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FASCICULATIN, A NOVEL SESTERTERPENE FROM THE SPONGE *IRCINIA FASCICULATA*

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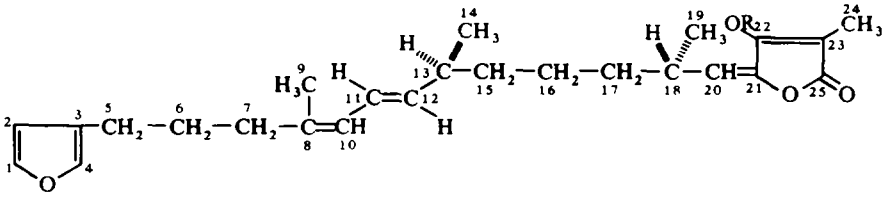
Abstract– A new furanosesterterpene, fasciculatin, has been isolated from the sponge *Ircinia fasciculata*. On the basis of physical and chemical evidence structure I is suggested for fasciculatin.

RECENTLY a number of C-21 furanoterpenes have been isolated from the marine sponges *Spongia nitens*, *S officinalis* and *Hippospongia communis*.[1] Biogenetically they could be derived either by degradation of higher terpenoids constructed from isoprene units linked head to tail or by coupling of two C-10 units with a C-1 unit. The first hypothesis is supported by the occurrence in the sponge *Ircinia oros* of two C-25 head to tail furanoterpenes, Ircinin-1 and -2.[2] We now report the isolation and structure determination of a further sesterterpene, fasciculatin (I) from the sponge *I fasciculata*.



I

I: R = H
II: R = CH_3
III: R = $COCH_3$

The ether-soluble fraction of the methanolic extract of *I fasciculata* was chromatographed on silica gel to give fasciculatin, $C_{25}H_{34}O_4$ (elemental analyses and mass spectrum), λ_{max} 232(s), 241, 250(s) and 268 (ε 24,650, 29,150, 24,530, 14,500) nm. The NMR spectrum (Fig 1a) shows three broad singlets at δ 7·22 (1H), 7·10 (1H) and 6·13 (1H) consistent with the presence in the molecule of a, β-substituted furan ring. This is confirmed by the positive Ehrlich test, by the characteristic pattern in the IR spectrum (liquid film, 1560, 1510, 1165, 1065, 1020, 875 and 755 cm^{-1}) and by the mass spectrum (ions at *m/e* 67, 81, 95, 109).

