Structural Study of a New Dialkylated Scalarane from a *Carteriospongia* sp.

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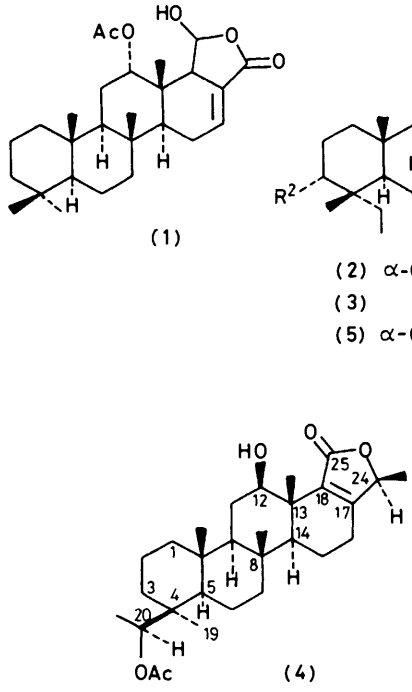
The structure of a new sesterterpene metabolite isolated from an unclassified *Carteriospongia* sp. has been assigned from *X*-ray crystallographic evidence. The compound has been indentified as (20*S*,24*S*)-20-acetoxy-12β-hydroxy-20,24-dimethyl-25-norscalar-17-ene-18,24-carbolactone (4). The absolute configuration is taken to be that found for other scalaranes from *Phyllospongia* spp.

Since the isolation of scalarin (1) was reported, [1] a number of tetracarbocyclic sesterterpenes have been isolated from marine sponges.[2] Recently it has been shown [3] that a specimen of *Dysidea herbacea* collected in the Gulf of Suez contains three C(24)-alkylated scalaranes† which occur as mixtures with corresponding homologues. The latter group could not be obtained in a pure form but from spectroscopic data were suggested to be 19,24-dimethylscalaranes. Further examples of these have been isolated from *Phyllospongia radiata* and *P. foliascens* ‡ collected on the Great Barrier Reef.[6] The evidence for the presence of a 4-equatorial ethyl group rests on an *X*-ray crystallographic analysis of compound (3), derived by oxidation of the C(12) hydroxy-group in the natural product (2) [6] and the absolute stereochemistry was deduced from c.d. studies. Interestingly, the sesterterpene metabolites from *Phyllospongia* differ from those of *Dysidea* in the relative stereochemistry of the substituents at C-(12) and -(16).[4]

In the course of our work on natural products from marine sources we examined the constituents of a *Carteriospongia* sp.§ collected from the Fiji Islands and we isolated another dimethylated scalarane derivative, compound (4). This paper described the structure and relative stereochemistry of this new compound as determined from a single-crystal *X*-ray diffraction analysis.

Extraction of the freeze-dried material (30 g) obtained from a small specimen of a *Carteriospongia* sp., and silica-gel chromatography of the extract, yielded the crystalline lactone (4), $C_{29}H_{44}O_5$, whose spectroscopic characteristics indicated the presence of three functional groups, *viz.* an equatorial, secondary hydroxy-group ($\nu_{max.}$ 3 400 cm^{-1}; δ_H 3.66, m, $w_{\frac{1}{2}}$ 14 Hz, X part of an ABX system), a secondary acetate [$\nu_{max.}$ 1 720 cm^{-1}; δ_H 2.00 (3 H)] which was shown by nuclear magnetic double resonance (n.m.d.r.) techniques to be part of a 1-acetoxyethyl moiety [7] [δ_H 5.43 (q, *J* 6.5 Hz) and 1.20 (d, *J* 6.5 Hz)], and a 2,3-disubstituted butenolide function [8] [$\nu_{max.}$ ($CHCl_3$) 1 740 cm^{-1}; $\lambda_{max.}$ (EtOH) 218 (ε 9 500)] further substituted at the 4-position by a methyl group [δ_H 1.40 (d, *J* 6.5 Hz) and 4.85 (q, *J* 6.5 Hz)]. The remaining degrees of unsaturation were attributed to four rings which contain four tertiary methyl groups [δ_H 1.11 (3 H), 0.94 (6 H), and 0.89 (3 H)].

