12-*epi*-Scalarin and 12-*epi*-Deoxoscalarin, Sesterterpenes from the Sponge *Spongia Nitens*

By **Guido Cimino, Salvatore De Stefano, Luigi Minale,* and Enrico Trivellone,** Laboratorio per la Chimica di Molecole di Interesse Biologico del C.N.R.-Via Toiano n.2, Arco Felice, Napoli, Italy, and Istituto di Chimica Organica dell'Università, Via A. Doria, Catania, Italy

Two minor terpenoid components of the *Spongia nitens* have been identified as the 12-epimers of scalarin (1) and deoxoscalarin (2), members of a new sesterterpene class recently discovered in sponges. With the aid of circular dichroism, and ^1H and ^{13}C n.m.r. spectroscopy, total stereochemistry has been assigned to the new compounds and their epimers.

TERPENES are the most abundant non-steroidal secondary metabolites so far isolated from marine sponges, and sesterterpenes, usually rare in nature, have been encountered frequently in species of the order Dictyoceratida.[1] They fall into two main groups: a linear series of sesterterpene molecules terminated by a furan ring at one end and by a tetraonic acid[1] or a lactone ring[2] at the other; and tetra- or penta-cyclic sesterterpene analogues of a new skeletal type, which seems biogenetically derivable from a geranylfarnesyl precursor by cyclization initiated the isopropylidene group, typical of triterpenes. Five members of the latter group have been reported to date: scalarin (1)[3] and scalradial (3)[4] from species of the *Cacospongia* genus, deoxoscalarin (2)[5] from *Spongia officinalis*, heteronemin (4)[6] from *Heteronema erecta*, and disidein (5)[7] (in which a pentacyclic sesterterpenoid moiety is combined with a hydroxyhydroquinone ring) from *Disidea pellscens*. Their structures were proposed without implied stereochemistry except for the AB-*trans* ring fusion in (1)—(3) inferred from degradative work on scalarin (1)[3] [which resulted in the known ester (6), and followed chemical correlation of (2) and (3) with (1)] and the configuration at C-18 and C-19 suggested for (4) from chemical and spectral data.[6] A re-examination of extracts of the sponge *Spongia nitens*, which contains a major terpenoids the linear C_{21} furan derivatives nitenin and dihydronitenin,[8] has now led to the isolation in small amounts of two additional members of this sesterterpene class, which we will show to be the 12-epimers, (1a) and (2a), of scalarin (1) and deoxoscalarin (2). During the structural work evidence on the stereochemistry of (1a) and (2a) and of the analogues (1) and (2) has been obtained, and the stereochemical proposals shown in the formulae have been confirmed by a ^{13}C n.m.r. study.

Extraction with acetone of fresh tissues of the sponge and fractionation on a silica gel column of the ether-soluble portion have been reported previously.[8] Crystalline 12-*epi*-scalarin (1a) (0.003% of dry material), m.p. 236—238°, $[\alpha]_D$ −57.0°, and 12-*epi*-deoxoscalarin (2a) (0.008% of dry material), m.p. 192—194°, $[\alpha]_D$ +13.9°, were obtained by column chromatography over silica gel and subsequent preparative t.l.c. of the chromatographic fractions more polar than those containing nitenin and dihydronitenin.

The key features of the spectra of (1a) and (2a) were strongly reminiscent of the spectra of the analogues (1) and (2).

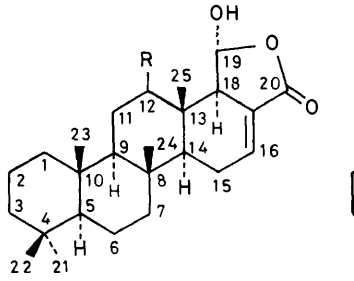
The mass spectra of each pair [(1) and (1a), and (2) and (2a)] were essentially identical; in all spectra the molecular ions were of low abundance or absent, but strong peaks corresponding to losses of water and acetic acid were observed [$C_{27}H_{38}O_4$ ($M^+ - H_2O$), $C_{25}H_{36}O_3$ ($M^+ - CH_3CO_2H$), and $C_{25}H_{34}O_2$ ($M^+ - H_2O - CH_3CO_3H$) for (1a); $C_{27}H_{40}O_3$ ($M^+ - H_2O$), $C_{25}H_{38}O_2$ ($M^+ - CH_3CO_2H$), and $C_{25}H_{36}O$ ($M^+ - H_2O - CH_3CO_2H$) for (2a)]. All spectra showed fragment ions at *m/e* 258, 205, and 191; the first was interpreted as corresponding to fragment *a*, arising by a retro-Diels–Alder process with associated loss of acetic acid, and the latter two, also observed in spectra of a number of diterpenes, are attributable to cleavage across ring C (8, 14- and 11,12-, and 8,14- and 9,11-cleavages, respectively) and associated loss of an additional hydrogen atom from the charge-retaining rings A and B.