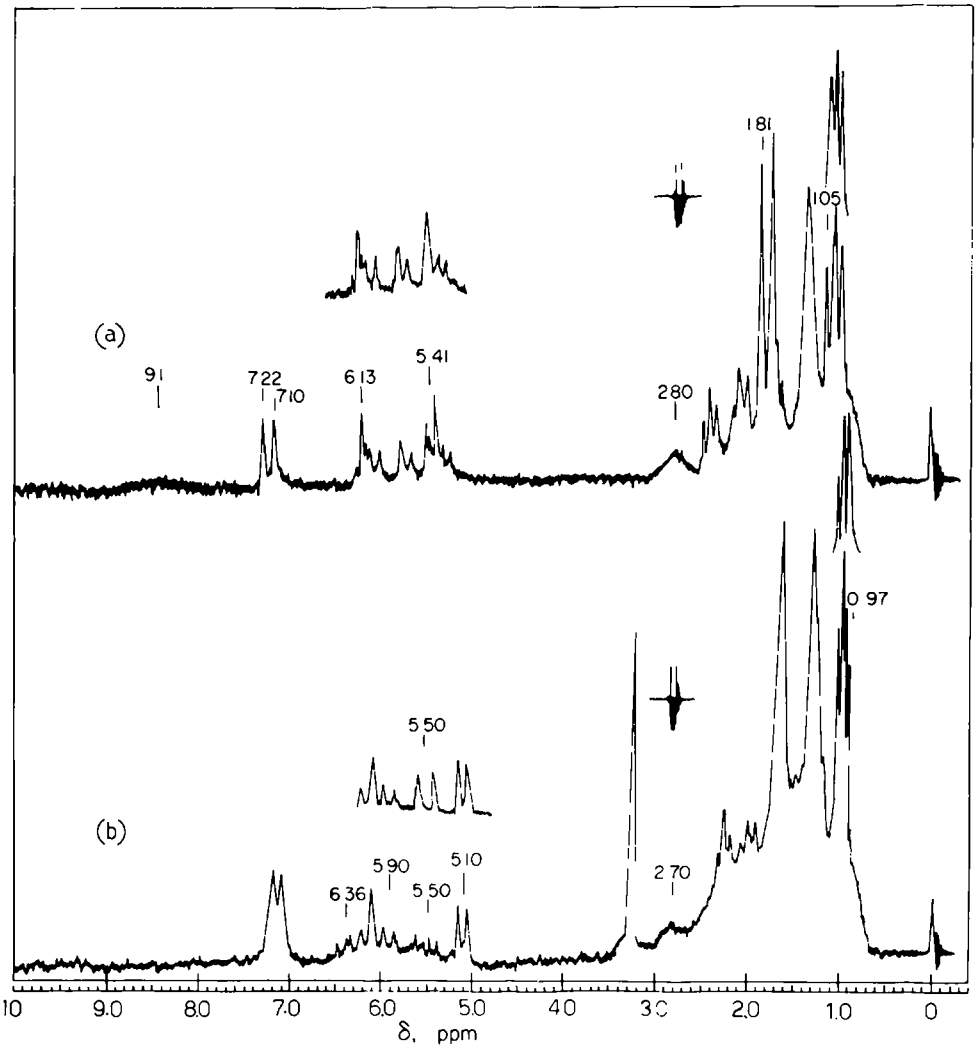


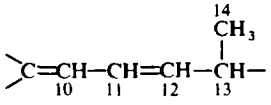
FIG 1. (a) 100 MHz NMR spectrum (CCl_4) of fasciculatin (I) (b) 100 MHz NMR spectrum (C_6D_6) of fasciculatin methyl ether (II)

Fasciculatin possesses an enolic group: in the IR spectrum a broad shallow band is present at 3600–2700 cm^{-1}, the NMR spectrum shows a broad signal, D_2O-exchangeable, at δ 9·1 (1H), and by methylation with diazomethane the metabolite afforded, as the main product, the methyl derivative II, $C_{26}H_{36}O_4$ (δ 4·07 3H, s), while acetylation provided the monoacetyl derivative III, $C_{27}H_{36}O_5$ (ν_{max} 1770 cm^{-1}: δ 2·22, 3H, s).

Further investigation of NMR spectrum of I and spin decoupling studies provided additional structural information consistent with the presence of the unit $-CH(CH_3)CH{=}C<$ (IV) in the molecule. A doublet at δ 5·41 indicates that this olefinic proton is coupled with the methine proton whose signal is superimposed on

the complex absorption between δ 3·0 and 2·5. Irradiation at δ 2·80 collapsed the doublet at δ 5·41 to a singlet and the 3H doublet at δ 1·05 to a sharp singlet (Fig 1a). On the other hand, comparison of NMR spectrum of II (Experimental) with that of I (Fig 1a) shows a down field shift of the CH_3—C= signal at δ 1·81 (3H, s) in I to δ 2·01 in II and a high field shift of the olefinic resonance from δ 5·41 in I to δ 4·98 in II: the latter shift is also observed after acetylation (from δ 5·41 in I to δ 4·82 in III). This indicates that very probably the olefinic bond of the unit IV is conjugated with another double bond bearing the enolic OH and a Me group.

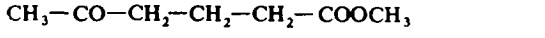
Analysis of the NMR spectrum of II in C_6D_6 (Fig 1b) suggests the presence in the molecule of the unit V. The broad doublet at δ 5·90 (J 10 Hz) can be assigned to the



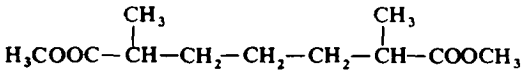
V

C-10 proton, the 1H double doublet at δ 6·36 ($J(H_{10}—H_{11})$ = 10 Hz; $J(H_{11}—H_{12})$ = 15 Hz) is ascribed to the proton at C-11 *trans* situated to H-12, while the double doublet at δ 5·50 ($J(H_{12}—H_{13})$ = 10 Hz; $J(H_{12}—H_{11})$ = 15 Hz) is due to the olefinic proton at C-12. Ir radiation of the H-13 proton signal at δ 2·70 simplifies the signal at δ 5·50 to a doublet (J = 15 Hz) and the doublet at δ 0·97 (3H, H_3—C—14) to a sharp singlet (Fig 1b).

Ozonolysis of fasciculatin, followed by oxidative decomposition of the ozonide with hydrogen peroxide and successive methylation with diazomethane afforded methyl-5-oxohexanoate (VI) and methyl 2,6-dimethylpimelate (VII).



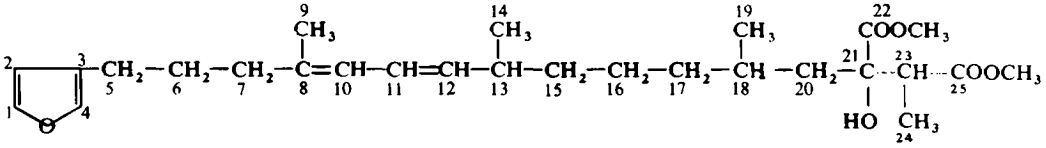
VI



VII

These results, taken together, account for all the skeletal atoms in I except the elements of the tetronic acid unit, whose presence is consistent with the IR spectrum (1720 cm^{-1}) and UV spectrum (λ_{max} 268 nm).