Given the origin of the compound, the discernible functional groups could be accommodated on a scalarane structure. The mass spectrum of compound (4) also could be interpreted in these terms and the major fragmentations were rationalized as arising from cleavage of the C(12)–C(13) and C(8)–C(14) bonds with H transfer to give the base peak at *m/z* 165, and from cleavage of the C(9)–C(11) and C(8)–C(14) bonds following the loss of a $CH_3CHOCOCH_3$ fragment (87 a.m.u.) to give an ion at *m/z* 177 (40%). These results suggest that the secondary hydroxy-group is located at C(12) and that the 1-acetoxyethyl moiety is on ring A or B.



Numbering scheme for compound (4) is that proposed by Kazlauskas *et al.*[4] for the hypothetical hydrocarbon scalarane

Owing to the small amount of material available and the difficulty in obtaining more of the sponge we established the structure and relative configuration of compound (4) by a single-crystal *X*-ray diffraction analysis. The results are reported below and show that the metabolite from the *Carteriospongia* sp. is (20S,24S)-20-acetoxy-12β-hydroxy-20,24-dimethyl-25-norscalar-17-ene-18,24-carbolactone (4).¶

† The numbering system used follows that proposed by R. Kazlauskas *et al.*[4]

‡ A number of *Phyllospongia* spp. have been reclassified recently [5] as *Carteriospongia* spp.

§ A specimen has been deposited with the Western Australian Museum (catalogue No. WAM 18–82).

¶ A more systematic name might be 4β-(1-acetoxyethyl)-12β-hydroxy-4α,8-dimethyl-D(17a)-homopregn-17-ene-17a,20-carbolactone.

Table 1. Non-hydrogen atom co-ordinates (crystallographic numbering scheme)

Atom	*x*	*y*	*z*
C(1)	0.870 3(3)	0 [a]	0.435 8(3)
C(2)	0.874 1(3)	0.099 3(5)	0.537 1(3)
C(3)	0.856 9(3)	0.255 1(5)	0.500 7(3)
C(4)	0.742 2(3)	0.285 0(4)	0.413 5(3)
C(19)	0.743 7(4)	0.443 4(5)	0.373 2(4)
C(20)	0.640 8(3)	0.264 6(4)	0.470 5(3)
O(20)	0.672 8(2)	0.343 7(3)	0.577 9(2)
C(201)	0.644 9(3)	0.283 3(5)	0.667 2(3)
C(202)	0.695 4(4)	0.365 4(6)	0.772 7(3)
O(201)	0.587 9(3)	0.176 9(4)	0.662 5(2)
C(203)	0.521 7(4)	0.315 7(7)	0.407 5(4)
C(5)	0.739 6(3)	0.180 4(5)	0.311 3(3)
C(6)	0.641 9(3)	0.202 0(4)	0.203 8(3)
C(7)	0.669 4(3)	0.129 0(4)	0.101 4(3)
C(8)	0.690 1(2)	−0.034 3(4)	0.116 4(3)
C(21)	0.572 2(3)	−0.107 5(4)	0.110 6(3)
C(9)	0.782 5(2)	−0.057 0(4)	0.230 7(2)
C(10)	0.759 4(2)	0.016 3(4)	0.339 5(2)
C(22)	0.660 1(3)	−0.058 8(5)	0.375 9(3)
C(11)	0.820 1(3)	−0.214 0(4)	0.245 7(3)
C(12)	0.873 9(3)	−0.266 2(4)	0.152 3(3)
O(12)	0.909 0(3)	−0.412 2(3)	0.182 1(2)
C(13)	0.789 6(2)	−0.252 1(4)	0.034 0(2)
C(23)	0.695 4(3)	−0.369 1(5)	0.016 5(3)
C(14)	0.744 9(3)	−0.091 4(4)	0.022 2(2)
C(15)	0.674 5(3)	−0.061 4(5)	−0.100 3(3)
C(16)	0.753 2(3)	−0.066 8(5)	−0.180 9(3)
C(17)	0.832 6(3)	−0.190 9(4)	−0.154 4(3)
C(18)	0.850 7(3)	−0.270 0(4)	−0.060 9(3)
C(25)	0.934 9(4)	−0.381 6(6)	−0.069 6(3)
O(25)	0.973 5(3)	−0.481 4(5)	−0.007 7(3)
C(24)	0.906 5(3)	−0.241 9(5)	−0.228 4(3)
O(24)	0.968 6(2)	−0.362 7(4)	−0.167 0(2)
C(241)	0.842 5(4)	−0.292 0(5)	−0.344 8(3)

[a] Defines origin.