Abundant ions at *m/e* 137 and 123 associated with the fragmentation of ring B (6,7- and 9,10-, and 5,6- and 9,10-cleavages, respectively) are also present in the spectra of all these compounds. An additional significant feature

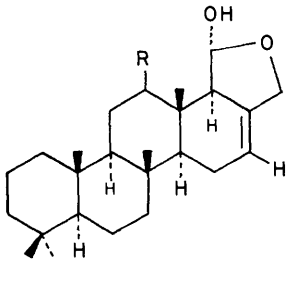
[1] L. Minale, G. Cimino, S. De Stefano, and G. Sodano, *Fortschr. Chem. org. Naturstoffe*, 1976, **33**, 1, and references therein.
[2] R. Kazlauskas, P. T. Murphy, R. J. Quinn, and R. J. Wells, *Tetrahedron Letters*, 1976, 2633.
[3] E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Tetrahedron*, 1972, **28**, 5993.
[5] G. Cimino, S. De Stefano, and L. Minale, *Experientia*, 1974, **30**, 846.
[5] G. Cimino, S. De Stefano, and L. Minale, *Experientia*, 1973, **29**, 934.
[6] R. Kazlauskas, P. T. Murphy, R. J. Quinn, and R. J. Wells, *Tetrahedron Letters*, 1976, 2631.
[7] G. Cimino, P. De Luca, S. De Stefano, and L. Minale, *Tetrahedron*, 1975, **31**, 271.
[8] E. Fattorusso, L. Minale, G. Sodano, and E. Trivellone, *Tetrahedron*, 1971, **27**, 3909.

of the spectrum of 12-*epi*-scalarin (1a) was an abundant ion at *m/e* 398 ($M^+ - HCO_2H$), also observed in the spectrum of scalarin (1).

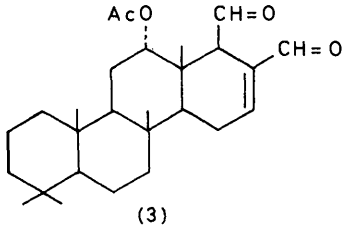
^1H N.m.r. spectral data of (1a) and (2a) along with those of (1) and (2) and derivatives are given in Table 1. Comparison indicates that (1a) differs significantly from (1), and (2a) from (2), only in the shape of the H-12 signal. In the spectra of (1a) and (2a) it appears as a double doublet, J 10 and 4 Hz, and is therefore representative of an axial proton, whereas in those of (1) and (2) it is a broad signal, $W_{\frac{1}{2}}$ *ca.* 4.5 Hz, typical of an equatorial proton.[9] Thus we conclude that the 12-acetoxy-group in (1a) and (2a) is equatorial whereas in (1) and (2) it is axial.



(1) R = α-OAc
(1a) R = β-OAc



(2) R = α-OAc
(2a) R = β-OAc



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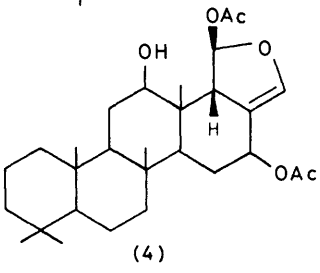

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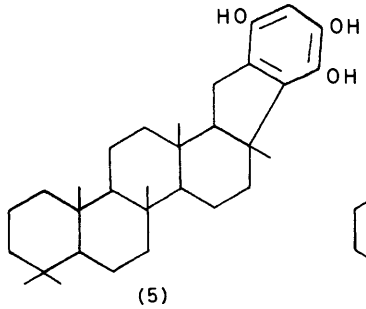

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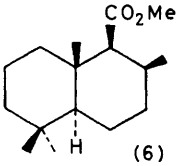


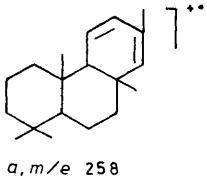
(6)

The two series of compounds can be also distinguished on the basis of the chemical behaviour of the corresponding 12-hydroxy-derivatives toward oxidation by chromic oxide: the scalarin-type compounds (12-OH axial) are oxidized rapidly by Jones reagent to the corresponding 12-ketones, whereas the episcalarin-type compounds (12-OH equatorial) resist Jones oxidation; this is in accord with the general observation that axial alcohols are oxidized more rapidly than equatorial alcohols.[10]

[9] E. R. H. Jones, Eighth International Symposium on the Chemistry of Natural Products (New Delhi, 6—12 February 1972), ed. T. R. Govindachari, Butterworths, London, 1973, p. 42.

Chemical correlation of 12-*epi*-scalarin (1a) and 12-*epi*-deoxoscalarin (2a) with scalarin (1) (Scheme) definitively confirmed their structures. Scalarin (1), previously isolated from *Cacospongia scalaris*,[3] and now re-isolated from *Spongia virgultosa*, which also contained scalaradial (3), was treated with sodium borohydride to give the lactone (7), m.p. 214—216°, $[\alpha]_D$ +66.4 (lit.,[3] 213—216°, $[\alpha]_D$ +65.2), which, on treatment with 10% potassium hydroxide in methanol–water afforded the hydroxy-lactone (8), m.p. 298—300° (lit.,[3] 297—300°). On oxidation with Jones reagent, the hydroxy-lactone gave the ketone (9), m.p. 260—262°, $\nu_{max.}$ 1 750 (lactone C=O) and 1 700 cm^{-1} (ketone C=O), which, on reduction with sodium borohydride and subsequent acetylation, gave a mixture from which the acetate (7a), m.p. 213—215°, $[\alpha]_D$ −11.5; $\nu_{max.}$ 1 750 (lactone C=O) and 1 735 cm^{-1} (acetate C=O), could be separated. The ester (7a) was identical (m.p., mixed m.p., $[\alpha]_D$, t.l.c., mass, and ^1H n.m.r. spectra) with the αβ-unsaturated lactone derived from 12-*epi*-scalarin (1a) on reduction with sodium borohydride and acetylation. The same acetate (7a) was also obtained from 12-*epi*-deoxoscalarin (2a) by treatment with sodium borohydride followed by chromium trioxide–pyridine.

