical change, mainly leading to two new compounds. A similar change was observed on leaving solutions of the di-*N*-oxide in various neutral

solvents, *e.g.* methanol, at room temperature for several days, the process being accelerated by heat. The two products were shown to be isomeric with the starting material and were identified as the *N*-benzyloxypyrazinones (26) and (27). In their i.r. spectra both products showed carbonyl absorptions at 1 660 and 1 655 cm^{-1}, absent from those of the starting material. Again ^1H n.m.r. spectroscopy was of value in these assignments. For isomer (26) the methyl group, being deshielded by both the adjacent *N*-oxide and carbonyl groups, resonates at lower field (δ 2.53) than in the starting di-*N*-oxide (25) (δ 2.20). In the isomer (27), the methyl signal occurs at δ 1.86, owing to shielding by the adjacent hydroxy-groups. Complementary shifts of the benzylic proton signals were also observed. Related migrations of benzylic groups have been recorded,[12] but the conditions needed for the current example are particularly mild. This rearrangement could also be catalysed by acid. Thus initial attempts to remove the benzyloxy groups, by treatment of the di-*N*-oxide (25) with methanolic hydrochloric acid at room temperature, gave, mainly, the rearrangement products (26) and (27), together with small amounts of the mono-debenzylated products (28) and (29). [Structural assignments to compounds (28) and (29) are tentative. ^1H N.m.r. chemical shifts of these products indicate the assigned structures rather than their isomeric counterparts (28A) and (29A), respectively.]

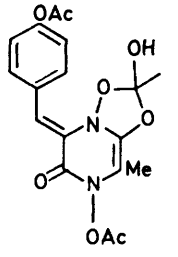
Removal of the benzyloxy groups from the di-*N*-oxide (25), or the rearrangement products (26) and (27), could be effected by treatment with anisole in trifluoroacetic acid. The product was the required phenol (9). Brief treatment of compound (25) with the cleavage reagent produced the mono-debenzylated compounds (28) and (29), which, on further treatment with the reagent also formed the phenol (9). Compound (9) behaved as a polar, water-soluble compound which gave a colour response to iron(III) chloride similar to that of degeranylmycelianamide (7). On t.l.c., compound (9) behaved as a slightly less polar material than its isomer (7), and its ^1H n.m.r. spectrum also differed. Thus, whereas degeranylmycelianamide showed a vinylic proton signal at δ 7.44 and the methyl signal as a doublet at 1.79 (*J* 6 Hz), the pyrazine (9) showed the benzylic methylene signal at δ 4.01 and the methyl signal as a singlet at 2.30. Prolonged treatment of the pyrazine (9) with trifluoroacetic acid had no effect, whilst exposure to bases, *e.g.* pyridine, rapidly caused its decomposition to a variety of products, none of which corresponded to the required degeranylmycelianamide (7). Compound (9) was also found to be sensitive to neutral protic solvents, such as methanol; solutions in such media resulted in disappearance of the pyrazine and the formation of more polar precipitates.

Since the instability of the pyrazine (9) precluded its direct isomerisation to degeranylmycelianamide, some indirect methods were explored. Initial attempts were made to use derivatives with the phenolic group protected by acetylation. Treatment of the bisbenzyloxyphenol (25) with acetic anhydride in pyridine gave the acetate (30). Debenzylation with the trifluoroacetic acid–anisole reagent produced the acetate (31). Like its parent phenol, this material was unaffected by prolonged treatment with trifluoroacetic acid, and bases catalysed its decomposition to complex mixtures.

The phenol (9) was next acetylated with acetic anhydride using imidazole as catalyst. It was rationalised that introduction of acetate functions into the heterocycle would enhance the acidity of the methylene protons and, conceivably, assist the required isomerisation. Two isomeric products resulted from the acetylation. The major compound, $C_{18}H_{18}N_2O_8$, was a triacetylated derivative. Its i.r. spectrum showed carbonyl peaks at 1 800, 1 755, and 1 710 cm^{-1} as well as a strong double bond absorption at 1 650 cm^{-1}, as found in enolic esters, and a weak hydroxy-absorption at 2 900—3 650 cm^{-1}. The ^1H n.m.r. spectrum showed four methyl signals, as singlets, in the region 1.87—2.33 but no benzylic methylene group, and a benzylidene singlet at δ 7.13. This suggests formation of the required arylmethylene system, and the assignment of structure (32) or the isomeric orthoester structure (33) to this compound. The *Z*-



(32)



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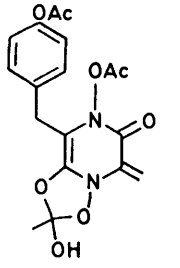

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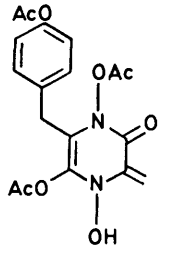