Table 2. Non-hydrogen molecular geometry. Bond lengths (Å) and bond angles (°)

Bond lengths

C(1)–C(2)	1.532(5)	C(9)–C(11)	1.517(5)
C(1)–C(10)	1.541(4)	C(10)–C(22)	1.539(5)
C(2)–C(3)	1.506(6)	C(9)–C(11)	1.517(5)
C(3)–C(4)	1.534(5)	C(11)–C(12)	1.525(5)
C(4)–C(5)	1.574(5)	C(12)–C(13)	1.544(4)
C(4)–C(19)	1.547(6)	C(12)–O(12)	1.433(4)
C(4)–C(20)	1.558(6)	C(13)–C(14)	1.573(5)
C(20)–O(20)	1.465(4)	C(13)–C(18)	1.530(5)
C(20)–C(203)	1.515(6)	C(13)–C(23)	1.540(5)
O(20)–C(201)	1.341(5)	C(14)–C(15)	1.544(4)
C(201)–O(201)	1.191(6)	C(15)–C(16)	1.528(5)
C(201)–C(202)	1.486(6)	C(16)–C(17)	1.474(5)
C(5)–C(6)	1.533(4)	C(17)–C(18)	1.326(5)
C(5)–C(10)	1.560(5)	C(17)–C(24)	1.494(6)
C(6)–C(7)	1.530(5)	C(18)–C(25)	1.466(6)
C(7)–C(8)	1.534(5)	C(25)–O(24)	1.362(5)
C(8)–C(9)	1.560(4)	C(25)–O(25)	1.208(6)
C(8)–C(14)	1.557(5)	C(24)–C(241)	1.506(5)
C(8)–C(21)	1.552(5)	C(24)–O(24)	1.442(5)
C(9)–C(10)	1.578(5)		

Bond angles

C(2)–C(1)–C(10)	113.6(2)
C(1)–C(2)–C(3)	111.2(3)
C(2)–C(3)–C(4)	114.1(3)
C(3)–C(4)–C(5)	105.9(3)
C(3)–C(4)–C(19)	108.1(3)
C(3)–C(4)–C(20)	109.3(3)
C(5)–C(4)–C(19)	109.2(3)
C(5)–C(4)–C(20)	114.8(3)
C(19)–C(4)–C(20)	109.3(3)
C(4)–C(20)–O(20)	106.5(3)
C(4)–C(20)–C(203)	118.5(3)
O(20)–C(20)–C(203)	107.3(3)
C(20)–O(20)–C(201)	117.3(3)
O(20)–C(201)–O(201)	124.4(4)
O(20)–C(201)–C(202)	111.2(4)
O(20)–C(201)–C(202)	124.4(4)
C(4)–C(5)–C(6)	117.1(3)
C(4)–C(5)–C(10)	116.8(3)
C(6)–C(5)–C(10)	110.9(3)
C(5)–C(6)–C(7)	111.5(3)
C(6)–C(7)–C(8)	113.5(3)
C(7)–C(8)–C(9)	107.2(2)
C(7)–C(8)–C(14)	109.3(3)
C(7)–C(8)–C(21)	107.8(3)
C(9)–C(8)–C(14)	106.2(3)
C(9)–C(8)–C(21)	114.4(3)
C(14)–C(8)–C(21)	111.7(3)
C(8)–C(9)–C(10)	117.3(2)
C(8)–C(9)–C(11)	111.0(2)
C(10)–C(9)–C(11)	114.8(3)
C(1)–C(10)–C(5)	108.5(2)
C(1)–C(10)–C(9)	107.4(2)
C(1)–C(10)–C(22)	108.4(3)
C(5)–C(10)–C(9)	106.3(2)
C(5)–C(10)–C(22)	114.6(3)
C(9)–C(10)–C(22)	111.3(3)
C(9)–C(11)–C(12)	112.5(3)
C(11)–C(12)–C(13)	111.9(3)
C(11)–C(12)–O(12)	105.2(3)
C(13)–C(12)–O(12)	113.4(3)
C(12)–C(13)–C(14)	107.0(3)
C(12)–C(13)–C(18)	112.0(3)
C(12)–C(13)–C(23)	110.6(3)
C(14)–C(13)–C(18)	104.5(3)
C(14)–C(13)–C(23)	115.5(3)
C(18)–C(13)–C(23)	107.1(3)
C(8)–C(14)–C(13)	116.8(3)
C(8)–C(14)–C(15)	115.2(3)
C(13)–C(14)–C(15)	110.3(3)
C(14)–C(15)–C(16)	110.2(3)
C(15)–C(16)–C(17)	110.7(3)
C(16)–C(17)–C(18)	125.0(3)
C(16)–C(17)–C(24)	124.2(3)
C(18)–C(17)–C(24)	110.8(3)
C(13)–C(18)–C(17)	126.0(3)
C(13)–C(18)–C(25)	127.1(3)
C(17)–C(18)–C(25)	106.8(3)
C(18)–C(25)–O(25)	130.7(4)
C(18)–C(25)–O(24)	109.7(4)
O(24)–C(25)–O(25)	119.6(4)
C(17)–C(24)–O(24)	103.7(3)
C(17)–C(24)–C(241)	115.5(3)
O(24)–C(24)–C(241)	108.9(3)
C(24)–O(24)–C(25)	108.9(3)

