722. *Studies in Relation to Biosynthesis. Part VIII.* The Structure of Mycelianamide.*

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Mycelianamide, $C_{22}H_{28}O_5N_2$, gives by the action of ammonia *p*-myceloxybenzamide which is very similar to, but not identical with *p*-geranyloxybenzamide. Hydrogenation produces deoxymycelianamide, $C_{22}H_{28}O_3N_2$, which is hydrolysed by acid to *p*-hydroxyphenylpyruvic acid and alanine. Reduction with sodium and liquid ammonia gives methylgeraniolene. Ozonolysis gives acetaldehyde and acetone. On the basis of this and previously published evidence, together with considerations based on the general properties of the substance, the formula

[VIII; R = CMe_2:$CH{\cdot}CH_2{\cdot}CH_2{\cdot}C$(:$CHMe){\cdot}CH_2{\cdot}$]

is suggested.

Mycelianamide, $C_{22}H_{28}O_5N_2$, from the mycelium of strains of *Penicillium griseofulvum* Dierckx was examined by Oxford and Raistrick,[1] who suggested the structural formula (I). They showed that ammonia gave *p*-myceloxybenzamide (II; R = NH_2) and mild alkaline hydrolysis gave *p*-myceloxybenzoic acid (II; R = OH). Acid hydrolysis gave ammonia and substance (III), together with an unsaturated hydrocarbon, $C_{10}H_{16}$, "mycelene," which contained a persistent oxygenated impurity. Mycelene, examined by Simonsen (quoted by Oxford and Raistrick [1]), appeared to be a monocyclic hydrocarbon, and ozonolysis gave formaldehyde and a diketone, $C_8H_{14}O_2$. It was suggested that the mycelyl radical is derived from an allyl alcohol because of the ease of fission of the ether link.

Mycelianamide gives a red ferric test, is soluble in carbonate solutions, and is non-basic. Oxford and Raistrick, therefore, suggested the partial formula (I), the asymmetric centre being marked by an asterisk.

We have shown that this formula is incorrect since the substance is recovered with unchanged optical rotation after dissolution in sodium carbonate solution. The enolic salt derived from (I) in such a reaction would be inactive.

The problem of the structure of mycelianamide resolves itself into two parts: determination of the structure of the nitrogen-containing portion of the molecule, and determination of the structure of the mycelyl radical.

The Nucleus.—*C*-Methyl analyses of mycelianamide probably indicate three such groups; myceloxybenzamide appears to contain two *C*-methyl groups; consequently the nitrogen-containing portion of the molecule must contain one. This portion confers the solubility in carbonate solutions and the positive ferric test. The formation of ammonia, carbon dioxide, and compound (III) on acid hydrolysis, and of (II; R = NH_2 or OH) under alkaline conditions suggests an acyldioxopiperazine, as type (VII) where the position of the hydroxyl group cannot be defined with certainty. Support for this was obtained by detection of alanine among the products of acid hydrolysis. However, mild reduction with zinc dust and acetic acid produced in good yield deoxymycelianamide, $C_{22}H_{28}O_3N_2$, lacking two oxygen atoms; this is insoluble in alkali, gives no ferric test, and has an ultraviolet absorption very similar to that of mycelianamide itself. There is only slight alteration in the chromophore on reduction (from $\lambda_{max.}$ 231, 321 mμ to $\lambda_{max.}$ 225, 317 mμ) the absorption being similar to that of *p*-methoxybenzylideneacetone ($\lambda_{max.}$ 232, 317 mμ). The slight shift to shorter wavelengths on reduction would be expected on removal of hydroxyl groups from nitrogen. Deoxymycelianamide has a strong band at 1675 cm.$^{-1}$ which is characteristic of unstrained cyclic amides, including dioxopiperazines. This suggests that mycelianamide is a cyclic bisacylhydroxylamine, a probable formula being (VIII; R = $C_{10}H_{17}$). Support for this assumption was obtained by examining the hydrolysis of deoxymycelianamide, which produced alanine and *p*-hydroxyphenylpyruvic acid. In order to explain the products of hydrolysis of mycelianamide itself the formation

* Part VII—Birch, Massy-Westropp and Moye, *Austral. J. Chem.*, 1955, **8**, 539.