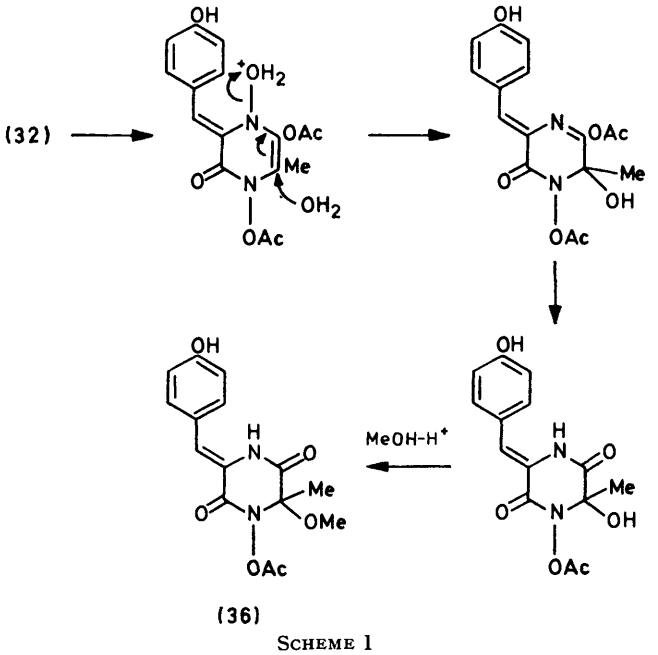
(34)

configuration of the benzylidene bond follows from the observed chemical shift of the benzylidene proton, which would resonate at considerably higher field in the *E*-isomer. Gratifyingly, acetylation of degeranylmycelianamide (7) under similar conditions also produces, as the major product, the triacetate (32), thus providing a point of congruence between the natural and synthetic derivatives.

The minor product from the acetylation of the dihydroxypyrazine di-*N*-oxide (9) was shown to be an isomer of the triacetate (32). Its ^1H n.m.r. spectrum contained three methyl singlets for the acetate groups at δ 2.05, 2.27, and 2.37. The benzylic methylene signal

was retained, although it appeared as an AB quartet (J 14 Hz), but the heterocyclic methyl signal had disappeared, being replaced by a vinylic methylene pattern at δ 4.83 and 5.55. Its i.r. spectrum showed absorptions at 2 800—3 650 cm^{-1} (indicating the presence of a hydroxy-function, as in the major isomer) and 1 800, 1 750, and 1 710 cm^{-1}. This substance can be assigned either structure (34) or (35). Since the benzylic methylene signal generally appears as a singlet, the observed AB quartet suggests a dissymmetric environment and the orthoester structure (35) is therefore preferred.

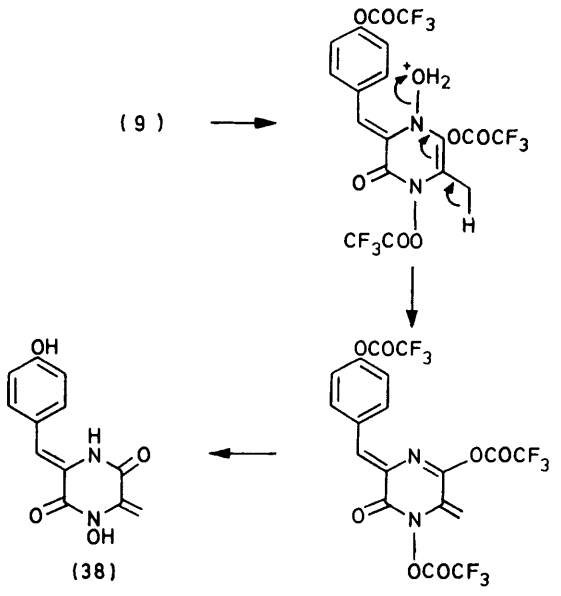
Some attempts at hydrolysing the ester groups from the acetate (32) commenced with its treatment with aqueous trifluoroacetic acid. A precipitate formed which showed loss of one acetic acid unit in its n.m.r. spectrum. Trituration with methanol transformed the precipitate into a new compound identified as the methanol adduct (36). That the remaining single acetyl group was attached to the hydroxamic acid function rather than the phenolic group was evident from its failure to give an immediate colouration with aqueous iron(III) chloride. The amide proton was evident in its i.r. spectrum (sharp band at 3 350 cm^{-1}). A possible route for the formation of compound (36) is given in Scheme 1.



SCHEME 1

An alternative hydrolysis of the triacetate (32), using aqueous pyridine, produced, amongst other products, one material which gave a violet-blue colouration with aqueous iron(III) chloride, identical with that produced by mycelianamide, indicating the presence of two hydroxamic acid functions. The polarity of this material was similar to that of mycelianamide, reflecting the presence of a blocked phenolic group; this material has thus been tentatively assigned structure (37).

