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SCALARIN, A NEW PENTACYCLIC C-25 TERPENOID FROM THE SPONGE *CACOSPONGIA SCALARIS**

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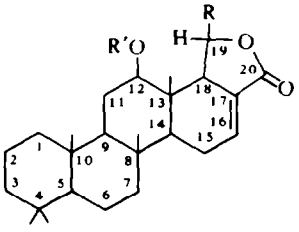
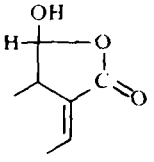
Abstract—On the basis of chemical and physico-chemical evidence, structure I has been assigned to scalarin, a new C-25 terpenoid isolated from the marine sponge *Cacospongia scalaris*.

IN PURSUING our research on the metabolites of Porifera[1] a new C-25 terpenoid, scalarin (I), was isolated from the sponge *Cacospongia scalaris*. Fresh material was extracted with methanol; the solvent was removed and the ether-soluble fraction, after chromatography on silica gel, afforded I (0·06% dry weight), $C_{27}H_{40}O_5$ (elemental analysis and mass spectrum).



I: R = OH R′ = $COCH_3$
III: R = H R′ = $COCH_3$
IV: R = H R′ = H



II

IR and NMR spectra suggest the presence of a $CH_3CO—OCH<$ group [ν_{max} 1733 and 1240 cm^{-1}; δ 2·09 (3H, s) and 4·91 (1H, m)].

Scalarin contains the unit II as shown by its UV [λ_{max} 220 nm (ε 8500)] and IR bands (3350, 3320, 1755 and 1690 cm^{-1}) and the signals in the NMR spectrum at δ 6·81 (1H, bm, H–C_{16}), 5·69 (1H, bd, H–C_{19}), and 3·14 (1H, bm, H–C_{18}). These assignments were supported by spin decoupling experiments: irradiation at δ 3.14 collapses the doublet at δ 5·69 into a singlet broadened by long range coupling and simplifies the broad multiplet at δ 6·81; on the other hand by irradiation at δ 6·81 or 5·69 the signal at δ 3·14 is simplified.

Further evidence for the presence of the lactol ring is obtained from the NMR spectrum of scalarin in pyridine-d_5/NaOD which shows a doublet at δ 9·81 (1H, J 5·5 Hz) in the region characteristic of aldehyde protons, instead of the doublet at δ 5·69

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present in the spectrum in $CDCl_3$ solution. This indicates that the basic solvent causes the expected opening of the lactol ring to give a γ-aldocarboxylic acid.

Mass spectrum of scalarin is in good agreement with the proposed structure I (Fig 2): ions at *m/e* 444 (0·8%, M^+), 426 (15%, M^+-H_2O), 398 (22%, a), 384 (25%, M^+-CH_3COOH), 370 (60%, b), 258 (22%, c–CH_3COOH), 206 (12%, d), 205 (70%, d–H), 192 (65%, e), 191 (100%, e–H), 124 (11%, f) and 123 (60%, f–H).

Additional proof for the structure of scalarin is described below. I by treatment with $NaBH_4$ afforded III, M^+ 428 *m/e*, λ_{max} 223 nm (ε 8500), ν_{max} 1760 and 1690 cm^{-1} (α,β-unsaturated γ-lactone), δ 3·98 (2H, complex series of signals, AB part of a ABX system, H_2–C_{19}), 3·15 (1H, bm, H–C_{18}).

III, by alkaline hydrolysis, gave IV, M^+ 386 *m/e*, λ_{max} 224 nm (ε 6600), ν_{max} 3500 (OH), 1755 and 1690 cm^{-1}; by comparison of its NMR spectrum with that of III it is possible to observe the disappearance of the acetyl singlet and the expected upfield shift of the H–C_{12} signal from δ 4·75 in III to δ 3·57 in IV.