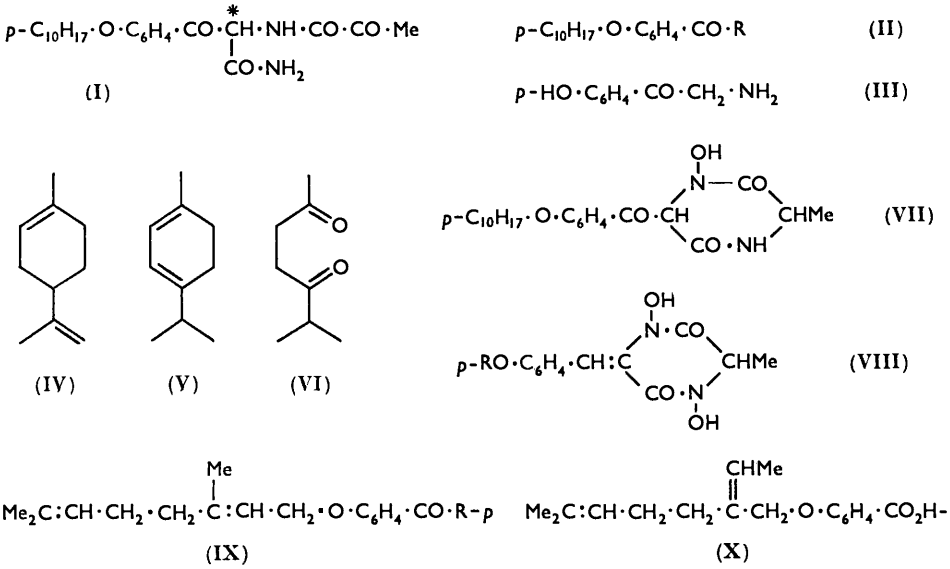
[1] Oxford and Raistrick, *Biochem. J.*, 1948, **42**, 323.

of an intermediate acyldioxopiperazine would appear to be necessary. The formula originally assigned to mycelianamide itself [2] is therefore rather that of such an intermediate. Formation of this would involve an internal rearrangement for which, so far as we are aware, no analogies exist although it has affinities with the rearrangement of 2-methylpyridine 1-oxides to 2-hydroxymethylpyridine derivatives.

A number of mould products are known which contain the acylhydroxylamine, or related groups, including mycobactin, cycloserine (oxamycin), nocardamin, and aspergillin.

The Side-chain.—The C_{10}-formula of the mycelyl radical suggests the presence of a terpenoid side-chain, which we particularly desire in a mould metabolic product for biosynthetic studies.

The presence of a geranyl side-chain as in (IX) is not incompatible with Simonsen's results, since geraniol is known to undergo cyclisation under the conditions used in the hydrolysis. Among the products of this cyclisation are dipentene (IV), which could give formaldehyde on ozonolysis, and α-terpinene (V) which could give a diketone $C_8H_{14}O_2$



(VI). The persistent oxygenated impurity could well be a tertiary alcohol like α-terpineol. Ozonolysis of mycelianamide gave rise chiefly to acetone and some acetaldehyde, and it was originally believed that the latter was derived from the alanine residue. The mycelyl radical would on the basis of these properties and its optical inactivity probably be identical with the geranyl radical.[2] Accordingly *p*-geranyloxybenzoic acid (IX; R = OH) and *p*-geranyloxybenzamide (IX; R = NH_2) were synthesised for comparison with the corresponding mycelyl compounds.

