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# FUROSPONGIN-1, A NEW C-21 FURANOTERPENE FROM THE SPONGES *SPONGIA OFFICINALIS* AND *HIPPOSPONGIA COMMUNIS*

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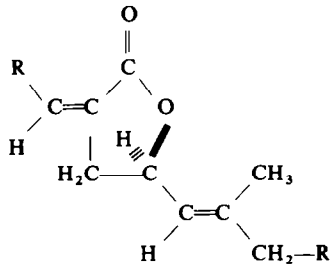
**Abstract**—A new C-21 furanoterpene, furospongin-1 (III), related to nitenin (I) and dihydronitenin (II), has been isolated from the sponges *Spongia officinalis* and *Hippospongia communis*. On the basis of physical and chemical evidence structure (III) is suggested for furospongin-1.

RECENTLY[1] we reported the occurrence in the sponge *Spongia nitens* of two unusual C-21 furanoterpenes, nitenin (I) and dihydronitenin (II). Investigation of two further species, *Spongia officinalis* and *Hippospongia communis*, which are both very common in the Mediterranean Sea, has resulted in the isolation, from the methanolic extracts, of a number of Ehrlich-positive substances. The minor components are being investigated and in this paper we report data which establish structure III for the major compound, which we have called furospongin-1, $C_{21}H_{30}O_3$ ($M^+$ 330), m.p. 35°, $[\alpha]_D$ +8·8°.

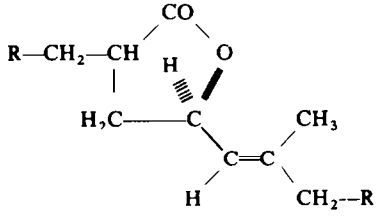
The presence of two β-methylene-substituted furan rings is deduced from the 100 MHz NMR spectrum given in Fig 1. Peaks at $\delta$ 7·28 (2H) and 7·16 (2H) are attributed to the four α-hydrogens; the peak at 6·16 (2H) is due to the two β-protons, while the triplet $\delta$ 2·40 (4H, $J$ = 6 Hz) must be assigned to the two methylene groups attached to the furan rings. The positive Ehrlich test, the IR spectrum (liquid film) $\nu_{max}$ 3140, 1570, 1510, 875 and 780 $cm^{-1}$),[2] the mass spectrum [*m/e* 67 ($C_4H_3O^+$), 81 (base peak, $C_4H_3OCH_2^+$) and 95 ($C_4H_3OCH_2CH_2^+$)][2] and the UV spectrum ($\lambda_{max}$ 222 nm, $\varepsilon$ = 9100 in cyclohexane) confirmed the presence of furan rings in the molecule.

The IR also reveals the hydroxyl group ($\nu_{max}$ 3400 $cm^{-1}$) which, on the basis of NMR data, must be secondary: the multiplet at $\delta$ 3·75 (1H, >C<u>H</u>OH) shifts downfield to $\delta$ 5·10 on acetylation.

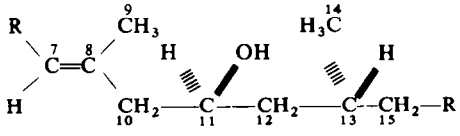
Furospongin-1 possesses a secondary methyl group: doublet ($J$ = 7 Hz) at $\delta$ 0·88 and a trisubstituted double bond: the vinyl Me group resonates as a doublet at $\delta$ 1·58 (Me *trans* to the olefinic proton in an isoprene residue)[3] and its coupling ($J$ = 0·8 Hz) to the olefinic proton, which resonates at $\delta$ 5·23 as a triplet ($J$ = 7 Hz; $H_2C$—C<u>H</u>=C<) broadened by long range coupling, was confirmed by double



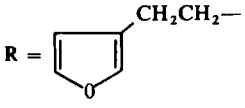
I



II



III



irradiation experiments. Moreover, irradiation at $\delta$ 3·75 (>C<u>H</u>OH) causes the doublet at $\delta$ 2·20 (2H, $J$ = 7 Hz) to collapse to a singlet, while irradiation at $\delta$ 2·20 simplifies the multiplet at $\delta$ 3·75 to a triplet ($J$ = 7 Hz). These irradiations are completely consistent with the presence in the molecule of the unit IV.