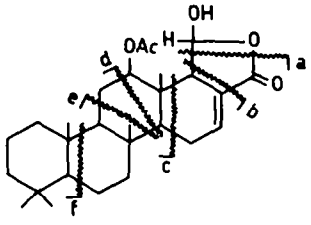
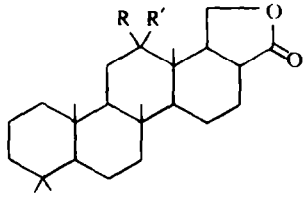


Fig. 1. Fragmentation of Scalarin (I).

Hydroxylactone IV was hydrogenated on Pd/C at room temp to give V, M^+ 388 *m/e*, ν_{max} 3430 (OH), 1770 cm^{-7} (CO γ-lactone). The lack of olefinic proton resonance in the NMR spectrum of this compound is consistent with the proposed structure V.

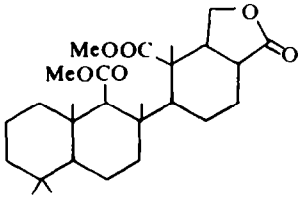
By treatment with Jones reagent V afforded VI M^+ 386 *m/e*, ν_{max} 1780 (CO γ-lactone), 1710 cm^{-1} (CO ketone). The NMR spectrum of VI, when compared with that of V, shows the disappearance of the H–C_{12} multiplet (δ 3·68 in V).



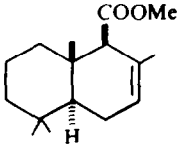
V: R = H R' = OH
VI: R,R' = O

Ketolactone VI, by oxidation with HNO_3 in acetic acid, after diazomethane methylation, gave diester VII, M^+ 462 *m/e*. IR spectrum shows bands at 1790 (CO γ-lactone) and 1735 (CO ester) cm^{-1}. In the NMR spectrum singlets at δ 3·65 (3H, OCH_3), 3·68 (3H, OCH_3) and 2·38 (1H, H–C_9) are present.

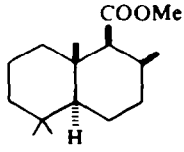
Diester VII was pyrolyzed at 350° for 25 min[2] and the crude mixture was successively hydrogenated on Pd/C at room temp. Ester IX (or its enantiomer), clearly arising from VIII (or its enantiomer) by reduction, was identified in the mixture by comparison of its chromatographic properties with those of authentic sample.



VII



VIII



IX

These results, taken together, account for all the skeletal atoms of scalarin; it still remains to assign the positions of the substituents on the ring C. From our results it could not be established whether the acetoxyl group is on C–11 or C–12 and whether the Me group is linked to C-13 or C-14. From an accurate analysis of the NMR spectrum of I, however, a greatly reduced half-band width (6 Hz) of the signal due to CH linked to acetoxyl group (δ 4·91) appears. This can be attributed to a combination of two factors: first its equatorial nature, and second the presence of only two vicinal protons. Hence the acetoxyl group must be linked to C-12 and the Me group to C-13.

Decisive proof was, however, provided by deuterium exchange of all the enolysable hydrogens of ketolactone VI. From the mass spectrum of deuterated compound it resulted that only three deuteria were incorporated. This indicates that in the α-positions of the ketonic carbonyl are present only two hydrogens, as the third exchangeable hydrogen must be that situated in α-position to the lactone carbonyl.

The stereochemistry of scalarin has not yet been determined. However, the formation of the ester IX (or its enantiomer) by pyrolysis and hydrogenation of VI (or its enantiomer) indicates that the fusion of rings A and B in scalarin must be *trans*; in addition the Me group on C–10 is *trans* to H-9.

EXPERIMENTAL

UV and IR spectra were recorded on Perkin–Elmer 402 and 157 spectrophotometers. NMR spectra in $CDCl_3$ solns unless otherwise stated were taken on a Perkin–Elmer R12A spectrometer, using TMS as internal standard with $\delta = 0$. Mass spectra were performed by Mr. A. Milone on a AEI MS9 spectrometer.