The sponge genus *Carteriospongia* (previously *Phyllospongia*) [5] (family Spongiidae) is restricted to the Indo-Pacific region and the metabolites of four species collected on the Great Barrier Reef have been examined in detail. Thus a number of 24-methylated scalaranes were isolated from *P. dendyi* and an unclassified *Phyllospongia* sp.[4] On the other hand several 19,24-dimethylated scalaranes were isolated from two other species, *e.g.* metabolite (2) from *P. radiata* and compound (5) from *P. foliascens*.[6] These metabolites are reported to contain an ethyl group at the 4-equatorial position

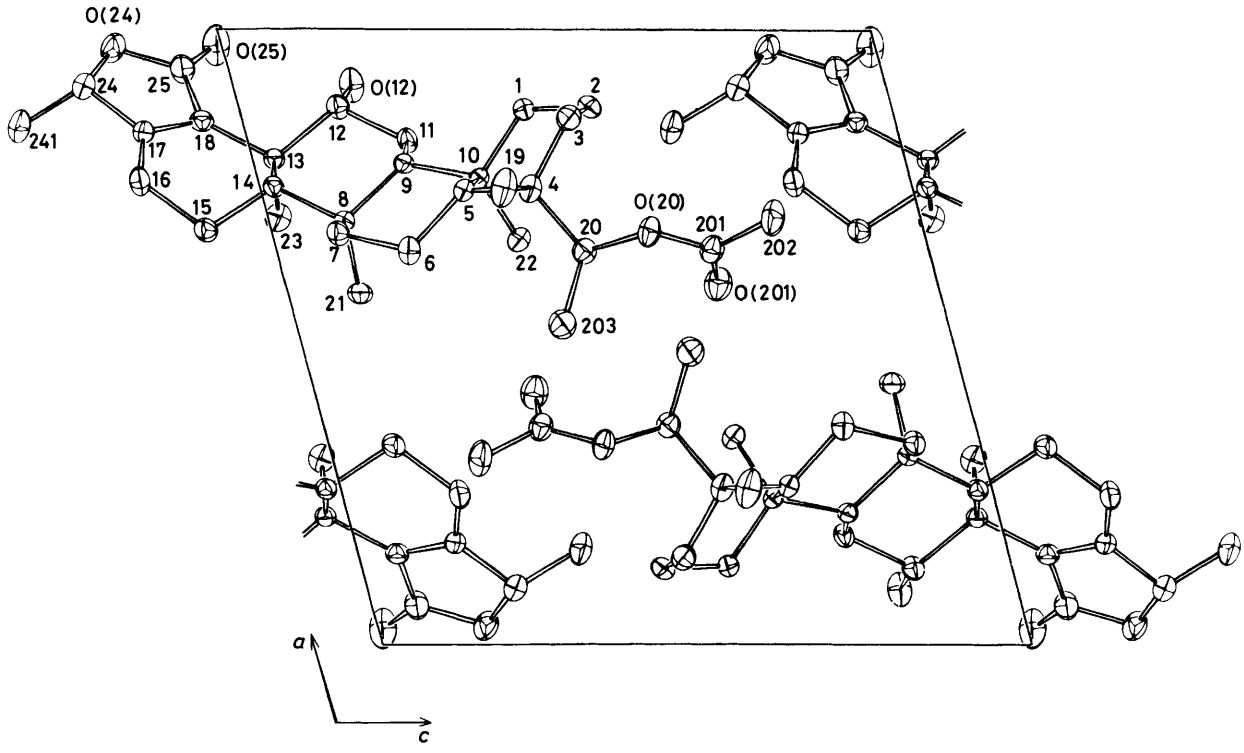


Figure 1. Unit-cell contents projected down *b*. Thermal ellipsoids are shown at the 20% probability level, together with non-hydrogen atom numbering

and 12α- or 12β-oxygenation. In contrast, metabolite (4) described in this report not only includes a novel 1-acetoxyethyl side chain but also contains a 4-axial ethyl group and 12β-oxygenation. The *X*-ray crystallographic evidence for the structure and relative stereochemistry of compound (4) is described below. The absolute stereochemistry of the metabolite (4) is taken to be that found for other scalaranes from *Phyllospongia* species.[4]

Experimental

All solvents used were distilled. For t.l.c. analysis Merck precoated silica-gel plates were used, with dichloromethane–5% ethyl acetate as developing solvent. The ^{1}H n.m.r. spectrum was measured with a Bruker WP80 Spectrospin instrument using tetramethylsilane as internal standard. The mass spectrum was obtained with a Varian MAT-CH7 instrument.