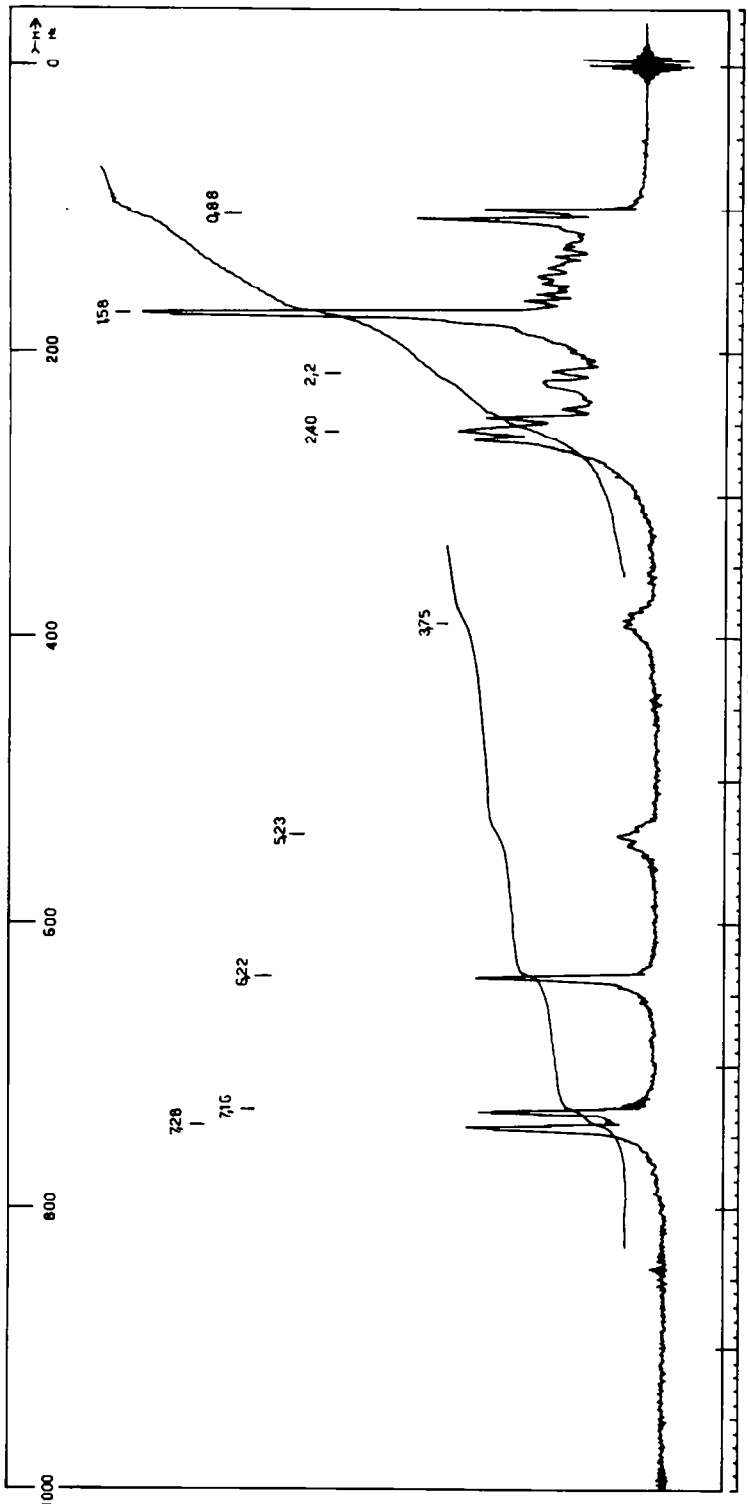
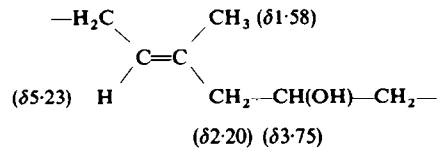
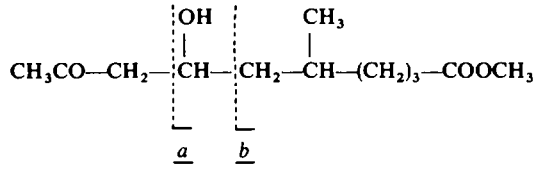