Elemental analyses were performed by Mr. S. De Rosa (Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico–Arco Felice, Napoli).

TLC and PLC were carried out on pre-coated plates of silica gel F and aluminium oxide F (Merck). GLC's were run using a C. Erba Fractovap model GV with a flame ionization detector.

Sponges (*Cacospongia scalaris*) collected in the bay of Naples were obtained from the supply department of the Zoological Station (Naples).

Isolation of Scalarin from Cacospongia scalaris. The fresh material (250 g, dry weight after extraction) was extracted 4 times with MeOH at room temp for 3 days. The combined methanolic extracts (6 l) were concentrated under red press and the remaining aqueous suspension was extracted with Et_2O (2 l in 3 portions). The combined extracts were evaporated to dryness and the residue (6 g) was chromatographed on a SiO_2 column (500 g) using C_6H_6–Et_2O 9:1 as eluent; fractions of 500 ml were collected. Fractions 13–20, on evaporation, left mg 145 of crude scalarin, which was crystallized from 80–100° light petroleum, m.p. 133–135°, $[\alpha]_D$ +43·2 (*c*, 1·5; $CHCl_3$). NMR spectrum is described in the general part; ν_{max} 3350, 3320, 1755, 1733, 1690 and 1240 cm^{-1} (CCl_4): λ_{max}^{MeOH} 220 nm (ε 8500). (Found: C, 73·00; H, 8·93. Calc. for $C_{27}H_{40}O_5$: C, 72·94; H, 9·07%).

$NaBH_4$ *Reduction of scalarin (I) to obtain* III. To a soln of 100 mg of I in EtOH (4 ml) $NaBH_4$ (40 mg) was added. The mixture was allowed to stand at room temp for 30 min. After acidification with AcOH and removal of the solvents the resulting residue was extracted with $CHCl_3$. Evaporation of $CHCl_3$ gave an amorphous solid, which was crystallized from 80–100° light petroleum to give mg 78 of III, m.p. 213–216°, $[\alpha]_D$ + 65·2 (*c*, 0·5; $CHCl_3$), λ_{max}^{MeOH} 223 nm (ε 8500), ν_{max} 1760, 1740, 1690, 1240 and 1210 cm^{-1} (CCl_4), M^+ 428 *m/e* δ 6·65 (1H, m, H–C_{16}), 4·75 (1H, m, H–C_{12}), 3·98 (2H, m, AB part of an ABX system, H_2–C_{19}), 3·15 (1H, bm, H–C_8), 2·06 (3H, s, CH_3–CO) and 0·95, 0·84 and 0·82 (15H, singlets, 5 CH_3).

Alkaline hydrolysis of III *to obtain* IV. A mixture of III (100 mg) and a soln of KOH (1 g) in H_2O–EtOH (1:1, 10 ml) was refluxed for 3 hr, concentrated, diluted with H_2O and washed with Et_2O to remove neutral products. The aqueous soln was acidified with 2N HCl and extracted with Et_2O (50 ml in 5 portions). The combined ethereal extracts were taken to dryness and the residue was purified by PLC on SiO_2 (C_6H_6–Et_2O 13:7). The band R_f 0·5 (UV light) was eluted with $CHCl_3$–MeOH 9:1 to give mg 80 of IV, m.p. 297–300° (from EtOH), $[\alpha]_D$ + 37·4 (*c*, 0·5; $CHCl_3$), M^+ 386 *m/e*, λ_{max}^{MeOH} 224 nm (ε 6600), ν_{max} 3500, 1755 and 1690 cm^{-1} ($CHCl_3$), δ 6·77 (1H, m, H–C_{16}), 4·13 (2H, m, AB part of ABX system, H_2–C_{19}), 3·57 (1H, m, H–C_{12}) and 0·92, 0·84 and 0·79 (15H, singlets, 5 CH_3).