Extraction of Carteriospongia *sp.*—A sample of the sponge (Specimen No. 04/11-4-79) collected near Namuka Island, Fiji, was freeze-dried. The material obtained (30 g) was extracted with dichloromethane (500 ml) to yield a dark-green extract (2.5 g) which was subjected to rapid silica-gel filtration (100 g, t.l.c.-grade silica gel) using gradient elution with dichloromethane with increasing amounts of ethyl acetate as eluant. The initial fractions containing fatty material and sterols were discarded. Fractions enriched in the major terpenoid constituent (80 mg content) were combined and purified by preparative t.l.c. to give the lactone (4) (30 mg) which was recrystallized from diethyl ether–light petroleum (b.p. 56—60 °C) as *prisms*, m.p. 252—254 °C; $[\alpha]_D$ 20° (*c*, 3.3 in $CHCl_3$) (Found: C, 73.1; H, 9.1. $C_{29}H_{44}O_5$ requires C, 73.7; H, 9.4%); $\nu_{max.}$ ($CHCl_3$) 3 400, 1 740, and 1 720 cm^{-1}; $\lambda_{max.}$ (EtOH) 218 nm (ε 9 500); δ_H (80 MHz; $CDCl_3$) 5.96br (1 H, s, OH), 5.43 (1 H, q, *J* 6.5 Hz, 20-H), 4.85 (1 H, q, *J* 6.5 Hz, 24-H), 3.66 (1 H, X part of ABX, $w_{\frac{1}{2}}$ 14 Hz, 12-H$_\alpha$), 2.00 (3 H, s, COMe), 1.40 (3 H, d, *J* 6.5 Hz, 24-Me), 1.20 (3 H, d, *J* 6.5 Hz, 20-Me), and 1.11 (3 H), 0.94 (6 H), and 0.89 (3 H) (4-, 8-, 10-, and 13-Me); *m/z* (%) 472 (M^+, 50), 457 (45), 454 (40) 412 (10), 394 (15), 385 (40), 367 (30), 205 (35), 203 (40), 177 (40), and 165 (100).