FIG 1. 100 MHz NMR spectrum of furospongin- 1 (III) in $CDCl_3$




(IV)

Ozonization of furospongin-1, followed by oxidative decomposition of the ozonide with $H_2O_2$ and successive diazomethane methylation gave methyl succinate and a methyl hydroxy-ketoester, $C_{12}H_{22}O_4$, characterized as methyl 9-oxo-7-hydroxy-5-methyldecanoate (V) from spectral data. The IR of V shows the hydroxyl band

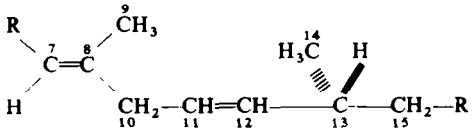



V

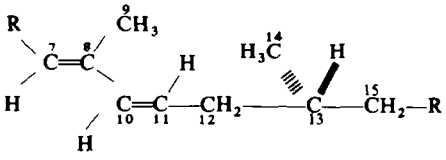
(3400 $cm^{-1}$) and two CO bands at 1730 (>C=O ester) and 1715 $cm^{-1}$ (>C=O ketone). In the NMR a one-proton multiplet at $\delta$ 4·10 (>C<u>H</u>OH) confirms the presence of the secondary hydroxyl group; two sharp singlets at $\delta$ 3·61 (3H) and 2·14 (3H) confirm the presence of the carbomethoxy and ketomethyl groups. Two methylene groups ($\delta$ 2·53, d, $J$ = 6 Hz and 2·23, t, $J$ = 7 Hz), one secondary methyl group at $\delta$ 0·88 ($J$ = 6 Hz) and seven other methine and methylene protons spread between $\delta$ 1·58–1·28 are the remaining signals. In spin decoupling experiments, irradiation at the resonance frequency of the >C<u>H</u>OH proton ($\delta$ 4·10) allowed the doublet at 2·53 to collapse into a singlet. This result, along with the NMR evidence on furospongin-1, indicates that the methylene group, the protons of which resonate at $\delta$ 2·53, must be situated between the $CH_3CO$— and >CHOH groups and, accordingly, the methylene group at $\delta$ 2·23 must be situated $\alpha$ to the —$COOCH_3$. Evidence about the position of the secondary methyl group on C-5 is obtained from the mass spectrum with peaks at *m/e* 74 [$CH_3OC(\overset{+}{O}H) = CH_2$], 87 [$H_3CO—C(OH(\phi))= CH—\overset{+}{C}H_2$], 101 ($H_3CO—COCH_2CH_2\overset{+}{C}H_2$) and 129 [$H_3CO—COCH_2CH_2CH_2CH(CH_3)^+$], while no peak at *m/e* 115 is observed. In addition the mass spectrum shows peaks at *m/e* 212 ($M^+—H_2O$; the molecular ion is not observed), *m/e* 173 and *m/e* 144 corresponding to cleavages *a* and *b* respectively (structure V), i.e. adjacent to the hydroxyl group (cleavage *b* occurring, as expected, with hydrogen rearrangement),[4] *m/e* 141 which derive from *a* by elimination of MeOH[4] and prominent peak at *m/e* 43 ($CH_3—CO^+$). Thus all the spectral data are consistent with structure V for the ozonolysis product, and consequently structure III, without stereochemical details, can be assigned to furospongin-1.