Some stereochemistry of the sesterterpenes (1), (1a), (2), and (2a), was inferred from the ^1H n.m.r. spectra of the hydroxy-αβ-unsaturated lactone (8) and the corresponding ketone (9), in which the signals for the methylene protons at C-19 formed a pair of triplets, both with J 9 Hz (see Table 1). The coincidence of the values of the coupling constants between the two geminal protons and between each geminal proton and the allylic methine



a, *m/e* 258

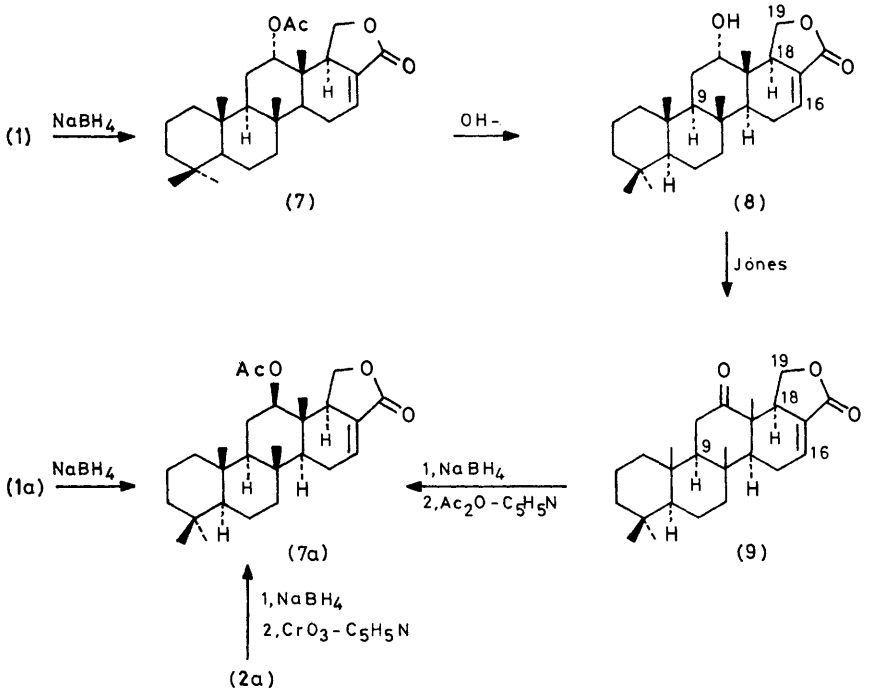
proton at C-18 is noteworthy, but significantly an identical pattern (two triplets, both J 9 Hz, δ 4.24 and 3.93) has been observed previously for the methylene protons at C-15 in the spectrum of isoagatholactone (10) [which can also be depicted as in (10a)], a diterpene isolated from *Spongia officinalis*, whose structure and absolute stereochemistry were ascertained by chemical correlation with a known diterpene, grindelic acid, which in turn was correlated with ambrein.[11] In addition the signal for the olefinic proton (H-16) in the spectra of both (8) and (9) formed a quartet with J 3.5 Hz, also observed in the spectrum of isoagatholactone [(10) or (10a)] for the corresponding proton (H-12). Furthermore the c.d. spectra of (7), (7a), and (8), $[\phi]_{244}$ +12 280, +14 980, and +13 430, respectively, were almost identical in appear-

[10] G. H. Rasmusson and G. E. Arth, in ' Organic Reactions in Steroid Chemistry,' vol. 1, J. Fried and J. A. Edwards, Van Nostrand–Reinhold Company, 1972, p. 222.

[11] G. Cimino, D. De Rosa, S. De Stefano, and L. Minale, *Tetrahedron*, 1974, **30**, 645.

ance and amplitude with that of isoagatholactone [(10) or (10a)], but opposite in sign ($[\phi]_{247}$ −12 090).

From these comparative n.m.r. and c.d. data, the sponge-derived sesterterpenes (1), (1a), (2), and (2a) appear to have a *trans-transoid-trans*-skeleton as in the diterpene isoagatholactone; on that assumption, the upfield shifts exhibited by their resonances in the ^1H n.m.r. spectrum of the ketone (9) on passing from $CDCl_3$ to C_6D_6 solution: in the spectrum of (9) in $CDCl_3$ the 13-methyl resonance occurs at δ 1.00, whereas the 8-methyl protons resonate at δ 1.15; in benzene solution the 13- and 8-methyl resonances occur at δ 0.56 and 0.76,



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positive Cotton effect exhibited by the αβ-unsaturated lactone sesterterpenes, when compared with the negative one exhibited by the αβ-unsaturated lactone [(10 or (10a)], leads to the assignment of the 18α-absolute configuration for the former. The stereochemistry at C-18 in the sesterterpene compounds and at C-14 in (10a) determines the chirality of the αβ-unsaturated lactone system, and therefore the sign of the Cotton effect.