Decisive proof for the proposed tetronic acid structure was provided by drastic alkaline treatment of I which, as expected,[3] by hydrolysis of the lactone followed by a



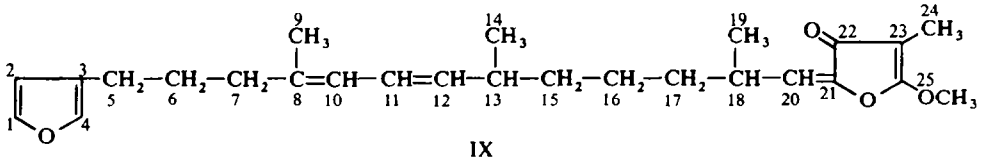
VIII

benzilic rearrangement gave, after diazomethane methylation, VIII (mixture of diastereoisomers), $C_{27}H_{42}O_6$ (elemental analyses and mass spectrum).

The structure of VIII was determined by inspection of its NMR spectrum. In comparison with that of I no signals were observed from H-20 and Me on C-23, but the spectrum showed signals due to OMe groups at δ 3·67 and 3·58 (each 3H, s), to the C-24 Me group at δ 1·24 (3H, d, J = 6 Hz) and to the C-23 proton at δ 2·68 (1H, q, J = 6 Hz).

Further evidence for presence of the tetronic acid unit was provided by the isolation of IX as a by-product of the diazomethane methylation of fasciculatin, according to the well-known behaviour of tetronic acids towards diazomethane.[4]

The characterization of IX is based on analysis of the spectral data and also ozonolysis. Comparison of the NMR signals of IX and II shows a down field shift of the doublet at C-20 from δ 4·98 in II to δ 5·54 (J = 10 Hz) in IX, and a high field shift of the Me protons at C-24 from δ 2·01 in II to δ 1·55 in IX. Supporting evidence was found in the IR spectrum which showed absorptions at 1720 and 1640 cm^{-1} (5-membered α,β-unsaturated ketone), whereas in II the carbonyl group absorbs at



IX

1750 cm^{-1}. Upon ozonization of IX and oxidative decomposition of the ozonide with hydrogen peroxide followed by diazomethane methylation, methyl 5-oxohexanoate (VI) and methyl 2,6-dimethylpimelate (VII) were obtained.

As far as the stereochemistry of fasciculatin is concerned, the configuration at C-13 and C-18 must be *S,S* as ozonolysis of I gave (2*S*,6*S*)-2,6-dimethylpimelic acid: the *trans* configuration at C-11 double bond is deduced from the coupling between H-11 and H-12 (J = 15 Hz). The stereochemistry at C-8 and C-20 double bonds has not been investigated.

EXPERIMENTAL

NMR spectra were determined on a Varian HA-100 spectrometer in CCl_4 solns (unless otherwise indicated) using TMS as internal reference with δ = 0. Abbreviations: s = singlet, d = doublet, q = quartet, m = multiplet, b = broad. Coupling constants are expressed in Hz. Mass spectra were determined with an AEI model MS 90 instrument. UV spectra were taken on Perkin–Elmer 402 spectrometer, IR spectra were recorded on a Perkin–Elmer 137 E instrument. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Elemental analyses were performed by Mr. S. De Rosa (Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R., Arco Felice-Napoli). TLC and PLC separations were effected using glass packed precoated silica gel plates obtained from E. Merck.

Isolation of fasciculatin (I). Fresh material (450 g) (weighed dry after extraction), which was collected by Supply Department of the Zoological Station (Naples) in the bay of Naples, was extracted with MeOH (2 l) for 1 day at room temp. This operation was repeated 3 times. The combined solns were concentrated (200 ml) under reduced press and extracted with ether. The ethereal soln was taken to dryness and the oily residue (930 mg) was chromatographed on 9 preparative silica gel plates (benzene–ether 7:3). The band R_f 0·3 (UV light) was eluted with ether to give 390 mg of I, as an oily product: M^+ 398; $[\alpha]_D$ −15·60 (*c*, 0·5: $CHCl_3$): UV λ_{max} 232(s), 241, 250(s), 268 nm, ε 24,650, 29,150, 24,530, 14,500 (n-hexane); IR (liquid film) 3600–2700 (OH), 1720 (C=O), 1625 (C=C), 1560, 1510, 1165, 1065, 1020, 875 and 755 (furan) cm^{-1}: NMR spectrum is reported in Fig 1a. (Found C, 75·15; H, 8·73. Calc. for $C_{25}H_{34}O_4$· C, 75·34; H, 8·60%).