The configuration at C-11 of III was determined by the method of Horeau:[5] following asymmetric esterification[6] with excess racemic α-phenylbutyric acid anhydride, (−)-α-phenylbutyric acid was recovered in an optical yield of 9·8%. Consequently the S-configuration must be assigned to the asymmetric centre C-11.

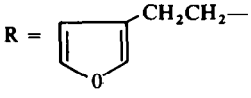
To determine the stereochemistry at C-13, furospongin-1 was treated with $POCl_3$ and pyridine to give two anhydroderivatives, VI $[\alpha]_D$ +5·0° and VII, $[\alpha]_D$ +2·40, separated with difficulty by reapeated chromatography on $SiO_2$. Elemental analyses and mass spectra of both anhydro-derivatives show that they are formed from III by



VI



VII



loss of one mole $H_2O$. The UV of VII ($\lambda_{max}$ 229 and 237 nm) clearly indicates the presence of a conjugated diene system; in VI ($\lambda_{max}$ 220 nm) the two double bands must be isolated. The NMR of both compounds (experimental) are fully consistent with the structures VI and VII, with double bond configurations as shown.

Oxidative ozonolysis of VI gave succinic acid and 2-methyladipic acid. This last compound, after purification by preparative paper chromatography, showed $[\alpha]_D$ = +1·30 (Lit 1·42), thus proving it to be the R isomer.[7]

Accordingly the asymmetric centre C-13 of furospongin-1 must have R configuration and consequently, the full structure III is proposed.

Biogenetically, nitenin I clearly arises by the further oxidation at C-9 of a compound such as furospongin-1, itself apparently an oxidised linear sesterterpenoid. Compound III has some antibacterial activity (*Diploococcus, Streptococcus*).[8]

## EXPERIMENTAL

UV and IR spectra were recorded on Bausch and Lomb Spectronic 505 and Perkin–Elmer 257 Infracord spectrophotometers. NMR spectra were taken on a Varian HA-100 spectrometer, (100 MHz) TMS as internal standard with $\delta = 0$. Mass spectra were taken on an AEI MS-9. GLC's were run using a Carla Erba Fractovap model GV with a flame ionization detector; carrier gas $N_2$, flow rate of 50 ml/min; column length 2 m ($\phi$ 0·5 cm). Column chromatography was carried out on silica gel 0·05–0·2 mm (Merck): TLC were carried out on pre-coated plates of silica gel F 254 (Merck).

Analyses were performed in the microanalytical service of our laboratory, under the direction of Mr S. De Rosa.

Sponges (*Spongia officinalis* and *Hippospongia communis*) collected in the bay of Naples, were obtained from the supply department of the Zoological Station (Naples).