The axial orientation for the angular methyl groups at C-8 and C-13 was substantiated by the pronounced respectively. The chemical shift assignments of the methyl protons followed from the europium-shifted spectra, which also gave further additional stereochemical information. In particular, the europium-expanded ^1H n.m.r. spectrum {0.5 mol. equiv. Eu([$^2H_9$]fod)$_3$ in C_6D_6} clearly showed the separate proton resonances of H-9, H-11*ax*, and H-11*eq* at δ 1.7, 3.12, and 3.41 as a broad doublet (*J* 14), a triplet (d of d, *J* 14), and a double doublet (*J* 3 and 14 Hz), respectively. Irradiation at the frequency of the doublet at δ 1.7 caused both the triplet

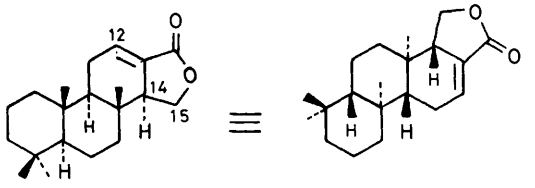
TABLE 1

^1H N.m.r. data [a] of the sponge-derived sesterterpenes (1), (1a), (2), (2a), and derivatives

Compound	C-CH_3	H-12	H-16	H-18	H-19	H-20	OAc
(1)	0.96, 0.90 (6 H), 0.83 (6 H)	4.91 (m, $W_{1/2}$ 4.5)	6.81br (q, *J* 3.5)	3.14br (m)	5.69br (d, *J* 5)		2.09
(1a)	0.96, 0.92, 0.83 (6 H), 0.80	4.69 (dd, *J* 4,10)	6.84br (q, *J* 3.5)	2.60br (m)	5.72br (d, *J* 5)		2.07
(2)	0.94, 0.87, 0.83 (6 H), 0.79	4.86 (m, $W_{1/2}$ 4.5)	5.36br (m)	2.66br (m)	5.12br (d, *J* 4)	4.39, 4.03 (d, *J* 12, 1 H each)	2.02
(2a)	0.96, 0.88, 0.84 (6 H), 0.80	4.67 (dd, *J* 4,10)	5.48br (m)	2.25br (m)	5.37br (d, *J* 4)	4.42, 4.10 (d, 12, 1 H each)	2.03
(7)	1.00, 0.87 (9 H), 0.80	4.92 (m, $W_{1/2}$ 6.5)	6.94 (q, *J* 3.5)	3.25br (m)	4.16 (complex, 2 H)		2.12
(7a)	0.98, 0.88 (9 H), 0.82	4.68 (dd, *J* 4, 10)	6.90br (q, *J* 3.5)	2.88br (m)	4.17 (complex, 2 H)		2.06
(8)	0.94, 0.86 (6 H), 0.82, 0.78	3.60 (m, $W_{1/2}$ 5)	6.78 (q, *J* 3.5)	3.38br (m)	4.44, 4.03 (t, *J* 9, 1 H each)		
(9)	1.15 (8-CH_3), 1.00 (13-CH_3), 0.91, 0.88, 0.85		6.82 (q, *J* 3.5)	3.14br (m)	4.78, 4.10 (t, *J* 9, 1 H each)		

[a] δ Values (Me_4Si as internal standard); solvent $CDCl_3$; *J* and $W_{\frac{1}{2}}$ in Hz.

and the double doublet to collapse to doublets with J 14 Hz. The magnitude of the coupling constants $J_{11ax,9}$ (14 Hz) and $J_{11eq,9}$ (3 Hz) clearly indicated that H-9 is axially oriented. Furthermore, the axial orientation of both H-9 and H-14 is also required to explain the relative magnitudes of the paramagnetic shifts induced by Eu([$^2H_9$]fod)$_3$ on the signals of H-9, H-11*eq*, and H-14 in the n.m.r. spectrum of the alcohol (8): all these signals were shifted by about the same amount, and with a 1 : 1 molar ratio of shift reagent to (8) they appeared as separated resonances at δ 5.85br (d, J 14, H-11*eq*), 5.36br (t, J 6, H-14), and 5.13br (d, J 12 Hz, H-9). Irradiation at δ 3.9 (3 H, complex m, H-15 and H-11*ax*) caused collapse of all three signals to broad singlets. These



(10) (10a)

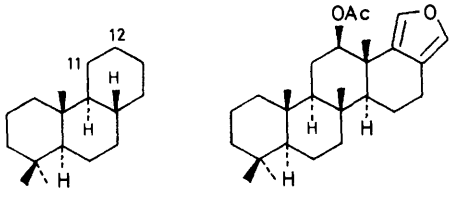
results are consistent with a *cisoid* relationship of H-11*eq*, H-9, and H-14 to the hydroxy-group, and accordingly an axial orientation for H-9 and H-14 is required.

All these spectral data together with the previous degradative results on scalarin (1),[3] indicating an AB-*trans* ring fusion, strongly favour the stereostructures (1a) and (2a) for the new sesterterpenes and (1) and (2) for their epimers, except that the configuration at C-19 is yet to be ascertained.

The ^{13}C n.m.r. spectra of (1), (1a), and (2a) and their derivatives (7) and (7a) were valuable in confirming these stereochemical proposals. The shielding data for this series of compounds are collected in Table 2. In each case three types of spectra were run: proton-resonance decoupled spectra for the determination of the ^{13}C chemical shift values, noise off-resonance decoupled spectra (NORD) [12] for the detection of non-protonated carbon sites, and single-frequency off-resonance decoupled spectra (SFORD) [13] for the differentiation of carbon species, the first step of the assignment. Further assistance was obtained by applications of known chemical shift rules such as the substituent effects to be expected of an axial and an equatorial acetoxy-group in cyclic systems,[14] by comparison of the spectra of different compounds, and by comparison with published data for structurally related compounds, especially diterpenes[14,15] and triterpenes [16] of known stereochemistry.