Trifluoroacetylation of the pyrazine di-*N*-oxide (9) proved too vigorous for the system. After hydrolysis the reaction afforded one product which was shown to be the dehydrated material (38). This gave a red-brown colour with Fe^{III} chloride, a colour change typical of such monohydroxamic acids. The benzylidene and methylene groups were indicated by the ^1H n.m.r. spectrum. Preferential loss of the 4-hydroxy-group (Scheme 2),



SCHEME 2

rather than the 1-hydroxy-group, is consistent with the known behaviour of this function in mycelianamide.[10]

EXPERIMENTAL

All m.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with a Perkin-Elmer PE 157G spectrophotometer for Nujol mulls unless otherwise stated. ^1H N.m.r. spectra were recorded with Varian A60 and HA100 spectrometers for solutions in deuteriochloroform (tetramethylsilane as internal reference); where stated, spectra of solutions in trifluoroacetic acid (TFA) were recorded with tetramethylsilane as external reference. Mass spectra were recorded with an A.E.I. MS9 instrument.

Thin-layer chromatography (t.l.c.) and preparative layer chromatography (p.l.c.) were carried out on Kieselgel GF_{254} (Merck); polar materials and hydroxamic acids were chromatographed on commercial polyamide-coated sheets distributed by B.D.H. Chemicals and located by spraying with freshly prepared aqueous iron(III) chloride. Column chromatography was carried out using either silica gel M.F.C. (Hopkin and Williams) or MN polyamide powder-CC6 (Macherey, Nagel and Co.). Solvents were generally distilled and dried before use; light petroleum refers to the fraction of boiling range 60—80 °C. Organic extracts were dried over anhydrous sodium sulphate and solvent mixtures are described in ratios of volumes used before mixing. The pyrazine *N*-oxides were sensitive to light; therefore all reaction flasks and vessels containing these substances were shielded with aluminium foil.

Degeranylmycelianamide (7).—Mycelianamide (100 mg) was treated with trifluoroacetic acid (5 ml) at room temper-

ature, with stirring for 0.5 h. The acid was distilled off *in vacuo* and the residue triturated with chloroform to give *compound* (7) (62 mg, 94%) as white needles, m.p. 161—163 °C, $\nu_{max.}$ 3 400, 3 250, 3 050—2 800, 1 660, 1 635, 1 605, 1 465, 1 420, 1 400, 1 385, 1 315, and 1 280 cm^{-1}; δ (TFA) 1.79 (3 H, d, *J* 6 Hz), 4.81 (1 H, q, *J* 6 Hz), 7.16 (4 H, as AA'BB' system), and 7.44 (1 H, s) (Found: M^+, 264.0755. $C_{12}H_{12}N_2O_5$ requires *M*, 264.0746). Aqueous solutions of this substance gave an immediate violet-blue colour with aqueous Fe^{III} chloride, followed by formation of a precipitate of the same hue.

3-(4-*Hydroxybenzyl*)-6-*methyl*-2,5-*dioxopiperazine* (2).—*N*-Benzyloxycarbonyl-DL-alanine (68.8 g) and L-tyrosine methyl ester (64.7 g), freshly liberated from its hydrochloride salt, were dissolved in dry dichloromethane (600 ml) at 0 °C before addition of a solution of dicyclohexylcarbodi-imide (66.7 g) in dichloromethane (250 ml), with stirring over 1 h. The mixture was allowed to warm to room temperature over 3 h and then stirred, under nitrogen, for a further 30 h. The insoluble urea was removed by filtration, and the filtrate washed with aqueous 0.5M-citric acid (2 × 500 ml), saturated aqueous sodium hydrogen carbonate (2 × 500 ml), and water (500 ml). The organic extract was dried, filtered, and evaporated under reduced pressure to afford *N*-benzyloxycarbonyl-DL-alanyl-L-tyrosine methyl ester (152 g). The compound was dissolved in methanol (1 l) and subjected to hydrogenolysis at atmospheric pressure in the presence of 10% Pd–C (6.0 g) and a few drops of glacial acetic acid. The catalyst was removed by filtration and washed with methanol. The combined methanol solutions were saturated with ammonia gas at 0 °C and then stirred overnight at room temperature. The resultant suspension was filtered and the solid combined with a second crop obtained by concentrating the filtrate. The product (2) [13] (46.0 g, 64%) showed m.p. 218—220 °C.