*Isolation of furospongin-1 from* spongia officinalis. The fresh material (350 g, dry weight after extraction) was extracted three times with MeOH for three days; the combined extracts (4 l) were conc *in vacuo* and the remaining aqueous soln extracted with ether (1·5 l, 3 portions). Evaporation of solvent gave a dark brown oil (11·5 g), chromatographed in $C_6H_6$ on silica gel (300 g). 100 ml fractions were collected and monitored by TLC. Fractions 2–3 contained traces of Ehrlich positive material; fractions 7–11 left, on evaporation, a residue (250 mg) which contained four other Ehrlich positive substances, fraction 12 (40 mg) yielded another Ehrlich positive compound and finally fractions 12–36 gave furospongin-1 (III; 3·6 g) colourless oil, crystallized on standing, m.p. 35° from 80–100° light petroleum, $[\alpha]_D = +8{\cdot}8$ (c, 1; $CHCl_3$); $\lambda_{max}$: 220 nm, $\varepsilon = 9100$ (cyclohexane); $\nu_{(liq.\,film)}$: 3400 (b), 3140, 1570, 1510, 1450, 1380, 1170, 1070, 1020, 875 and 780; NMR see Fig 1; MS *m/e* (%) 330 [10%; $M^+$], 312 [2%, $M^+-H_2O$], 181 [15%; $C_4H_3O(CH_2)_3-\overset{+}{C}H(CH_3)-CH(OH)$]; 150 [50%; $C_4H_3OCH_2CH_2CH=\overset{+}{C}(CH_3)-\overset{+}{C}H_2 + 1H$], 135 [20%; $C_4H_3OCH_2CH_2-CH=C(CH_3)^+$]. 109 (5%; $C_4H_3OCH_2CH_2\overset{+}{C}H_2$), 95 (15%; $C_4H_3OCH_2\overset{+}{C}H_2$), 94 (20%; $C_4H_3OCH=\overset{+}{C}H_2$), 81 (100%; $C_4H_3OCH_2^+$), and 67 (10%; $C_4H_4O^+$). (Found: C, 75·92; H, 9·10. Calc. for $C_{21}H_{30}O_3$: C, 76·33; H, 9·15%).

Further elution of column with $C_6H_6/Et_2O$ ether (8:2) gave another Ehrlich positive substance (300 mg).

*Isolation of furospongin-1 from* hippospongia communis. Extraction with MeOH of fresh material (110 g, dry weight after extraction) and chromatography on silica gel of crude extract (4 g) as above gave furospongin-1 (0·35 g) with minor less polar components. The more polar Ehrlich positive substance, eluted from the column of *Spongia officinalis* extract with $C_6H_6/Et_2O$ ether, (8:2), was not observed.

*Furospongin-1 acetate.* Furospongin-1 (III) (55 mg) was acetylated with $Ac_2O$ (1 ml) pyridine (0·1 ml) for 20 min under reflux. Addition of MeOH and evaporation *in vacuo* gave a crude acetate, chromatographed on a $SiO_2$ (5 g) column; elution with $C_6H_6$ yielded the acetate (47 mg) as an oil. ($M^+$ 372): $\nu_{max}$: ($CHCl_3$) 1720 (>C=O, acetate) and 1250 (C—O, acetate) $cm^{-1}$; NMR $\delta$ ($CCl_4$) 5·06 (2H, bm; >C<u>H</u>OAC and —C<u>H</u>=C<), 1·88 (3H, s; $CH_3CO_2$—).

*Ozonolysis of furosponging-1* (III). Furospongin-1 (250 mg), dissolved in EtOAc (40 ml) was ozonized (2% $O_3$) for three hr at −15°. After evaporation of solvent *in vacuo*, the ozonide was decomposed with $H_2O$ at 100° for 1 hr in the presence of a few drops of $H_2O_2$. The mixture was extracted continuously for 5 hr with $Et_2O$. The extract concentrated and treated with $CH_2N_2$. After solvent removal, the degradation products (150 mg) were analysed by GLC (10% DEGS and 15% Reoplex −400 at 175° and 190°, respectively) and found to comprise methyl succinate and a second compound.

The former was removed by heating *in vacuo* and the residue, after chromatography on $SiO_2$ (5 g) column ($\phi$ 0·5 cm, eluent: $C_6H_6$), (68 mg) was analysed by MS, IR and NMR, as above. (Found: C, 62·34; H, 9·51. Calc. for $C_{12}H_{22}O_4$: C, 62·68; H, 9·63%).

