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A REVISED STRUCTURE FOR CAULERPICIN FROM *CAULERPA RACEMOSA*

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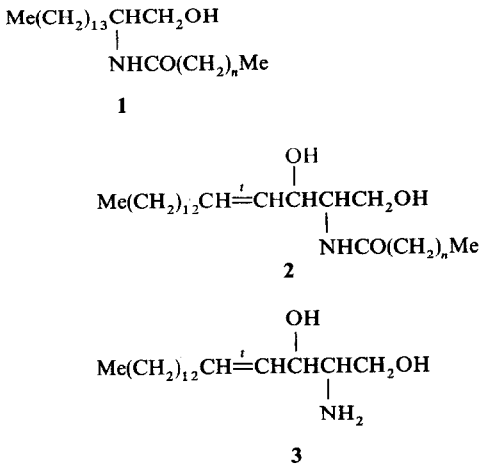
Key Word Index—*Caulerpa racemosa*; Chlorophyta; caulerpicin; *N*-acylsphingosine.

Abstract—Caulerpicin, from the green alga *Caulerpa racemosa*, is shown to be a mixture of *N*-acylsphingosines.

We recently revised [1] the structure of caulerpin, a pigment from *Caulerpa* green algae, and now report a revised structure for caulerpicin from the same source.

Based mainly on spectroscopic evidence, caulerpicin was regarded [2] as a mixture of homologous hydroxyamides (**1**; $n = 23$, 24, and 25), the highest being $C_{43}H_{87}NO_2$ (M, 649). We have isolated a similar mixture from *C. racemosa* but find that the homologue of M, 649 has the molecular formula $C_{42}H_{83}NO_3$. We confirm a hydroxyamide structure (IR) but the NMR spectrum shows signals for two vinyl protons, and agrees very closely with that of the ceramides (**2**).‡ Hydrolysis of caulerpicin yielded sphingosine (**3**), identified as its triacetyl derivative, and a mixture of fatty acids shown by GC–MS of their methyl esters to be *n*-C_{14}, C_{16}, C_{22} and C_{24} saturated acids, and C_{22} and C_{24} monosaturated acids. Our sample of caulerpicin is thus a mixture of the sphingosine derivatives (**2**; $n = 12$, 14, 20, and 22) with unsaturated analogues.



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Sphingosine (**3**) is the dominant long chain base in all the animal sphingolipids [3] and has hitherto been regarded as a product solely of animal metabolism. However, recently very similar material (**2**; $n = 12$, 14, 16, 21, 22, and 23) was isolated [4] from the red alga *Amansia glomerata*, and indeed Cardellina and Moore [4] noted the similarity of their product to caulerpicin. They also found a dihydrosphingosine derivative in *Laurencia nidifica*.

‡ From bovine brain sphingomyelin (Sigma).

EXPERIMENTAL

Isolation of caulerpicin. Air-dried powdered plants of *Caulerpa racemosa* (170 g) collected at Jaffna (Pannai), Sri Lanka were extracted successively with petrol (bp 60–80°), Me_2CO and MeOH. The combined Me_2CO and MeOH extracts were concd under red. pres. and extracted with Et_2O. The Et_2O-soluble material (3.2 g) was chromatographed on an alumina column eluting with CH_2Cl_2, $CHCl_3$ and MeOH, increasing the polarity gradually; 34 fractions were collected. Fractions 24, 25 and 26 eluted with 1% MeOH in $CHCl_3$ were combined and evapd to give the *N*-acylsphingosines (**2**) as a white amorphous solid (30 mg), which was crystallized from MeOH; mp 95°; IR (KBr): cm^{-1}: 3370, 3290, 1620; MS *m/e*: 649, 631, 621, 603, 590, 365, 363, 338, 281, 270 and 256; high resolution MS, *m/e* 649.6337 ($C_{42}H_{83}NO_3$ requires: 649.6372); ^1H NMR: δ 0.88 (*bt*, C-Me), 1.24 (*bs*, $-(CH_2)_n-$), 2.0 (*m*, $=CHCH_2-$), 2.15 (*m*, $-COCH_2-$), 2.7 (*br*, OH), 3.6–3.96 (*bm*, CH_2OH and $>CHNHCO-$), 4.04 (*m*, $=CH-CH(OH)-$), 5.33 (*t*, $-CH=CH-$), 6.3 (*br*, NH or OH).

Hydrolysis of caulerpicin. The mixture of *N*-acylsphingosines (30 mg) was hydrolysed [4] with 1.2 M H_2SO_4 in 85% MeOH at reflux for 4 hr. The fatty acid esters were extracted from the reaction mixture with hexane, and the aq. phase was then basified and extracted with CH_2Cl_2 to obtain 2 mg of crude sphingosine. This was converted to the triacetate (Ac_2O–NaOAc) which was crystallized from MeCN–EtOAc. It had mp 100–101° and was identical (mmp, TLC, NMR) with authentic sphingosine triacetate. Evapn of the hexane extract and GC–MS analysis (25 m capillary column coated with OV 101, gas flow (He) 2 ml/min, 200°) showed the presence of Me esters of *n*-C_{14} (*m/e* 242), C_{16} (270), C_{22} (354) and C_{24} (382), and mono-unsaturated C_{22} (352) and C_{24} (380) acids in approximately equal amounts.