Crystallography *

Crystal Data.—$C_{29}H_{44}O_5$, M = 472.7. Monoclinic, space group $P2_1$ (C_2^2, No. 4), a = 11.987(5), b = 9.246 (3), c = 12.217(5) Å, β = 104.77(3)°, U = 1309.3(9) Å^3. D_m = 1.20(1), D_c = 1.20 g cm^{-3}, Z = 2. F(000) = 516. Monochromatic Mo-K_α radiation, λ = 0.7106$_9$ Å. μ = 0.86 cm^{-1}. Specimen: needle, 0.17 × 0.55 × 0.13 mm.

Structure Determination.—A unique data set was measured within the limit $2\theta_{max.}$ = 45° using a Syntex $P2_1$ four-circle diffractometer in the conventional 2θ–θ scan mode. 1 845 Independent reflections were obtained, 1 577 of which with $I > 3\sigma(I)$ were considered 'observed' and were used in the least-squares refinement without absorption correction after the solution of the structure by direct methods. Block-diagonal least-squares refinement was used, parameters being divided among three blocks: C(1—8) and associated atoms, C(9—13) (similarly), and C(14—24) (similarly). Anisotropic thermal parameters were used for the non-hydrogen atoms; for the hydrogen atoms, $(x, y, z, U)_H$ were constrained at idealized estimates. At convergence, R, R' were 0.039, 0.051, respectively, reflection weights being $[\sigma^2(F_o) + 0.0003(F_o)^2]^{-1}$. At this juncture, the refinement had proceeded very satisfactorily and it was apparent that the data were of rather better quality than is commonly encountered for structures of this type. Accordingly, it was decided to ascertain whether the quality of the data

* Crystallographic numbering used in this section.

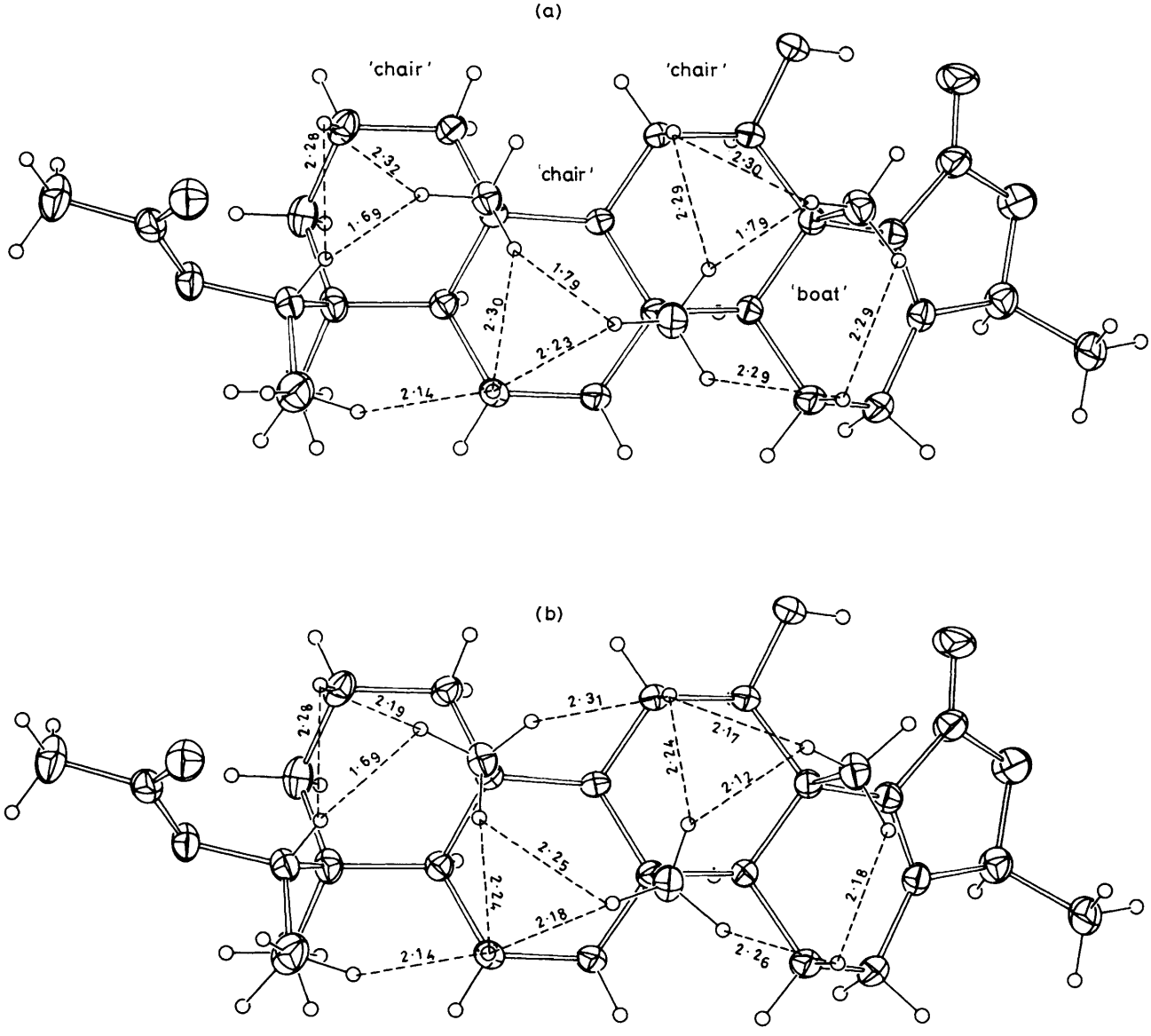
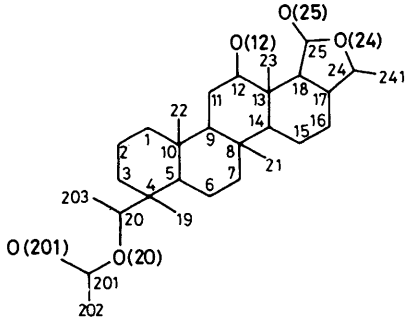