Peaks corresponding to the carbon atoms of rings A and B except for C-9, having chemical shift values virtually identical in all the spectra, were assigned by comparison with published data for podocarpane (11),[14] bearing in mind the expected consequence of the introduction of a C-8 axial angular methyl group on the basis of chemical shift theory.[17] Differentiation of the pairs C-2 and -6, C-3 and -7, and C-8 and -10 is difficult because of the closeness of their resonances. Assignments for C-8 and C-10 are tentative and are based on the fact that the C-10 signal should not be shifted throughout this series of compounds.

Comparison of the spectra of (1a), (2a), and (7a) with those of (1) and (7) allowed ready recognition of the signals corresponding to the functionalized carbon atoms and also assignment of the other signals due to carbon atoms in rings C and D. In particular the resonances associated with the acetoxy-substituted carbon atoms (α-carbons) exhibited large differences between the two series, in accord with the axial orientation of the acetoxy-group in (1) and (7) and the equatorial orientation in (1a), (2a), and (7a); as expected, large differences between the two series were also discernible for the γ-carbons, C-9, -14, and -18, the signals at higher field being observed in the spectra of (1) and (7), having the 12-acetoxy-group axially oriented. The signals for the methine carbon atoms C-9, -14, and -18 were differentiated in the following way. The C-18 signal was distinguished in the spectra of (1) and (1a) by selective decoupling of H-18 (δ 3.14 and 2.66, respectively), and in the spectrum of (2a) the C-18 resonance may be identified by comparison with the spectrum of (1a), since introduction of the oxo-group at C-20 is expected to produce an upfield shift of γ-carbon signals. In the spectra of (7) and (7a) the C-18 resonance may be assigned by comparison with the spectra of (1) and (1a), respectively, since the absence of the 19-hydroxy-group is expected to produce a pronounced upfield shift (*ca.* 8—9 p.p.m.) of β-carbon signals. In all the spectra the C-9 and -14 resonances were differentiated on the assumption that the presence of the double bond in ring D would result in shielding of the homoallylic carbon atoms of *ca.* 3—4 p.p.m.,[18] and on the consideration that in each individual compound C-9 and -14 have very similar geometrical environments. The off-resonance doublets associated with C-9 and -14 exhibited in each spectrum differences of *ca.* 4 p.p.m.; accordingly we assigned the lowfield



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[12] E. Wenkert, A. O. Clouse, D. W. Cochran, and D. Doddrell, *J. Amer. Chem. Soc.*, 1969, **91**, 6879.

[13] G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley–Interscience, New York, 1972.

[14] I. Wahlbery, S. Almqvist, T. Nishida, and C. R. Enzell, *Acta Chem. Scand.*, 1975, **B29**, 1047.

[15] E. Wenkert and B. L. Buckwalter, *J. Amer. Chem. Soc.*, 1972, **94**, 4367.

[16] G. Lukacs, F. Khuong-Huu, C. R. Bennett, B. L. Buckwalter and E. Wenkert, *Tetrahedron Letters*, 1972, 3515.

[17] J. B. Stothers in 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York, 1972.

[18] E. Wenkert, D. W. Cochram, E. W. Hagaman, F. M. Schell, N. Neuss, A. S. Katner, P. Poitier, C. Kan, M. Plot, M. Koch, M. Meheri, J. Poisson, N. Kunesch, and Y. Rolland, *J. Amer. Chem. Soc.*, 1973, **95**, 4990.

signals to C-9. Comparison of the spectra of the two series of compounds also revealed, as expected, the smallest differences for the β-carbons C-13 and -11, which should experience an upfield shift in (1) and (7) (12α-yl acetate) as compared with (1a) and (7a) (12β-yl acetate), as observed for C-11 (methylene carbon atoms) of podocarpan-12α- and -12β-yl acetate.[14] Furthermore the C-11 resonance is expected to be essentially unshifted in (1a) as compared with (2a) and (7a). These considerations allowed us to distinguish definitively C-13 from the quaternary sites C-8 and -10, and led to the assignments for C-11 and -15, although the differentiation between the signals of these two carbon atoms in the spectra of (1a) and (7a) remains uncertain because of their proximity (δ_C 23.4 and 23.5—23.7). Finally, significant differences between the two series of compounds were also observed, as expected, for the C-25 (methyl) resonances.[19] In the spectra of (1a), (2a), and (7a) the C-25 angular methyl group is shielded by its *gauche* interaction with the 12β-acetoxy-group, as compared with (1) and (7) in which this *gauche* interaction is lacking because the acetoxy- and the angular methyl group are *trans*-oriented. This ^{13}C n.m.r. study provides strong support for the stereochemistry proposed for the sponge-derived sesterterpenes (1), (1a), (2), and (2a). Particularly diagnostic for the *trans-transoid-trans*-skeleton of these molecules are the high-field shifts of the signals for the methyl groups at the ring junctions, similar to those observed for *trans*-4a-methyldecalin and related systems, which are reported to be less than 20 p.p.m.[20]