*Dehydration of furospongin-1* (III) *to* VI *and* VII. A soln of (III) (800 mg) in pyridine (2·5 ml) was stirred with $POCl_3$ (1 ml) at room temp for 30 min and then 10 min at 100°. After cooling, the mixture was poured onto ice–water and ether extracted. After removal of solvent, the product was carefully chromatographed 3× on $SiO_2$ (80 g) column ($\phi$ 2 cm), in 40–70°–light petroleum. Thus we obtained: VI, (170 mg), $[\alpha]_D$ +5·0° (c, 2; $CHCl_3$); $\lambda_{max}$ 220 nm $\varepsilon$ 9800 (cyclohexane); $M^+$ 312 *m/e*; $\delta$ ($CCl_4$) 7·26 (2H, bs; α-furano-protons) 7·10 (2H, bs; α-furanoprotons), 6·15 (2H, bs, β-furanoprotons), 5·34 (3H, bm; H—C-7, H—C-11 and H—C-12), 2·58 (2H, d, $J = 5$ Hz; $H_2$—C-10), 1·53 (3H, s: $CH_3$ on C-8) and 0·95 (3H, d, $J = 6$ Hz, $CH_3$ on C-13). (Found: C, 80·47; H, 8·89. Calc. for $C_{21}H_{28}O_2$: C, 80·73; H, 9·03%). Also VII, (220 mg), $[\alpha]_D$ +2·4° (c, 5; $CHCl_3$); $\lambda_{max}$ 229 and 237 nm, $\varepsilon$ 22,830 and 22,850 (cyclohexane); $M^+$ 312 *m/e*; $\delta$ ($CCl_4$) 7·24 (2H, bs; α-furanoprotons), 7·10 (2H, bs; α-furanoprotons), 6·14 (2H, bs; β-furanoprotons), 5·92 (1H, d, $J = 16$ Hz; part A of an $ABX_2$ system, $J$ AB = 16 Hz, $J$ AX = O, H—C-10), 5·46 (1H, dt, part B

of an $ABX_2$ system; $J$ AB = 16 Hz; $J$ BX = 6 Hz; H—C-11), 5·34 (1H, m; H—C-7), 1·67 (3H, s, $—CH_3$ on C-8) and 0·85 (3H, d; $J$ = 6 Hz; $CH_3$ on C-13). (Found: C, 80·61; H, 9·12. Calc. for $C_{21}H_{28}O_2$: C, 80·73; H, 9·03%).

*Ozonolysis of* VI *and* VII. Ozonolysis of VI (100 mg) and VII (50 mg) were as previously described for furospongin-1.

The ozonolysis product of VI, after treatment with $CH_2N_2$ showed two major peaks on GLC (10% DEGS, oven temp 175°, and 15% Reoplex −400, oven temp 190°), identified as methyl succinate and methyl 2-methyladipate by co-chromatography with authentic materials. Preparative paper chromatography (Whatmann 3 MM, eluent: EtOH −32% $NH_3$–$H_2O$, 80:5:15) of the free acids resulted in separation; the isolated 2-methyl adipic acid (21 mg), after passing through a 50W-X2 Dowex column (0·5 × 5 cm), showed $[\alpha]_D$ +1·3 (c, 2; $CHCl_3$); its NMR was identical to synthetic material.

Among the ozonolysis products of (VII), after methylation with $CH_2N_2$, methyl succinate and methyl 3-methyl pimelate were identified by direct comparison with authentic materials on GCL (−10% DEGS, oven temp 175° and 15% Reoplex −400, oven temp 190°).

*Determination of the absolute configuration of* C-11 *in furospongin-1* III *by the method of Horeau.* A soln of I (200 mg; 0·606 mM) and racemic α-phenylbutyric anhydride (585 mg; 1·88 mM) in pyridine (5 ml) was left at room temp for 16 hr. Work up as usual afforded crystalline α-phenylbutric acid, (310 mg) $[\alpha]_D$ −1·8° (c, 10; $C_6H_6$). Fully stereospecific esterification should yield $[\alpha]_D$ 95·6/[2(3·1) − 1] = −18·4°; therefore the optical yield is 9·8%. The neutral fraction contained no starting material, as demonstrated by NMR.

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