Figure 2. (a) Molecular projection at R 0.039. Hydrogen atoms are shown as circles of radius 0.1 Å. (b) As for (a), but at R 0.033, showing the displacement of the refined hydrogen atom positions for the central methyl substituents relative to the idealized positions depicted in (a)

would support meaningful refinement of the parameters of some of the more interesting hydrogen atoms in the system, in particular those in close contact between the central methyl substituents. Accordingly, (x, y, z, U) for the hydrogen atoms attached to C(203, 21, 22, 23, and 12) were allowed to refine, and appeared to do so in a significant manner, the residuals falling significantly in the process and converging to 0.033, 0.042 respectively. Neutral-atom scattering factors were used, those for C and O being corrected for anomalous dispersion (f', f'').[9] The X-RAY 76 program system [10] and GENTAN [11] were used, implemented on a Perkin-Elmer 3240 computer. Material deposited comprises tables of structure factor amplitudes, thermal parameters, and hydrogen parameters.* The non-hydrogen atom crystallographic numbering scheme is as follows for the skeleton; hydrogen-atom numbering follows that of the parent C, O atom, with H atoms labelled A, B, C as necessary.



* Supplementary Publication No. SUP 23451 (10 pp.). For details see Notice to Authors No. 7, *J. Chem. Soc., Perkin Trans. 1*, 1981, Index issue.

Structural Commentary.—The structure determination establishes the molecular stoicheiometry and stereochemistry of compound (4) to be as discussed above and as shown in the Figures; the asymmetric unit of the cell is a single molecule of assumed chirality.

The molecular geometry has a number of features of interest; the structure is one of the most precise so far determined for the present skeleton and affords an opportunity to examine in some detail the nature of the steric strain involved by the disposition of the methyl substituents on the fused ring system. [For the purposes of the discussion, C(20), attached to C(4), is considered to be a ' methyl ' substituent.]