At this point the stereochemistry at C-19 remained to be discovered. Some indication was derived from the results of acetylation (with heating) of 12-*epi*-deoxoscalarin (2a), which gave essentially the furan derivative (12), M^+ 412, m.p. 122—125°, $[\alpha]_D$ −9.9°. Similar behaviour was also observed with deoxoscalarin (2), which, on treatment with acetic anhydride–pyridine at reflux, afforded an analogous furan derivative.[4] Heteronemin (4) is reported to generate, on controlled pyrolysis, a monoacetylated furan along with a vinyl furan by stepwise elimination of two molecules of acetic acid; this led to the suggestion of a *cis*-relationship of the C-18 proton with the 19-acetoxy-group as required for a pyrolytic *cis*-elimination.[6]

The furan (12) was also generated by mild pyrolysis of 12-*epi*-deoxoscalarin acetate, obtained by acetylation of (2a) in the cold. On this basis we suggest that in both (2) and (2a), as in heteronemin, the C-18 proton and the 19-acetoxy-group are in a *cis*-relationship. The same stereochemistry at C-19 may also be tentatively advanced for scalarin (1) and 12-*epi*-scalarin (1a) on the basis of the similarity in pattern of the H-19 signals in the spectra of all four compounds [(1), (1a), (2), and (2a)] and in view of the magnitude of the deshielding influence (*ca.* 1 p.p.m.) of the 19-hydroxy-group on the C-25 angular methyl carbon atom in (1) and (1a), which is similar to that observed for (2a). These are examples of deshielding trends caused by δ-interaction [*cf.* the C-25 resonances of (1) and (7) and of (1a) and (7a)]; if in (1) and (1a) the 19-hydroxy-group were β-oriented the *cisoid* δ-OH,CH_3 interaction should shift the C-25 resonance much further downfield.[21]

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Column chromatography was carried out on silica gel (0.05—0.2 mm; Merck). Preparative t.l.c. was carried out on silica gel layers (Merck F_{254}; 20 × 20 cm). I.r. spectra were measured for solutions in chloroform with a Perkin–Elmer 257 Infracord, u.v. spectra for solutions in methanol with a Bausch and Lomb Spectronic, and ^1H n.m.r. spectra for solutions in [^2H]chloroform with a Varian XL-100 spectrometer (tetramethylsilane as internal reference) unless otherwise indicated. Mass spectra were measured with an A.E.I. MS30 instrument at 70 eV; accurate mass measurements were performed with an A.E.I. MS902 instrument. C.d. curves were recorded for solutions in methanol with a Fica Spectropolarimeter. Rotations were measured for solutions in chloroform. Light petroleum had b.p. 40—70 °C.

Isolation of 12-epi-*Scalarin* (1a) *and* 12-epi-*Deoxoscalarin* (2a).—Fresh material (3 kg dry weight after extraction) was extracted with acetone (× 3) for 3 days; after concentration the aqueous residue was extracted with ether. The combined ethereal extracts were taken to dryness and the oily residue (41 g) was chromatographed on a column of silica gel (600 g) [elution with benzene containing increasing amounts of ether]. Fractions eluted with benzene–ether (6 : 4) contained a mixture of (1a) and (2a) (1.8 g), which was rechromatographed over silica gel in ether–light petroleum (6 : 4) to give 12-epi-*deoxoscalarin* (2a); the product was crystallized from ethanol; yield 235 mg; m.p. 192—194°; (Found: $M^+ - H_2O$, 412.297 2; $M^+ - CH_3CO_2H$, 370.287 6; $M^+ - H_2O - CH_3CO_2H$, 352.276 9. $C_{27}H_{40}O_3$ requires 412.297 7. $C_{25}H_{38}O_2$ requires 370.287 2. $C_{25}H_{36}O$ requires 352.276 6); $[\alpha]_D$ +13.9 (*c* 2); $\nu_{max.}$ 358 0, 3 400, 1 720, and 1 240 cm^{-1}; *m/e* 412 ($M^+ - H_2O$, 14%), 370 ($M^+ - CH_3CO_2H$, 14), 352 ($M^+ - CH_3CO_2H - H_2O$, 9), 337 (10), 324 (19), 309 (14), 258 (10), 231 (10), 205 (14), 191 (100), 137 (60), and 123 (61); and 12-epi-*scalarin* (1a), which was further purified by preparative t.l.c. [ether–light petroleum (8 : 2)] and crystallization from ethanol; yield 31 mg; m.p. 236—238° (Found: $M^+ - H_2O$, 426.276 5; $M^+ - CH_3CO_2H$, 384.266 9; $M^+ - CH_3CO_2H - H_2O$, 366.255 2. $C_{27}H_{38}O_4$ requires 426.277 0. $C_{25}H_{36}O_3$ requires 384.266 4. $C_{25}H_{34}O_2$ requires 366.255 9); $[\alpha]_D$ − 57.0 (*c* 1); $\nu_{max.}$ 3 560, 3 330, 1 750, 1 730, 1 690, and 1 240 cm^{-1}; $\lambda_{max.}$ 222 (ε 7 814); *m/e* 426 ($M^+ - H_2O$, 4%), 398 ($M^+ - HCO_2H$, 16), 384 ($M^+ - CH_3CO_2H$, 20), 366 ($M^+ - CH_3CO_2H - H_2O$, 100), 356 (30), 351 (30), 341 (40), 275 (10), 258 (12), 231 (40), 205 (30), 191 (90), 137 (45), 123 (50); ^1H n.m.r. data of both compounds are in Table 1.