3-(4-*Acetoxybenzyl*)-2,5-*dichloro*-6-*methylpiperazine* (12).—The dioxopiperazine (2) (20 g) and imidazole (1.0 g) were suspended in acetic anhydride (300 ml) at room temperature, with shaking, for 16 h. The white suspension was filtered and the solid washed with a little chloroform, ether, and water before drying *in vacuo* to yield 3-(4-*acetoxybenzyl*)-6-*methyl*-2,5-*dioxopiperazine* (11) (18.5 g, 78%) as a mixture of the L,L- and L,D-isomers, m.p. 210—230 °C. A sample recrystallised from acetonitrile showed $\nu_{max.}$ (KBr) 3 220, 3 100—2 850, 1 775, 1 690, 1 520, 1 475, 1 380, 1 340, and 1 260—1 199 cm^{-1} (Found: C, 60.8; H, 5.8; N, 10.3. $C_{14}H_{16}N_2O_4$ requires C, 60.9; H, 5.8; N, 10.1%).

The acetate (11) (20.0 g) was stirred with phosphoryl chloride (125 ml) at room temperature for 20 h. The resulting red solution was slowly poured into an ice-cooled, neutral buffer solution (18) prepared by adding 2N-NaOH to aqueous 1.5M-sodium dihydrogen phosphate until pH 7 was reached. The oily aqueous solution was extracted with chloroform; the extracts were washed with water and then dried before evaporation to afford a brown oil. Chromatography through SiO_2, eluting with chloroform, afforded *compound* (12) (6.6—7.8 g; 29—35%) as a white crystalline solid, m.p. (light petroleum) 85—86 °C; $\nu_{max.}$ (CCl_4) 3 050, 2 950, 1 672, 1 515, 1 440, 1 442, 1 375, 1 330, 1 230—1 190, and 1 170 cm^{-1}; δ (CCl_4) 1.5 (3 H, s), 2.52 (3 H, s), 4.09 (2 H, s), and a multiplet centred around 7.04 (4 H, AA'BB' system) (Found: C, 54.3; H, 4.1; Cl, 23.0; N, 9.1. $C_{14}H_{12}Cl_2N_2O_2$ requires C, 54.0; H, 3.9; Cl, 22.5; N, 9.1%).

Further elution of the column gave a mixture of two compounds (0.2—0.5 g) isolated as a white solid which possessed physical properties consistent with the monochloropyrazines (14) and (15), not further characterised. The most polar fraction, also eluted by chloroform, was the deacetyl derivative (13) (1.8—2.0 g; 9—10%). Reacetylation of this, with acetic anhydride–imidazole, readily afforded more of the acetate (12).

Oxidation of the Dichloride (12).—Trifluoroacetic acid (100 g) was cooled to 0 °C before addition of hydrogen peroxide (20 ml; 80%) and the free radical inhibitor 2,6-di-t-butyl-4-methylphenol (0.15 g); the mixture was stirred at 0—5 °C for 0.5 h. The dichloropyrazine (3.0 g) was then added in one portion and the mixture stirred at 0—5 °C for 24 h. The mixture was poured into a neutral phosphate buffer solution (600 ml) and extracted with chloroform (4 × 200 ml). The extract was washed with water, dried, and evaporated *in vacuo* to give a yellow solid. Trituration with several small portions of methanol afforded 3-(4-*acetoxybenzyl*)-2,5-*dichloro*-6-*methylpyrazine* 1,4-*dioxide* (16) (1.2 g, 36%), as an off-white solid, m.p. (MeOH) 214—215 °C; $\nu_{max.}$ ($CHCl_3$) 3 100—2 850, 1 750, 1 603, 1 520, 1 490, 1 335, and 1 270—1 170 cm^{-1}; δ 2.22 (3 H, s), 2.65 (3 H, s), 4.40 (2 H, s), 6.8—7.5 (4 H, AA'BB') [Found: C, 49.1; H, 3.7; N, 8.1%; M^+, 342.0165. $C_{14}H_{12}Cl_2N_2O_4$ requires C, 49.0; H, 3.5; N, 8.2%; *M*, 342.0174 (for ^{35}Cl)].

The methanolic triturate was evaporated *in vacuo* and the residue crystallised once from methanol to give a mixture of the isomeric 3-(4-acetoxybenzyl)-2,5-dichloro-6-methylpyrazine 4-oxide (17) and 1-oxide (18) (1.76 g), m.p. 98—101 °C. The ^1H n.m.r. spectrum of this mixture showed peaks at δ 2.18 (3 H, s), 2.52 (3 H, s), 4.12 and 4.28 (total 2 H, as singlets), and 6.8—7.5 (4 H, m). From the mother liquors, left in a refrigerator for an extended period, were deposited colourless crystals of the 1-*oxide* (18), δ 2.18 (3 H, s), 2.52 (3 H, s), 4.12 (2 H, s), 6.8—7.5 (4 H, AA'BB') (Found: C, 51.6; H, 4.0; Cl, 21.55; N, 8.6. $C_{14}H_{12}Cl_2N_2O_3$ requires C, 51.4; H, 3.7; Cl, 21.7; N, 8.6%).

