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FURANOSESQUITERPENOIDS IN SPONGES - V* :
SPINIFERINS FROM PLERAPLYSILLA SPINIFERA

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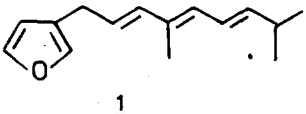
A series of biogenetically interesting furanosesquiterpenes has been recently isolated from sponges[1-3]. In particular, the investigation of Pleraplysilla spinifera[1-2] has led to the isolation of dehydrodendrolasin (1), pleraplysillin-1 (2) and -2 (3).



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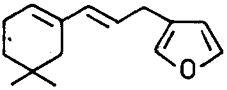

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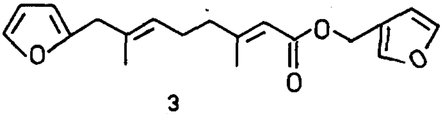

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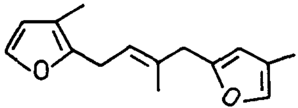
The sponge was collected in the bay of Naples and the more recent collections were separated into two types on the basis of slight morphological differences. Although both of these have been identified (spicule analysis) as a single species, Pleraplysilla spinifera, we have now found their sesquiterpenoid content to be remarkably different. In the second type, 1, 2 and 3 are not detectable, but it contains spiniferin-1 and spiniferin-2 accompanied by minor amounts of longifolin (4; 0.05% of the dry weight of animal), previously found in a terrestrial plant, Actinodaphne longifolia[4]. These differences in chemical constitutions strongly suggest that the two forms represent distinct species of Pleraplysilla and in its connection we must note that sponges are exceptionally difficult to classify.



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The instability of both spiniferins prevented extensive chemical investigation; on the basis of spectral arguments (mainly n.m.r.), which follow, we propose the

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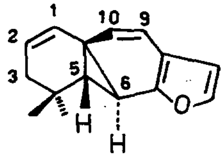
[*] Part IV: G. Cimino, S. De Stefano, A. Guerriero and L. Minale, preceding paper.

alternative structures (5 or 6) and (7 or 8), spiniferin-1 and -2, respectively.



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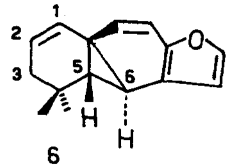

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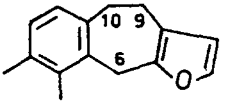

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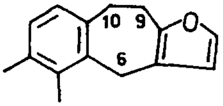

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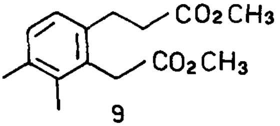
Spiniferin-2 (7 or 8, 0.2% of dry animal), $C_{15}H_{16}O$ (M^{+}/e 212.12004; calc. 212.12011), is an optically inactive oil showing u.v. sbsorptions at 222, 224 and 264 nm (ε, 10,200; 13, 200 and 790 in MeOH). The band centered at 264 nm shows the shape of a benzenoid absorption curve. In the 'H-n.m.r. spectrum (CCl_4) the presence of a 2,3-disubstituted furan is indicated by doublets at δ 7.03 and 5.96 ppm coupled to each other (J 2Hz). Two benzenoid protons are seen as a singlet at δ 6.82 and two aromatic methyl groups resonate at δ 2.21 and 2.25 ppm. An isolated methylene group between the aromatic and furan rings is indicated by a low field broad singlet at δ 4.02, while a C_2 saturated chain is suggested by an A_2B_2 system with line positions at δ 2.61 and 2.92 ppm. The multiplet at δ 2.61 (C-9 methylene protons) is "long-range" coupled with both the furanoid protons, while the multiplet at δ 2.92 (C-10 methylene protons) couples to the benzenoid protons (benzylic coupling). Decoupling experiments also revealed benzylic couplings between the C-6 methylene protons (δ 4.02) and both the furanoid protons and established the existence of "homobenzylic" couplings between the C-6 and C-9 methylene protons ($J_{6,9}$ 1Hz). Smaller but observable couplings of C-6 protons with the C-10 protons and the methyl protons at δ 2.25 have also been noted. Oxidative ozonolysis, followed by methylation with diazomethane, afforded a dicarboxylic acid methyl ester, M^{+}/e 264, whose n.m.r. properties fully agreed with the structure 9: the two aromatic protons now appear as an AB quartet a δ 6.86 with J of 8Hz. Now only two alternative structures (7 and 8), both equally compatible with above evidence, appear to be possible for spiniferin-2. Attempts to obtain a maleic anydride adduct to obtain a criterion to distinguish between the two possibilities failed, giving a complex mixture, including polymeric material. Oxidations in different conditions (peracid , DDQ) to convert the furan ring to the corresponding



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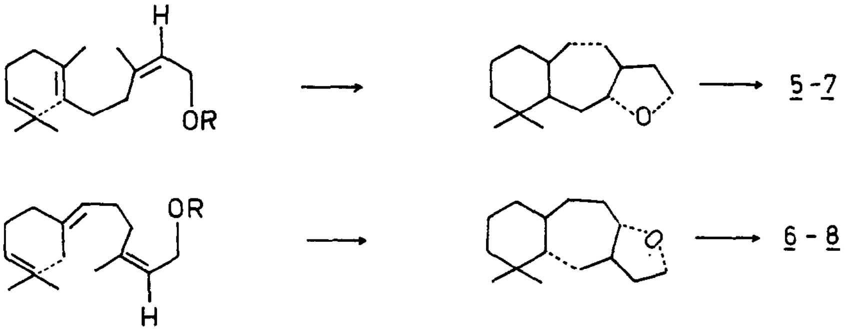
α,β-unsaturated-γ-lactone also gave intractable material.