Lauer and Labriola [3] record m. p. 113—114° for *p*-geranyloxybenzoic acid, compared with m. p. 118—120° for *p*-myceloxybenzoic acid. The action of geranyl bromide on methyl *p*-hydroxybenzoate in the presence of potassium carbonate, and hydrolysis, gave several fractions of acid, m. p. 117—118° and m. p. 119—120°, which could not be appreciably altered by recrystallisation. Both fractions were undepressed in m. p. by *p*-myceloxybenzoic acid. Some difficulty was experienced in preparing pure *p*-geranyloxybenzamide. The acid chloride, prepared by the action of thionyl chloride, reacted with ammonia to give specimens melting between 105° and 114°, which could not be purified further by crystallisation. However, phosphorus tribromide and pyridine, followed by

[2] Birch, Massy-Westropp, and Rickards, *Chem. and Ind.*, 1955, 1599.
[3] Lauer and Labriola, *J. Amer. Chem. Soc.*, 67, 1254.

ammonia, gave an amide, m. p. 117—119° undepressed by *p*-myceloxybenzamide, m. p. 119—120·5°. It was originally considered [2] that this evidence proved the identity of the radicals. However, we were later able to measure the infrared spectra, and although the resemblances between the spectra of the synthetic substances and of the hydrolysis products are great there exist some slight differences in positions and intensities of bands in the region 8—14 μ which are greater than would be expected if the substances are in fact identical. We have accordingly examined the ozonolysis of *p*-myceloxybenzamide. From this reaction were obtained acetone and acetaldehyde, and none of the expected lævulaldehyde or acid. Control experiments ensured that the acetaldehyde could not be derived from the solvents used. Reduction of mycelianamide with sodium and methanol in liquid ammonia should remove the side-chain by hydrogenolysis to give a hydrocarbon [4] probably identical with methylgeraniolene (2 : 6-dimethylocta-2 : 6-diene). Reduction gave an oil which, from its infrared spectrum, appears to be identical with this hydrocarbon obtained by similar reduction of linalool. Some minor differences in the spectra may be due to *cis-trans* isomerism, but the crystalline tetrabromide, m. p. 88—89°, was undepressed in m. p. by an authentic specimen, and had an identical infrared spectrum. We therefore suggest (X) as the most probable formula for *p*-myceloxybenzoic acid, this formulation being supported by the close resemblance to *p*-geranyloxybenzoic acid and by the general properties of the mycelyl radical including its ready removal by dilute acid and its optical inactivity. Mycelianamide is, therefore, probably [VIII; R = CMe_2:$CH{\cdot}CH_2{\cdot}CH_2{\cdot}C(:CHMe){\cdot}CH_2{\cdot}$]. Further work is in progress.

Biochemical studies with isotopically labelled acetic acid and labelled tyrosine show that both of these substances are readily incorporated by the mould into the mycelianamide molecule.[5] The results of degradations of these labelled compounds will be reported.

Experimental

M. p.s are uncorrected.

Mycelianamide was obtained from the mycelium of *Penicillium griseofulvum* Dierckx (Raistrick's strain P.68) by Oxford and Raistrick's method; it had m. p. 171° (Found: *C*-Me, 10·3. Calc. for $C_{22}H_{28}O_5N_2$: 3*C*-Me, 11·25%).

p-Myceloxybenzamide, m. p. 119—120·5°, was obtained as described [1] (Found: *C*-Me, 7·9. Calc. for $C_{17}H_{23}O_2N$: 2*C*-Me, 11·0%). Hydrolysis with refluxing saturated methanolic potassium hydroxide for 3·5 hr. gave *p*-myceloxybenzoic acid, m. p. 117—119° after crystallisation from light petroleum (b. p. 60—80°).