Further oxidation of the mono-*N*-oxides with trifluoroperacetic acid under the conditions described above produced more of the di-*N*-oxide (16) (39% yield).

Hydrolysis Experiments on the 1,4-*Dioxide* (16.)—(a) *With excess of base.* To the dioxide (0.6 g) in tetrahydrofuran (200 ml) was added aqueous 2N-NaOH (200 ml). The mixture immediately turned a light brown and, after 1 h, t.l.c.[14] indicated that all the starting material had been converted into a more polar material, with only traces of hydroxamic acids at the origin. After a further 2 h no further change was observed in the reaction mixture, which was diluted with chloroform and acidified with 4N-HCl. The chloroform extract was then washed with water, dried, and evaporated, *in vacuo*, to afford a crystallizing oil. The product was filtered through polyamide powder, using light petroleum as solvent, in order to remove the polar products. Removal of the solvent from the filtrate gave 2,5-*dichloro*-3-(4-*hydroxybenzyl*)-6-*methylpyrazine* 4-*oxide* (19) (435 mg, 87%), m.p. (MeOH) 219—221 °C; $\nu_{max.}$ 3 500—3 050, 1 620, 1 565, 1 520, 1 480, 1 430, 1 370, 1 275, 1 240, 1 220, 1 180, 1 100, 1 090, and 1 010 cm^{-1}; δ (TFA) 2.33 (3 H, s), 4.10 (2 H, s), and a multiplet centred at 6.68 (4 H, AA'BB') (Found: C, 50.6; H, 3.8; Cl, 25.05; N, 9.7. $C_{12}H_{10}Cl_2N_2O_2$ requires C, 50.55; H, 3.5; Cl, 24.9; N, 9.8%).

(b) *With one equivalent of base.* The dioxide (16) (0.5 g) was suspended in methanol (10 ml) and N-sodium methoxide (1.5 ml) was added with stirring and ice-cooling. After 1 h the solution was acidified with N-HCl (1.6 ml) and the methanol removed *in vacuo*. The solid product was filtered

off, washed with a little methanol, and dried *in vacuo* to yield *2,5-dichloro-3-(4-hydroxybenzyl)-6-methylpyrazine* 1,4-*dioxide* (21) (320 mg, 73%), m.p. (dioxan) 250—251 °C; $\nu_{max.}$ (KBr) 3 350, 3 100—2 900, 1 618, 1 595, 1 525, 1 490, 1 450, 1 425, 1 335, 1 270, 1 230—1 200, 1 105, 2 060, and 1 000 cm^{-1}; δ (TFA) 2.86 (3 H, s), 4.56 (2 H, s), and 6.8—7.3 (4 H, AA'BB' m) (Found: C, 47.7; H, 3.5; Cl, 23.7; N, 9.4. $C_{12}H_{10}Cl_2N_2O_3$ requires C, 47.8; H, 3.35; Cl, 23.6; N, 9.3%).

Hydrolysis of 3-(4-Acetoxybenzyl)-2,5-dichloro-6-methylpyrazine 1-Oxide (18).—To the mono-*N*-oxide (100 mg) in tetrahydrofuran (10 ml), 2N-NaOH (4 ml) was added, with stirring. After 1.5 h the solution was acidified with N-HCl before extraction with chloroform. The extract was washed with water, dried and evaporated *in vacuo*. The residual oil on trituration with methanol gave colourless crystals of *2,5-dichloro-3-(4-hydroxybenzyl)-6-methylpyrazine 1-oxide* (20) (60 mg, 69%), m.p. (aq. MeOH) 177—179 °C, considerably depressed on admixture with the 4-oxide (19); $\nu_{max.}$ 3 500—3 100, 1 620, 1 595, 1 570, 1 520, 1 465, 1 450, 1 390, 1 355, 1 270, 1 225, 1 180, 1 080, 840, and 835 cm^{-1}; δ (TFA) 2.45 (3 H, s), 4.00 (2 H, s), and a multiplet centred at 6.69 (4 H, AA'BB') (Found: C, 50.5; H, 3.6; Cl, 24.65; N, 9.6. $C_{12}H_{10}Cl_2N_2O_2$ requires C, 50.55; H, 3.5; Cl, 24.9; N, 9.8%).