(i) *Hydrogen-atom dispositions.* Location of the hydrogen atoms on the methyl substituents geometrically results in a series of hydrogen contacts involving unrealistically short H · · · H distances (Figure 2a). Refinement of these hydrogen-atom positions as described above displays a consistent picture of concerted motion of the methyl groups; as depicted, the C(22) and C(23) methyl groups rotate about their carbon–carbon bonds in a clockwise direction, while C(21) undergoes anti-clockwise rotation [about C(8)—C(21)], in consequence of which a less strained array of H · · · H contacts is observed (Figure 2b). It is of interest to note (SUP 23451; H-atom parameters), that, whereas a C–H distance of 0.95 Å is usually considered to be a reasonable norm in an *X*-ray structure determination, in the present determination, although it would be unwise to draw any conclusion about a single bond, nine out of twelve refined methyl hydrogen atoms have C–H distances $\geqslant$1.0 Å, and all are $\geqslant$0.95 Å, suggesting either diminished CH_3–C torsional motion and/or that a further mode of strain relief may involve C–H bond lengthening. There does not appear to be any noticeable concomitant lengthening of the associated (methyl) C–C distances.

(ii) *Skeletal distortion.* This, however, is noticeable, especially with regard to the ' spine ' of the molecule defined by the atoms C(4, 5, 10, 9, 8, 14, 13, 18), successive distances in this sequences being 1.574(5), 1.560(5), 1.578(5), 1.560(4), 1.557(5), 1.573(5), and 1.530(5) Å), all bonds except the last (which lies outside the region of strain) being appreciably greater than the usual $C(sp^3)$–$C(sp^3)$ value of 1.54 Å. In addition, notable angular distortions are observed about those carbon atoms in the spine which do not carry substituent methyl groups, but lie between those atoms which do, *i.e.* C(5, 9, 14), where the three angles subtended by the neighbouring carbon atoms of fused ring system are 117.1(3), 116.8(3), 110.9(3); 117.3(2), 111.0(2), 114.8(3); and 116.8(3), 115.2(3), 110(3)° respectively. We note further that in each case *two* of the three angles are greatly enlarged above the tetrahedral value, and that of these two angles, one is the exocyclic angle, while the other lies between the two carbon atoms which carry the methyl substituents (Figure 3).

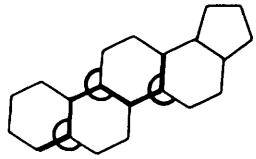


Figure 3. Diagram of the ring skeleton showing the unusually enlarged ' spinal ' bond lengths and angles of the fused-ring system

Acknowledgements

We gratefully acknowledge receipt of a grant from the Australian Research Grants Committee in support of this work. We also acknowledge receipt, from Dr. W. L. Duax and Dr. M. D. Fronckowiak of the Medical Foundation of Buffalo, of a preprint of their report of a neutron-diffraction determination of the structure of 20-methylpregnenediol, in which the effect of steric strain on the disposition of the methylene hydrogen atoms is examined. We are grateful to Miss J. Fromont for identification of the sponge as belonging to the genus *Carteriospongia.*

References

1 E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Tetrahedron*, 1972, **28**, 5993.
2 L. Minale in ' Marine Natural Products,' ed. P. J. Scheuer, Academic Press, 1978, vol. 1.
3 Y. Kashman and M. Zviely, *Tetrahedron Lett.*, 1979, 3879.
4 R. Kazlauskas, P. T. Murphy, R. J. Wells, and J. J. Daly, *Aust. J. Chem.*, 1980, **33**, 1783.
5 P. R. Bergquist, *N. Z. J. Zool.*, 1980, **7**, 443.
6 J. J. Daly and W. Hofheinz, unpublished data reported in ref. 4.
7 M. Fetizon, G. Moreau, and N. Moreau, *Bull. Soc. Chim. Fr.*, 1969, 1614.
8 C. J. W. Brooks and G. H. Draffan, *Tetrahedron*, 1969, **25**, 2887.
9 ' International Tables for X-ray Crystallography, Vol. IV,' eds. J. A. Ibers and W. C. Hamilton, Kynoch Press, Birmingham, 1974.
10 ' The X-RAY System, Version of March 1976,' Technical Report TR.446, ed. J. M. Stewart, Computer Science Center, University of Maryland, U.S.A.
11 S. R. Hall, ' Generalized Application of Invariants of Automatic Solutions,' Paper 15, 1–2, Abstracts of 11th I.U.Cr. Congress, Warsaw, Poland, 1978.

Received 2nd June 1982; *Paper* 2/895