Ozonolysis of fasciculatin. A soln of 300 mg fasciculatin in 50 ml EtOAc was ozonized (2% O_3) at $-20°$. After removal of the solvent under reduced press at room temp, water was added to the residue and the mixture was kept at 100° for 1 hr in the presence of a few drops of H_2O_2. The mixture was extracted continuously for 5 hours with ether; the ether extract was concentrated and treated with excess CH_2N_2. The soln was taken to dryness *in vacuo* and the residue (135 mg) was chromatographed on a SiO_2 (18 g) column using C_6H_6–Et_2O 9:1 as eluent to give 40 mg of VI identified by comparison (IR and NMR) with an authentic sample and 61 mg of VII: M^+ 216, NMR 3·55 (6H, s, 2 —OCH_3), 1·8–1·15 (6H, bm, 3 =CH_2), 1·05 (6H, d, J = 7, 2 —CH_3). VII was hydrolysed in acidic medium thus obtaining 46 mg of (2*S*,6*S*)-2,6-dimethylpimelic acid.

Acetylation of fasciculatin (I). A soln of fasciculatin (60 mg) in Ac_2O (2·5 ml) and pyridine (0·4 ml) was allowed to stand for 16 hr at room temp. Addition of MeOH and evaporation *in vacuo* afforded a crude product, which was purified by PLC (C_6H_6: R_f 0·6) thus obtaining an oily substance (III, 49 mg): M^+ 440; $[\alpha]_D$ −12·0° (*c*, 0·5: $CHCl_3$): UV λ_{max} 241, 275 nm, ε 37,000, 15,500, (MeOH): IR (CCl_4) 1770 (C=O enol acetate), 1740 (C=O α,β-unsaturated γ-lactone) cm^{-1}; NMR δ 4·82 (1H, d, J = 9, H—C—20), 2·22 (3H, s, CH_3CO—).

Treatment of fasciculatin (I) *with* CH_2N_2. To a soln of 95 mg fasciculatin in MeOH excess of ethereal CH_2N_2 was added. The soln was evaporated to give a mixture of II and IX which were separated on PLC (C_6H_6–Et_2O 8:2: R_f of II 0·7 and R_f of IX 0·5).

II (oily substance, 70 mg): M^+ 412; $[\alpha]_D$ −11·1° (*c*, 0·5: $CHCl_3$): UV λ_{max} 243, 274 nm, ε 34,700, 18,000 (MeOH); IR (CCl_4) 1750 (C=O) cm^{-1}; NMR δ 4·98 (1H, d, J = 9, H—C—20), 4·07 (3H, s, OMe), 2·01 (3H, s, H_3—C—24).

IX (oily substance, 10 mg): M^+ 412; $[\alpha]_D$ +34·7° (*c*, 0·5; $CHCl_3$): UV λ_{max} 240 nm, ε 34,700; IR (CCl_4) 1720 and 1640 (C=O, C=C) cm^{-1}; NMR δ 5·54 (1H, d, J = 10 Hz, H—C—20), 3·98 (3H, s, OCH_3), 1·55 (3H, s, H—C—24).

IX (60 mg) was ozonized in the same experimental conditions used for fasciculatin. Working up as previously described afforded, as the main products, VI (9 mg) and VII (11 mg).

Alkaline treatment of fasciculatin (I). Fasciculatin (400 mg) in 30% KOH (H_2O–MeOH 1:1) was refluxed under stirring for 18 hr. After cooling the soln was concentrated, acidified with AcOH and extracted with ether. The ethereal extract was dried on Na_2SO_4 and taken to dryness *in vacuo*. The residue was dissolved in MeOH and treated with excess of ethereal CH_2N_2. After evaporation of the solvents the residue was purified by PLC (4 plates) using C_6H_6–EtOH 9:1 as eluent to give VIII (mixture of diastereoisomers) as an oily product (R_f 0·5; 210 mg): M^+ 462; UV λ_{max} 239 nm, ε 35,400 (MeOH); IR (liquid film) 3500, 3300 (OH), 1745 (C=O) cm^{-1}; NMR 7·25 (1H, bs α-furanoproton), 7·13 (1H, bs, α-furanoproton), 6·17 (1H, bs, β-furanoproton), 6·12–5·12 (3H, complex series of signals, olefinic protons), 3·67 and 3·50 (6H, s, 2 —OMe), 2·68 (1H, q, J = 6, H—C_{23}), 1·72 (3H, s, H_3—C—9), 1·24 (3H, d, J = 6, H_3—C_{24}), 2·5–0·9 (23H, complex series of signals, remaining protons). (Found: C, 70·23; H, 8·96. Calc. for $C_{27}H_{42}O_6$: C, 70·10; H, 9·15%).

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