Reaction of 3-(4-Acetoxybenzyl)-2,5-dichloro-6-methylpyrazine 1,4-Dioxide (16) *with Sodium Benzyloxide*.—Benzyl alcohol (1.3 ml) in tetrahydrofuran (60 ml) was treated with sodium hydride (360 mg of 60% w/w dispersion in mineral oil) under nitrogen for 1.5 h at room temperature. When hydrogen evolution ceased the pyrazine di-*N*-oxide (0.9 g) was added, in one portion, and the mixture stirred at room temperature for 50 min. Chloroform and water were added and the mixture was neutralised with 0.5M-citric acid. The organic layer was separated and the aqueous layer re-extracted with chloroform (total 250 ml). The combined organic phases were washed with water, dried, and evaporated *in vacuo* to afford a mobile oil. On t.l.c. (1 : 9 Me_2CO–$CHCl_3$) this material ran as a single compound, slightly less polar than the phenol (21). With aqueous iron(III) chloride a sample slowly produced an orange-red colour, which gradually darkened to blue-black. The crude oil, generally used without further purification for further reactions, was purified by column chromatography [SiO_2 (100 g); chloroform] to give *2,5-bisbenzyloxy-3-(4-hydroxybenzyl)-6-methylpyrazine 1,4-dioxide* (25) (600 mg, 52%) as a colourless solid, m.p. 160—180 °C (decomp.); $\nu_{max.}$ 3 600—2 700, 1 610, 1 520, 1 460, 1 350, 1 220, 1 175, 1 140, 1 080, 1 000, 760, 710, and 675 cm^{-1}; δ 2.22 (3 H, s), 4.00 (2 H, s), 5.43 (2 H, s), 5.48 (2 H, s), and 7.0—7.4 (14 H, m); *m/z* 444 (M^+), 478 (M^+ − 16), and 412 (428 − 16).

Acid Treatment of the Pyrazine Di-N-oxide (25).—Freshly prepared oxide (150 mg) was dissolved in methanol (20 ml) and stirred with 4N-HCl (1.5 ml) at room temperature overnight. T.l.c. indicated the formation of two new products, in approximately equal quantities, together with a small quantity of very polar material which gave an immediate red colour with Fe^{III} chloride. The mixture was diluted with chloroform, washed with water, dried, and evaporated *in vacuo* to give a yellow amorphous solid. This was filtered through a column of polyamide powder (5 g; 1 : 1 light petroleum–$CHCl_3$) and the eluate (85 mg) was separated by preparative t.l.c. (SiO_2; $CHCl_3$). The less polar compound was characterised as *1,5-bisbenzyloxy-3-(4-hydroxybenzyl)-6-methyl-2-oxo-1,2-dihydropyrazine 4-oxide* (27), m.p. ($CHCl_3$–Me_2CO) 217—219 °C; $\nu_{max.}$ 3 650—3 100, 1 660, 1 610, 1 600, 1 520, 1 470, 1 430, 1 380, 1 260, 1 230, 1 225, 1 170, 1 105, 1 090, and 1 075 cm^{-1}; δ 1.86 (3 H, s), 4.25 (2 H, s), 5.20 (2 H, s), 5.26 (2 H, s), multiplet centred at 7.07 (4 H, AA'BB'), and 7.28—7.37 (10 H, m); *m/z* 444 (M^+) and 428 (M^+ − 16) (Found: C, 70.1; H, 5.4; N, 6.25. $C_{26}H_{24}N_2O_5$ requires C, 70.3; H, 5.4; N, 6.3%).

The more polar compound was the isomeric *1,5-bisbenzyloxy-6-(4-hydroxybenzyl)-3-methyl-2-oxo-1,2-dihydropyrazine 4-oxide* (26), m.p. (MeOH) 189—190 °C; $\nu_{max.}$ 3 450—3 100, 1 655, 1 610, 1 590, 1 525, 1 465, 1 380, 1 350, 1 280, 1 250, 1 220, 1 170, 1 090, and 1 055 cm^{-1}; δ 2.53 (3 H, s), 3.67 (2 H, s), 4.95 (2 H, s), 5.21 (2 H, s), multiplet 6.87 (4 H, AA'BB'), and 7.42 (10 H, m); *m/z* 444 (M^+) and 428 (M^+ − 16) (Found: C, 69.9; H, 5.3; N, 6.0. $C_{26}H_{24}N_2O_5$ requires C, 70.3; H, 5.4; N, 6.3%).

Further elution of the polyamide column, with $CHCl_3$, afforded an oil (20 mg), which crystallised on addition of methanol. This polar material gave an immediate orange-red colour with Fe^{III} chloride, which slowly darkened to blue-black. Analytical t.l.c. (polyamide; $CHCl_3$) separated the material into two components. The substance was identified as an approximately 1 : 1 mixture of the isomers 5-benzyloxy-1-hydroxy-3-(4-hydroxybenzyl)-6-methyl-2-oxo-1,2-dihydropyrazine 4-oxide (29) [or its isomer (29A)] and 5-benzyloxy-1-hydroxy-6-(4-hydroxybenzyl)-3-methyl-2-oxo-1,2-dihydropyrazine 4-oxide (28) [or its isomer (28A)]. The mixture showed $\nu_{max.}$ 3 450—2 700, 1 640, 1 590, 1 520, 1 455, 1 380, 1 335, 1 280, 1 245, 1 220, and 1 150 cm^{-1}; *m/z* 354 (M^+) and 338 (M^+ − 16) (Found: M^+, 354.1222. $C_{19}H_{18}N_2O_5$ requires *M*, 354.1216). After preparative t.l.c., samples of the two compounds were subjected to ^1H n.m.r. analysis. Compound (28) showed δ 2.36 (3 H, s), 3.80 (2 H, s), 5.18 (2 H, s), and 6.3—7.4 (9 H, m), and compound (29) showed δ 2.06 (3 H, s), 4.10 (2 H, s), 5.18 (2 H, s), and 6.3—7.4 (9 H, m).