p-*Geranyloxybenzoic Acid.*—The preparation was essentially as described by Lauer and Labriola,[3] with methyl instead of ethyl *p*-hydroxybenzoate. After hydrolysis of the ester by refluxing in methanolic potassium hydroxide (8%) for 1·5 hr., and acidification with hydrochloric acid the oily precipitate was only partly soluble in hot benzene. The insoluble portion (undecomposed sodium salt) on treatment with acetic acid gave *p*-geranyloxybenzoic acid, m. p. 119—120° (Found: C, 74·2; H, 8·1. Calc. for $C_{17}H_{22}O_3$: C, 74·4; H, 8·1%). The benzene solution on similar treatment gave a less pure material, the m. p. of which was raised to 117—118° by repeated crystallisation from light petroleum (b. p. 60—80°). Both fractions, which had almost identical infrared spectra, were undepressed in m. p. on admixture with *p*-myceloxybenzoic acid, m. p. 117—119°.

p-*Geranyloxybenzamide.*—*p*-Geranyloxybenzoic acid (200 mg.) in pyridine (0·5 c.c.) was treated slowly at 0° with phosphorus tribromide (100 mg.) and left for 30 min. at room temperature. Concentrated aqueous ammonia was then added and the mixture extracted with benzene. Several crystallisations from benzene–light petroleum (b. p. 40—60°) yielded p-*geranyloxybenzamide* (50 mg.), m. p. 117—119° (Found: C, 74·3; H, 8·5. $C_{17}H_{23}O_2N$ requires C, 74·7; H, 8·5%). Its m. p. was undepressed by *p*-myceloxybenzamide, m. p. 119—120·5°.

Ozonolysis of Mycelianamide.—Mycelianamide (1 g.) in glacial acetic acid (purified by refluxing with chromic oxide) was ozonised at 20° until ozone emerged freely, zinc dust and a little water were added, and the mixture was slowly distilled into 2 : 4-dinitrophenylhydrazine

[4] Birch, *J.*, 1945, 809.
[5] Birch, Massy-Westropp, and Moye, unpublished work.

in 2N-hydrochloric acid. The resulting mixture was chromatographed on alumina in ethyl acetate–light petroleum (b. p. 60—80°), and arbitrary fractions collected. After several crystallisations from ethanol fractions of m. p. 124° and m. p. 160° were obtained, undepressed by authentic specimens of the derivatives of acetone (m. p. 126°) and acetaldehyde (m. p. 166°), respectively. Paper chromatography with heptane–methanol also confirmed these identifications: R_F (acetone derivative) 0·54; R_F (acetaldehyde derivative) 0·37. A blank test on the solvent gave neither of these derivatives.

Ozonolysis of p-*Myceloxybenzamide.*—*p*-Myceloxybenzamide (40 mg.) in glacial acetic acid was ozonised as above, and paper chromatography of the 2-4-dinitrophenylhydrazone in heptane–methanol showed the presence of acetone and acetaldehyde derivatives (R_F 0·54 and 0·37).

Acid Hydrolysis of Mycelianamide.—Mycelianamide (0·5 g.) was refluxed under nitrogen for 6 hr. with hydrochloric acid (0·5N., 80 c.c.) with slow distillation to remove mycelene. The residue was extracted with ether several times and the aqueous solution evaporated to dryness under reduced pressure at a low temperature. The solid residue was submitted to paper chromatography,[6] phenol saturated with water and containing ammonia (3%) being used. The R_F values of natural alanine and this material were the same: 0·62. Similarly, *iso*butyric acid saturated with water being used, an R_F of 0·35 was obtained for both alanine and the hydrolysis product.

Deoxymycelianamide.—Zinc dust (2 g.) was added gradually during 15 hr. with occasional shaking and warming to mycelianamide (0·5 g.) in glacial acetic acid (30 c.c.). After a further 2 days the mixture was warmed and filtered, the zinc residues being extracted three times with boiling ethanol (*ca.* 10 c.c.). The combined solutions were evaporated to dryness under reduced pressure and the residue washed several times with water and crystallised from ethanol. The deoxymycelianamide (280 mg.) was obtained as colourless needles, m. p. 170°, $[\alpha]_D$ −5° ($CHCl_3$) (Found: C, 71·5; H, 7·6. $C_{22}H_{28}O_3N_2$ requires C, 71·7; H, 7·7%). It was insoluble in sodium carbonate solution and gave no ferric test. The ultraviolet absorption in ethanol: $\lambda_{max.}$ 225 mμ (ε 14,000), 317 mμ (ε 2200), resembles that of mycelianamide: $\lambda_{max.}$ 231 mμ (ε 11,000), 321 mμ (ε 23,000).