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OXYSPORONE, A NEW METABOLITE FROM *FUSARIUM OXYSPORUM*

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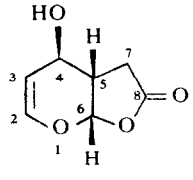
Key Word Index—*Fusarium oxysporum*; mould; oxysporone; fusaric acid; dehydrofusaric acid.

A finely ground mixture of earthworm casts and salt to taste is used in some parts of Nigeria to treat chronic dysentry. (D. S. Adoun, former Headmaster of E.K.A. gave us this anecdote. His mother experienced rapid cures by its use; he has since been using it with consistently good results). On the assumption that the alleged activity was in the casts, we decided to investigate the antibiosis of earthworm casts (collected in 7 Laird Place, University of Ibadan) by looking at secondary metabolites from its microorganisms. We have been able to establish antibiotic activity in the casts as a whole and found that the activity was dependent on the presence of living microorganisms within the casts (Alo, B. I. and Adesogan, E. K., unpublished results). One of the fungi isolated was *Fusarium oxysporum* var. Schelecht (accession No. IMI 211881 at the Commonwealth Mycological Institute). On Czapek-Dox medium, there was no pigmentation and the fungus gave fusaric acid and its dehydro-analogue. The fungus pigmented heavily however on Raulin–Thom medium. Most of the pigments on the fourth day was due to non-volatile naphthazarins [1]. On the eighth day, a red oil was isolated from which oxysporone was separated (*ca* 15 mg per l. culture fluid—pure by GLC) by elution with ethyl acetate–petrol (2:3) on a Si gel column.



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We propose the structure **1** for oxysporone on the basis of the following spectroscopic and chemical evidence. Oxysporone had IR absorptions at $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1806 (γ-alkoxy-γ-lactone [2]; cf. [3–5]), 1654 (C=C) and UV absorptions at λ_{max} nm: 215 (ε 1277) and 284 (ε 417). These assignments were in agreement with the NMR spectra of **1**. The ^1H NMR spectrum (100 MHz, δ) exhibited signals at 6.37 (1H, *d*, *J* = 6Hz, H2); 5.06 (1H, *ddd*, J = 6, 5.5, 1.0 Hz, H-3 coupled to H-2, H-4 and one of the high field protons possibly H-5); 5.82 (1H, *d*, *J* = 4.5 Hz, H-6); 4.16 (1H, *dd*, *J* = 5.5 and 2.0 Hz, H-4) and four high field protons between δ2 and 3, one of which was OH (exchange with D_2O). The ^{13}C NMR spectrum had absorptions at δ 175.3 (C-8), 143.5 (C-2), 100.1 (C-3), 96.0 (C-6), 60.0 (C-4), 41.8 (C-7) and 29.5 (C-5). The high resolution electron impact MS gave an M^+ ion at *m/e* 156.042, $C_7H_8O_4$. The major peaks in the MS at *m/e* (ret. int.) 43 (100, $[CH_2CHO]^+$); 84 (100; $[C_4H_4O_2]^+$. $-\beta\gamma$-unsaturated γ-lactone radical cation); 73 (87, $[CHO - CH_2 - CH_2O]^+$) and 55 (64), were attributed principally to a retro-Diels–Alder fragmentation, with the *m/e* 43 ion conceivably arising from α-cleavage of the malonyl dialdehyde and the *m/e* 73 ion by H-transfer to the dialdehyde, while the fragment at *m/e* 55 could arise by the loss of CHO from the *m/e* 84 fragment ion.

Oxysporone formed a monoacetate, $\nu_{max}^{CHCl_3}$ cm^{-1}: 1807 (γ-alkoxy-γ-lactone), 1738 (ester) and 1656 (C=C), whose ^1H NMR spectrum showed that the proton formerly at 4.16 had shifted to 5.08. On hydrogenation over Pt at NTP, oxysporone lost the IR absorption at ν_{max} 1654 cm^{-1}, but that at 1806 was not shifted.

In view of the fact that H-2 was a clean doublet and therefore not allylically coupled to H-4, we infer that the conformation of the molecule is one that gives them an allylic angle, of zero or almost zero. Furthermore, the 2 Hz coupling constant between H-4 and H-5 was indicative of a dihedral angle of *ca* 90° between these two protons. The Dreiding model of oxysporone, which incorporates these facts as well as giving a dihedral angle which is consistent with the coupling constants between H-5 and H-6, gives the structure shown in **1**. Oxysporone is therefore assigned the structure and relative stereochemistry shown in **1**.

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