Thermolysis of the Bisbenzyloxypyrazine (25).—The freshly prepared pyrazine di-*N*-oxide (50 mg) was heated in toluene (5 ml) at reflux. After 3 h no starting material remained and two new products had been formed in an approximately 1 : 1 ratio. The compounds were identified, by chromatographic and ^1H n.m.r. comparison, as the rearrangement products (26) and (27).

2,5-Dihydroxy-3-(4-hydroxybenzyl)-6-methylpyrazine 1,4-Dioxide (9).—The freshly prepared pyrazine dioxide (25) (1.2 g) in anisole (30 ml) was stirred with trifluoroacetic acid (33 ml) for 2 h. The dark red solution was then concentrated *in vacuo* to a small volume (20 ml) and the precipitate formed was collected and washed thoroughly with $CHCl_3$, MeOH, and then ether to yield a yellow solid (530 mg). The filtrate gave a further batch of the same solid (140 mg). Thus was obtained *compound* (9) (670 mg, 94%), m.p. 178—180 °C; $\nu_{max.}$ 3 430, 3 300—2 400, 1 650w, 1 585, 1 525, 1 460, 1 380, 1 340, 1 290, 1 260—1 120, 1 100, 1 090—1 040, and 985 cm^{-1}; δ (TFA) 2.30 (3 H, s), 4.01 (2 H, s), and 6.37—7.03 (4 H, AA'BB' m) (Found: M^+, 264.0750. $C_{12}H_{12}N_2O_5$ requires *M*, 264.0746). Attempted recrystallisation of this material caused extensive degradation. Exposure to light rapidly caused its colour to turn brown.

When the pyrazine (25) was treated with 10% trifluoroacetic acid in anisole for 0.5 h the major product was a mixture of the monobenzyloxy-derivatives (28) and (29) (70%). Further treatment of this mixture with the 1 : 1 anisole–trifluoroacetic acid reagent, as described above, gave the completely debenzylated compound (9). The latter gave a blue-violet colouration with Fe^{III} chloride.

Compound (9) was also formed by debenzylation, under the above conditions, of compounds (26) and (27).

The Acetate (31).—The bisbenzyloxypyrazine (25) (600 mg) was stirred in acetic anhydride (15 ml) and pyridine (30 ml) overnight at room temperature. The solution was poured onto ice–water (200 ml) and stirred for 1 h before extraction into chloroform. The extract was washed with water, dried, and evaporated *in vacuo*. The residue (30) was immediately dissolved in anisole (15 ml) and trifluoroacetic acid (15 ml) and stirred at room temperature for 3 h. The solution was concentrated *in vacuo*, resulting in the formation of a precipitate, which was collected, washed with $CHCl_3$, MeOH and acetone and dried to give a yellow solid, identified as 3-(4-*acetoxybenzyl*)-2,5-*dihydroxy*-6-*methylpyrazine* 1,4-*dioxide* (31) (328 mg, 79%); $\nu_{max.}$ 3 300—3 040, 1 760, 1 670, 1 585, 1 520, 1 475, 1 385, 1 360, 1 340, 1 220, 1 140, 1 100, 1 060, and 1 030 cm^{-1}; δ (TFA) 1.85 (3 H, s), 2.15 (3 H, s), 3.93 (2 H, s), and multiplet centred at 6.73 (4 H, AA'BB') (Found: M^+, 306.0846. $C_{14}H_{16}N_2O_6$ requires M, 306.0852).

Acetylation of the Dioxide (9).—The dioxide (100 mg) was shaken in acetic anhydride (10 ml) containing imidazole (10 mg) at room temperature overnight. The solvent was distilled off *in vacuo* (below 45 °C) and the residue subjected to preparative t.l.c. (SiO_2; $CHCl_3$, multiple elution) to afford two compounds.