Acid Hydrolysis of Deoxymycelianamide.—Deoxymycelianamide (50 mg.) was refluxed with N-hydrochloric acid in an atmosphere of nitrogen for 7 hr. A negligible amount of carbon dioxide was evolved. After filtration the solution was treated with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid, and the derivative extracted with ether and removed therefrom with sodium hydrogen carbonate solution. Crystallisation from aqueous acetic acid gave yellow needles, m. p. 171° undepressed by *p*-hydroxyphenylpyruvic acid 2 : 4-dinitrophenylhydrazone, m. p. 172°. Paper chromatography with butanol : ethanol : water (4 : 1 : 4) [7] confirmed the identification, giving R_F 0·75 for the authentic substance and that obtained above. The infrared spectra were also identical, with the following bands: 3470 m, 3390 m, 3170 w, 3070 w, 1680 w, 1615 s, 1570 m, 1520 s, 1420 m, 1340 s, 1310 s, 1270 m, 1215 m, 1135 m, 1110 s, 1090 m, 930 w, 860 w, 840 w, 800 w cm.$^{-1}$.

The original hydrolysis solution on evaporation and paper chromatography with phenol–water (see above) was shown to contain alanine, R_F 0·52.

Reduction of Mycelianamide.—Mycelianamide (3 g.) in warm methanol (30 c.c.) was added to liquid ammonia (200 c.c.), followed by sodium (6 g.) in small pieces. The initial precipitate rapidly dissolved. After addition of water (100 c.c.) small droplets of oil could be observed. These were collected by the addition of very small quantities of ether. The solution was well washed with water and evaporated finally at 100° for some time leaving an oil with a characteristic odour. Distillation (bath temp. 190°) gave an oil (0·57 g.). The infrared spectrum showed the following bands: 2900 s, 2710 w, 1840 w, 1670 m, 1640 w, 1460 s, 1387 s, 1350 m, 1332 w, 1310 w, 1265 w, 1215 m, 1200 w, 1157 m, 1112 s, 1098 m, 1150 m, 1135 m, 990 m, 960 w, 912 m, 882 w, 827 s, 790 m, 777 m, 740 m cm.$^{-1}$ (liquid film).

Authentic methylgeraniolene from linalool [4] showed the following bands: 2900 s, 2700 w, 1830 w, 1667 m, 1635 w, 1450 s, 1380 s, 1350 m, 1330 w, 1312 w, 1210 m, 1200 w, 1155 m, 1112 s, 1095 m, 1055 w, 1035 w, 987 m, 965 w, 912 w, 885 s, 790 m, 775 m, 740 w cm.$^{-1}$.

The tetrabromide of the substance from mycelianamide was prepared by the method of Schimmel and Co.[8] and had m. p. 88—89°, undepressed by the derivative similarly prepared from authentic methylgeraniolene. The infrared spectra (C in CS_2) were identical, with the

[6] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.
[7] Cavallini, Frontali, and Toshi, *Nature*, 1949, **163**, 568.
[8] Schimmel and Co., *Chem. Centr.*, 1912, **33**, 245.

following bands : 2942 s, 2900 s, 1380 s, 1370 s, 1330 w, 1280 m, 1240 s, 1210 s, 1160 m, 1140 m, 1100 s, 1060 s, 1020 w, 990 m, 955 m, 940 m, 915 w, 855 w, 795 s, 750 w cm.$^{-1}$.

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