Isolation of Scalarin (1) *from* Spongia virgultosa.—Fresh material (400 g dry weight after extraction) was extracted

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as before. The ether-soluble material (17 g) was chromatographed on silica gel in light petroleum containing increasing amounts of ether. Elution with 1 : 1 ether–light petroleum gave two principal fractions: (i) a fraction containing scalaradial (3) (580 mg), and (ii) a more polar fraction, consisting principally of scalarin (1) (1.05 g). Both fractions were then subjected to preparative t.l.c. [ether–light petroleum (6 : 4)] to give (3) (100 mg after crystallization; R_F 0.6), m.p. 110—113°, $[\alpha]_D$ +45.8 (lit.,[5] m.p. 111—113°, $[\alpha]_D$ +47.3); and (1) (600 mg after crystallization; R_F 0.3), m.p. 134—136°, $[\alpha]_D$ +44.5 (lit.,[3] m.p. 133—135°, $[\alpha]_D$ +43.2).

Acetate (7) was obtained from scalarin (1) by using the conditions of Fattorusso *et al.*;[3] m.p. 214—216°, $[\alpha]_D$ +66.4 (lit.,[3] m.p. 213—216°, $[\alpha]_D$ +65.2), c.d. $[\phi]_{244}$ +12.280.

Lanthanide-induced Shifts for the Alcohol (8).—The alcohol (8) was obtained from scalarin (1) by the procedure of Fattorusso *et al.*; m.p. 298—300°, $[\alpha]_D$ +38.5 (lit.,[3] 297—300°, $[\alpha]_D$ +37.4), c.d. $[\phi]_{244}$ +13 430; n.m.r. data (unshifted) are given in Table 1; with a 1 : 1 molar ratio of Eu($[^2H_9]$fod)$_3$ to (8), methyl singlets were observed at δ 2.60 (13-CH_3), 2.12 (8-CH_3), 1.64 (10-CH_3), and 1.10 and 0.98 [4-$(CH_3)_2$], and other signals at 10.5br (m, H-18), 10.0 (q, *J* 3.5 Hz, H-16), 8.68br (s, H-12), 8.18 and 6.58 (each t, *J* 9 Hz, H_2-19), 5.85br (d, *J* 14 Hz, H-11α), 5.36br (t, *J* 6 Hz, H-14), 5.13br (d, *J* 12 Hz, H-9), and 3.90 (complex m, H-11β and H_2-15).

Oxidation of the Alcohol (8) *to the Ketone* (9).—An excess of Jones reagent was added dropwise to a solution of (8) (68 mg) in acetone (5 ml). The mixture was left for 30 min at room temperature, and then diluted with water. The product was extracted with ether and the combined extracts were washed with water, aqueous sodium hydrogen carbonate, and water again, dried ($MgSO_4$), and evaporated. Preparative t.l.c. [benzene–ether (9 : 1)] and crystallization from ethanol gave the *ketone* (9) (53 mg), m.p. 260—262° (Found: M^+, 384.266 7. $C_{25}H_{36}O_3$ requires *M*, 384.266 4); $[\alpha]_D$ +150 (*c* 0.5); $\nu_{max.}$ 1 750 and 1 700 cm^{-1}; $\lambda_{max.}$ 221 (ε 11 700); *m/e* 384 (M^+, 10%), 369 (16), 342 (6), 205 (11), 192 (32), 191 (50), 179 (67), 177 (100), 137 (32), and 123 (84); c.d. $[\phi]_{245}$ +12 290, $[\phi]_{287}$ +9 600; ^{1}H n.m.r. data for solution in $CDCl_3$ are in Table 1; with 0.7 : 1 molar ratio of Eu($[^2H_2]$fod)$_9$ to (9) methyl singlets were observed at δ 1.99 (13-CH_3), 1.55 (8-CH_3), 1.09 (10-CH_3), and 0.92 [4-$(CH_3)_2$], and other signals at 8.40 (q, *J* 3.5 Hz, H-16), 6.16 and 5.52 (each t, *J* 9 Hz, H_2-19), 4.90br (m, H-18), 3.94 (dd, *J* 2 and 13 Hz, H-11α), and 3.78 (t, *J* 13 Hz, H-11β); in C_6D_6 (9) exhibited resonances at δ 6.56 (q, *J* 3.5 Hz, H-16), 4.07 and 3.88 (each t, *J* 9 Hz, H_2-19), 2.82br (m, H-18), 0.82 (s, 4-CH_3), 0.76 (s, 8-CH_3 and 4-CH_3), 0.66 (s, 10-CH_3), and 0.56 (s, 13-CH_3); with 0.5 : 1 molar ratio of Eu($[^2H_9]$fod)$_9$ to (9) in C_6D_6; δ 8.71 (q, *J* 3.5 Hz, H-16), 6.25 and 5.52 (each t, *J* 9 Hz, H_2-19), 4.52br (m, H-18), 3.41 (dd, *J* 3 and 14 Hz, H-11β), 3.12 (t, *J* 14 Hz, H-11α), 1.7br (d, *J* 14 Hz, H-9), 1.54 (3 H, s, 13-CH_3), 1.14 (3 H, s, 8-CH_3), and 0.88 [9 H, s, 10-CH_3 and 4-$(CH_3)_2$].