Catalytic hydrogenation of IV *to obtain* V. IV (100 mg) in MeOH (50 ml) was overnight hydrogenated over 10% Pd/C (20 mg) at room temp and atm press. After removal of the catalyst by filtration, the soln was evaporated to dryness and the residue was crystallized from EtOH to give 80 mg of V, m.p. 284–287°, $[\alpha]_D$ + 18·5 (*c*, 0·5; $CHCl_3$), ν_{max} 3430 and 1770 cm^{-1} ($CHCl_3$), M^+ 388 *m/e*, δ 4·10 (2H, m, H_2–C_{19}), 3·68 (1H, m, H–C_{12}), 0·84 (15H, singlet, 5 CH_3).

Oxidation of V *to obtain* VI. The hydroxyketone V (100 mg) in acetone soln (5 ml) was treated with Jones reagent for 30 min at room temp. Following the usual work-up the ketolactone VI was obtained and recrystallized from EtOH (70 mg), m.p. 200–204°, $[\alpha]_D$ +67·1 (*c*, 0·5; $CHCl_3$), M^+ 386 *m/e*, ν_{max} 1780, 1710 and 1260 cm^{-1} (CCl_4), δ 4·29 (2H, m, H_2–C_{19}).

HNO_3 *Oxidation of* VI. A soln of VI (60 mg) in AcOH (0·5 ml) and 99% HNO_3 (0·5 ml) was kept at room temp for 24 hr and, successively, at 60° for 2 hr. After addition of H_2O, the suspension was extracted with Et_2O (30 ml in 5 portions). The combined ethereal extracts were extracted with 2N Na_2CO_3 and the aqueous layer acidified and extracted with Et_2O. The organic phase was concentrated and treated with an excess of ethereal CH_2N_2. After removal of the solvent, the oily residue was chromatographed on a SiO_2 column (5 g; C_6H_6-Et_2O 49:1); fractions of 15 ml were collected. The fractions 10–15 taken to dryness gave a oily residue (30 mg, VII) characterized without further purification, $[\alpha]_D$ + 13·4 (*c*, 0·5; $CHCl_3$), M^+ 462 *m/e*, ν_{max} 1790 and 1735 cm^{-1} (CCl_4), δ 4·07 (2H, m, H_2–C_{19}), 3·68 (3H, s, OCH_3), 3·65 (3H, s, OCH_3) and 2·38 (1H, s, H–C_8).

Pyrolysis of VII. 30 mg of VII, after drying at 100° *in vacuo* for 24 hr over P_2O_5, were kept at 350° for 25 min in a sealed tube. After cooling the residue was dissolved in AcOH and hydrogeneated overnight over 10% Pd/C at room temp and atm press. After removal of the catalyst by filtration, the residue was shown to comprise IX (or its enantiomer) by comparison of its chromatographic properties [GLC: Carbowax 1540 at 180°, 190° and 200°, column length 2 m, 0·5 cm, flow rate of N_2 25 ml/min; capillary column prepared from 10% Dexsil and 2% FFAP in CH_2Cl_2, length 13 m, at 110°, 150° and 170°, flow rate of N_2 1 ml/min; TLC on silica gel (80°–100° light petroleum-C_6H_6 7:3; 30–50° light petroleum-C_6H_6 1:1) and on aluminium oxide (C_6H_6 and 80–100° light petroleum-$CHCl_3$ 49:1)] with those of authentic sample[3].

Deuteration of enolyzable hydrogens of VI. VI (5 mg), MeOD (1 ml), D_2O (0·5 ml) and Na (20 mg) were heated at 70° for 24 hr. After removal of MeOD under red press, the suspension was diluted with D_2O

and acidified with N DCl in D_2O. After extraction with ether the organic phase was taken to dryness to give VI-d_3 (4 mg) of 87% isotopic purity, M^+ 389 *m/e*.

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