Spiniferin-1 (*5* or *6* 0.32% of dry animal) $C_{15}H_{16}O$ (M^+/e 212.12024; calc. 212.12011), isomeric with spiniferin-2, $\{\alpha\}_D$ -4.2°, showed u.v. absorptions, λ_{max} 240 and 302 nm (ε, 14,900 and 8,500 in MeOH), indicating the presence of a conjugated chromophore. The resonances at relatively low field of the two furanoid protons, δ 7.25 and 6.50 (doublets with J 2Hz coupled each to other) were consistent in indicating further unsaturation conjugated with the heterocyclic ring. The signal centered at δ 6.30 with the apparent feature of the central bands of an AB quartet (the lateral bands are overlapped by other signals) was in agreement with the presence of a conjugated CH=CH. The n.m.r. spectrum also displayed signals for two *tert*-methyl groups (δ 0.8 and 1.4) an isolated $-CH=CH-CH_2-$ unit{ABXY system with line positions at 6.26 (H-1), 5.34 (H-2), 2.88 (H-3ax) and 2.02 (H-3eq) and coupling constants of 16 Hz ($J_{3eq,3ax}$), 10 Hz ($J_{1,2}$), 8 Hz ($J_{2,3eq}$) 3 Hz ($J_{2,3ax}$) and 3 Hz (J1,3ax) and also significantly two doublets at δ 3.62 and 0.75 with a coupling constant of 10 Hz, attributable to cyclopropane protons[5] (H-6 and H-5, respectively). All these assignments were confirmed by double irradiation experiments. Moreover decoupling experiments also revealed small couplings beetween the 0.8 δ methyl signal and the H-3ax at δ 2.86 (*trans* methyl-proton interaction). The proton H-6 (δ 3.62) also showed small couplings with the furan protons and the vinyl-H-9. All the atoms of spiniferin-1 are accounted for and bearing in mind the structures proposed for the co-occurring spiniferin-2, all the above evidence can be reasonably interpreted in terms of the two equally plausible alternative structures *5* and *6*. Molecular models show that the cyclopropyl hydrogens in *5* and *6* must be *trans*-oriented: the value of the coupling constant is 10 Hz and the expected value for J_{trans} is 4.0-9.6 Hz[6].

Catalytic hydrogenation of spiniferin-2 gave a dihydroderivative M^+/e 214, whose n.m.r. spectrum had lost the olefinic signals at δ 6.26 and 5.34 for H-1 and H-2. The u.v. spectrum of dihydrospiniferin-2 (λ_{max} 230 and 266; ε, 16,000 and 4,900 in MeOH) is in agreement with the presence of a furan conjugated with a double bond[7]. By comparison the absorption of the parent compound (λ_{max} 240 and 302 nm) is consistent with the further conjugation of the double bond in the dimethylcyclohexene ring through the spiro carbon of cyclopropane (spiroconjugation)[8]. ^{13}C-n.m.r. spectra on spiniferin-1 showed signals from the furan moiety, and from four further sp^2 carbons with an alkyl substituent (off-resonance doublets at 129.03, 123.98, 111.38 and 108.31 ppm). In the region characteristic of sp^3 C atoms, the proton-resonance decoupled spectrum of spiniferin-1 showed six signals. Seemingly, there is one carbon "missing". The proton off-resonance decoupled spectrum showed two quaternary 39.33 and 29.60 ppm), one methylene (43.98) and two methyl carbons (30.50 and

28.01 ppm), the strong signal at 33.88 ppm was split into a pair of doublets with apparent separation of 20 and 40 Hz, thus allowing identification of the two remaining methine carbons. The accidental coincidence of the chemical shift of the two methine carbons in a molecule without any local symmetry is unexpected. The ^{13}C-n.m.r. data accord with the proposed structures for spiniferin-1, even if they do not allow a choice between them (5 or 6).

Both pairs 5 - 7 and 6 - 8 appear biogenetically feasible, even trough they are based on carbon skeletons so far unique amongst sesquiterpenoids. A possible biogenetic scheme for the formation of both pairs starting from a *cis*-farnesyl precursor is shown below.



REFERENCES

1. G. Cimino, S. De Stefano, L. Minale and E. Trivellone, Tetrahedron, 28, 4761 (1972).
2. G. Cimino, S. De Stefano and L. Minale, Experientia, 30, 846 (1974).
3. G. Cimino, S. De Stefano, A. Guerriero and L. Minale, Tetrahedron Letters, 1417-1429 (1975).
4. N. Hayashi, H.Komae, E.Eguchi, M. Nakayama, S. Hayashi and T. Sakao, Chemistry and Industry, 572 (1972).
5. N. Morita, Y. Kitahora and T. Asao, Tetrahedron Letters, 869 (1972).
6. L. M. Jackman and S. Sternhell "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd edition, p. 286, Pergamon Press (1969).
7. K. Takeda, H. Minato and I. Horibe. Tetrahedron, 19, 2307 (1963).
8. J. M. Mellor, Annual Reports, section B Organic Chemistry, The Chemical Society, p. 590 (1973).