The major, less polar product was characterised as (*Z*)-1,5-*diacetoxy*-3-(4-*acetoxybenzylidene*)-4-*hydroxy*-6-*methyl*-3,4-*dihydropyrazin*-2(1H)-*one* (32) (63 mg, 43%), m.p. 178—180 °C (decomp.); $\nu_{max.}$ ($CHCl_3$) 3 650—2 900, 1 800, 1 755, 1 710, 1 650, 1 520, 1 450, 1 390, and 1 375 cm^{-1}; δ 1.87 (3 H, s), 2.08 (3 H, s), 2.33 (6 H, s), 7.13 (1 H, s), multiplet at 7.30 (4 H, AA'BB'), and 8.05 (1 H, br, s, exchanged by D_2O); *m/z* 390 (M^+) (Found: C, 55.6; H, 4.8; N, 7.2. $C_{18}H_{18}N_2O_8$ requires C, 55.4; H, 4.65; N, 7.2%).

The more polar, minor product was characterised as 7-*acetoxy*-8-(4-*acetoxybenzyl*)-2-*hydroxy*-2-*methyl*-5-*methylene*-5H-1,3,4-*dioxazolo*[4,5-a]*pyrazin*-6(7H)-*one* (35) (24 mg), obtained as a viscous oil, $\nu_{max.}$ ($CHCl_3$) 3 650—2 800, 1 800, 1 750, 1 710, 1 640, 1 520, 1 490, 1 450, and 1 380 cm^{-1}; δ 2.05 (3 H, s), 2.27 (3 H, s), 2.37 (3 H, s), 3.39 and 3.58 (2 H, ABq, *J* 14 Hz), 4.83 and 5.55 (2 H, br, s, vinylic methylene), and 6.98—7.45 (4 H, AA'BB' m); *m/z* 390 (M^+).

Acetylation of the acetate (31), under the above conditions, also formed the triacetates (32) and (35).

Acetylation of degeranylmycelianamide (7) (20 mg), under the above conditions, followed by preparative t.l.c. of the product, gave two products. The less polar component (6.6 mg) was identical (n.m.r. and mass spectroscopy and t.l.c. behaviour) with the triacetate (32).

Hydrolysis of the Triacetate (32).—(a) *Aqueous trifluoroacetic acid.* The ester (150 mg) was stirred with 1 : 2 aqueous trifluoroacetic acid (3 ml) at room temperature for 40 min. The chloroform-insoluble suspension was collected and dried (99 mg). Trituration, and crystallisation from methanol converted this chloroform-insoluble product into a soluble substance, which only slowly produced a red colour with Fe^{III} chloride. Purification, by preparative t.l.c. (SiO_2; MeOH–$CHCl_3$) afforded (*Z*)-1-acetoxy-3-(4-hydroxybenzylidene)-6-methyl-6-methoxypiperazine-2,5-dione (63 mg), $\nu_{max.}$ (film) 3 600—3 100, 3 050, 2 950, 1 760, 1 715, 1 675, 1 640, 1 600, 1 500, 1 440, 1 375, 1 320, 1 300, 1 270, 1 200, 1 170, 1 115, and 1 025 cm^{-1}; δ 2.05 (3 H, s), 2.28 (3 H, s), 3.85 (3 H, s), multiplet centred at 7.40 (4 H, AA'BB'), 7.43 (1 H, s), and 8.38 (1 H, br, s, exchanged with D_2O); *m/z* 329 (M^+) and 278 ($M^+ - CH_2CO$).

(b) *Aqueous pyridine.* A small quantity of the triacetate (10 mg) was stirred with 2 : 1 aqueous pyridine (5 ml) in a two-phase system with chloroform (5 ml). The mixture was stirred at room temperature, and hydrolysis was monitored by t.l.c. (polyamide; chloroform). A new product was formed after several hours that gave a violet-blue colour with the Fe^{III} chloride spray [12] and which had an R_F value similar to that of mycelianamide. Prolonged hydrolysis times led to general decomposition and the product was not isolated.

Trifluoroacetylation of the Pyrazine Di-N-oxide (9).—The di-*N*-oxide (190 mg), trifluoroacetic anhydride (5 ml), and imidazole (5 mg) were stirred at room temperature overnight. The solution was evaporated to dryness *in vacuo* and water (5 ml) and trifluoroacetic acid (1 ml) were added. The precipitate was stirred in the acid medium for 3 h before collection, washing with chloroform, and drying. Purification by elution through a column of polyamide powder ($CHCl_3$ and $CHCl_3$–MeOH mixtures) gave one main product, (*Z*)-1-hydroxy-3-(4-hydroxybenzylidene)-5-methylenepiperazine-2,5-dione (38) (64 mg, 36%), as a tan-coloured solid, $\nu_{max.}$ 3 400—3 050, 1 680, 1 655, 1 620, and 1 610 cm^{-1}; δ (TFA) 5.86 and 6.04 (2 H, br, s, methylene), multiplet centred at 7.20 (4 H, AA'BB'), and 7.32 (1 H, s); *m/z* 246 (M^+) and 230 ($M^+ - 16$).

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