The Acetate (7a).—(a) *From the ketone* (9). The ketone (9) (50 mg) was stirred with an excess of sodium borohydride in ethanol for 1 h. Acetic acid was added and the mixture was evaporated to dryness. The residue was extracted with chloroform. The extract was evaporated and the residue was kept at room temperature in acetic anhydride–pyridine (10 : 1;. 2 ml) for 18 h. The product, obtained as usual, gave several spots on t.l.c. The mixture was separated by t.l.c. in light petroleum–ether (6 : 4); the band with R_F 0.2 corresponded to the *acetate* (7a), which was crystallized from ethanol (yield 9 mg); m.p. 213—215° (Found: M^+, 428.292 9. $C_{27}H_{40}O_4$ requires *M*, 428.292 6), $[\alpha]_D$ −11.4° (*c* 0.8), $\nu_{max.}$ 1 750, 1 735, and 1 690 cm^{-1}; *m/e* 428 (M^+, 1%), 386 (3), 368 (8), 353 (6), 258 (12), 205 (20), 191 (100), 176 (31), 137 (30), and 123 (35); ^{1}H n.m.r. data are in Table 1.

(b) *From* 12-epi-*scalarin* (1a). 12-*epi*-Scalarin (50 mg) was dissolved in ethanol (5 ml) and an excess of sodium borohydride was added to the cooled solution. After agitation (1 h), the mixture was worked up as before. The product was purified by preparative t.l.c. and subsequent crystallization to give a dehydroxy-compound (32 mg) identical with the acetate (7a).

(c) *From* 12-epi-*deoxoscalarin* (2a). 12-*epi*-Deoxoscalarin (70 mg) was reduced with sodium borohydride as above. The crude product dissolved in pyridine (5 ml) was added to chromium trioxide (80 mg) in pyridine (2 ml); the mixture was stirred at room temperature for 24 h, treated with ethanol (1.5 ml) for 15 min, and then diluted with water. Extraction with ether followed by t.l.c. afforded the acetate (7a) identical with the material obtained before.

TABLE 2

^{13}C N.m.r. chemical shifts * for compounds (1), (1a), (2a), (7), and (7a)

	(1)	(1a)	(2a)	(7)	(7a)
C-1	39.8	39.8	39.6	39.8	39.8
C-2	18.1 [b]	18.0 [a]	18.1 [a]	18.0 [a]	18.0 [a]
C-3	41.6 [b]	41.6 [b]	41.5 [b]	41.6 [b]	41.5 [b]
C-4	33.3	33.3	33.2	33.3	33.3
C-5	56.5	56.5	56.4	56.5	56.5
C-6	18.5 [a]	18.5 [a]	18.5 [a]	18.5 [a]	18.5 [a]
C-7	42.1 [b]	42.1 [b]	42.0 [b]	42.0 [b]	42.0 [b]
C-8	37.9	37.3 [c]	37.3 [c]	37.9	37.4
C-9	52.5	58.5	58.3	52.5	58.4
C-10	37.4	37.5 [c]	37.5 [c]	37.5	37.4
C-11	22.4	23.4 [d]	23.5	22.5	23.4 [c]
C-12	74.6	82.1	82.7	74.7	82.7
C-13	36.9	38.6	37.7 [c]	36.9	38.8
C-14	49.9	53.6	54.0	50.3	53.6
C-15	24.2	23.6 [d]	22.2	24.3	23.7 [c]
C-16	135.3	135.3	116.3	135.3	135.6
C-17	128.1	128.0	136.2	126.8	126.5
C-18	50.8	58.8	61.2	43.1	49.7
C-19	98.9	99.8	99.8	66.9	67.7
C-20	167.8	167.6	68.2	169.8	169.4
C-21	33.3	33.3	33.2	33.3	33.3
C-22	21.4	21.4 [e]	21.4 [d]	21.3	21.4 [d]
C-23	16.1 [c]	16.5	16.5	16.1 [c]	16.6
C-24	16.3 [c]	16.5	16.5	16.2 [c]	16.6
C-25	15.1	10.2	9.8	14.1	9.0
CH_3CO	171.0	171.6	171.4	170.1	170.1
CH_3CO	21.4	21.3 [e]	21.3 [d]	21.3	21.3 [d]

* Solutions in $[^2H]$chloroform; 25.20 MHz; Varian XL-100 Fourier transform spectrometer; chemical shifts in p.p.m. from internal Me_4Si. [a–e] Assignments may be reversed.

Treatment of 12-epi-*Deoxoscalarin* (2a) *with Acetic Anhydride*.—12-*epi*-Deoxoscalarin (50 mg) was refluxed in acetic anhydride (2 ml)–pyridine (3 drops) for 15 min. The product, homogeneous on t.l.c., was chromatographed on a silica gel column in benzene to give the *furan derivative* (12) (32 mg), m.p. 122—125° (from ethanol) (Found: M^+, 412.296 9. $C_{27}H_{40}O_3$ requires *M*, 412.297 7); $[\alpha]_D$ −9.9° (*c* 1); δ 7.02 (2 H, s, furan protons), 4.75 (1 H, dd, *J* 11 and 4 Hz, H-12), and 2.17 (3 H, s, OAc); *m/e* 412 (M^+, 26%), 362 (28), 347 (30), 191 (100), 161 (50), 147 (75), 137 (45), and 123 (40).

Compound (12) was also obtained in the following way.

Treatment of (2a) with acetic anhydride–pyridine at room temperature for 18 h gave a crude diacetate, δ 2.06 and 2.11 (OAc), R_F [benzene–ether (2 : 1)] 0.23, which, without further purification, was heated at 100 °C for few minutes to give the furan derivative (12), R_F [benzene–ether (2 : 1)] 0.8, which was purified by preparative t.l.c.

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