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SARCOPHINE, A NEW EPOXY CEMBRANOLIDE FROM MARINE ORIGIN

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Abstract—From the soft bodied coral *Sarcophytum glaucum* a crystalline compound-sarcophine (1), which is believed to be one of the repellents protecting the coral against predators, was isolated. The functional groups of 1 have been deduced from detailed NMR study and the whole molecular structure has been determined by a single-crystal 3-dimensional X-ray diffraction study proceeding from direct methods. The compound crystallizes in orthorhombic crystals, space group $P2_12_12_1$, and there are four molecules in each unit cell of dimensions $a = 12{\cdot}429(3)$, $b = 13{\cdot}766(3)$ and $c = 10{\cdot}750(1)$. The structure was found to be a new epoxy cembranolide. The mass spectrum and CD of 1 and several simple derivatives as well as the conformation of 1 in solution are discussed.

While working on the ecology of coral reefs in the Red Sea[1] it was observed that many of the soft-bodied animals found exposed on rocky surfaces, were not attacked by predators whereas others were immediately devoured. From one of such common species, *Sarcophytum glaucum*, that forms large patches on the reefs, a crystalline compound, which is believed to be one of the repellents protecting the soft-coral against predators, was isolated in remarkably high concentrations (up to 3% dry weight).[2] The structure of this toxin, found to have interesting pharmacological properties and which we named *sarcophine* (1), is described herewith.

Mass spectral and elemental analysis of 1, m.p. 133°–134°, α_D^{25} +92°, indicated a formula of $C_{20}H_{32}O_3$, M. wt. 316. The IR absorptions at 1750, 1270, 1250 cm^{-1} seemed to propose an $\alpha\beta$-unsaturated γ-lactone corresponding to two out of the three molecules' O atoms, and, in the absence of an OH and another CO group, an ethereal linkage is suggested for the third O atom. Catalytic hydrogenation over $Pd/CaCO_3$ in ethanol led to the uptake of 3 mol of hydrogen. This fact indicated the presence of three double bonds and/or rings that are easily hydrogenated. Fig 1 illustrates the NMR spectrum of 1 and Chart 1 summarizes the structual features deduced from this spectrum.

A singlet for three hydrogens at $\delta 1{\cdot}28$, suggesting a somewhat deshielded Me group such as a Me β to an ethereal oxygen, together with a triplet at $\delta 2{\cdot}68$ ($J = 4$ Hz, 1H),* which is in a suitable place for an oxirane methine,[3] agree with the proposed epoxide. A 3-proton broadened singlet at $\delta 1{\cdot}63$ suggests a vinyl Me, the broadening of which originates from

*The δ-value of 2·68 agrees with the chemical shift of a *trans*-epoxide in fatty acids rather than the *cis*-one.[3]

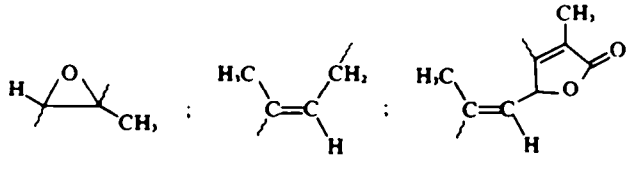


CHART I.

Structural Components

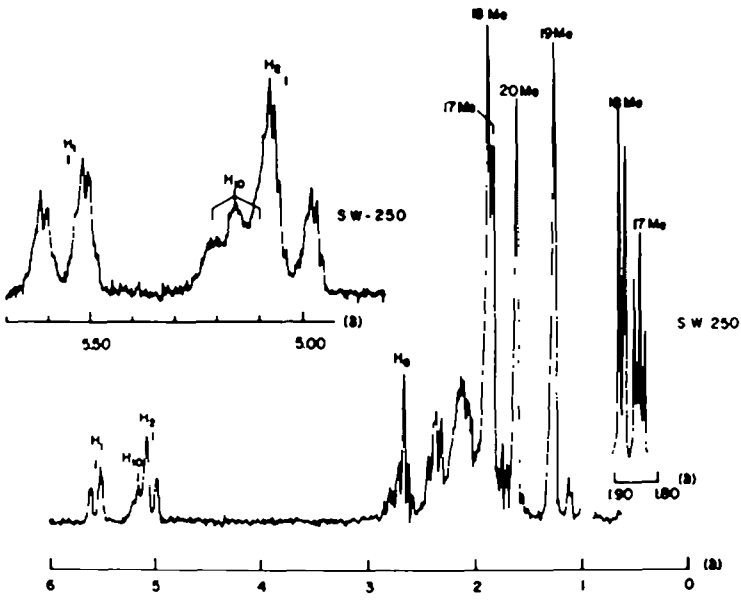


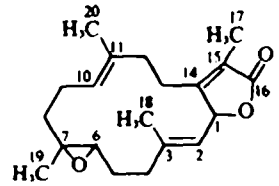
Fig 1. 100 MHz NMR spectrum of sarcophine.

allylic coupling with a vinylic proton appearing at δ5·15 br.t (J = 5 Hz). Irradiation at δ5·15 in a decoupling experiment sharpened the singlet at 1·63 and simultaneously caused a change in the multiplet at δ2·15 (6H); *vice versa* irradiation of these allylic protons changed the triplet at 5·15 into a broad singlet thus pointing to two neighbouring allylic protons. In a pair of deshielded vinyl Me groups at δ1·89 d (J = 1 Hz) and δ1·85 t (J = 1·5 Hz), one Me represents an αβ-unsaturated lactone α-Me group, while the other as yet unexplained will still be accounted for. The fact that no β-proton of the lactonic double bond could be seen suggests that it is a tetrasubstituted one. Two additional protons gave rise to an AB pattern at δ5·05 dq (J = 10 and 1 Hz, 1H) and δ5·55 dq (J = 10 and 1·5 Hz, 1H) each one of these protons being further coupled in a long range coupling to the vinyl methyls at δ1·89 and δ1·85 respectively. The multiplicities and chemical shifts of these low field signals demonstrate that one of them is located on the ethereal lactone-bearing C atom, while the second one must be a vicinal vinyl proton of an additional trisubstituted double bond, to which the second out of the vinyl Me pair, above mentioned, belongs. Irradiation of the signal at δ5·55 changed the triplet at 1·85 to a broad singlet (while still coupled to another homoallylic proton), while irradiation at 5·05 altered the 1·89 doublet into a singlet, confirming thereby their relationship. The δ5·05 signal is attributed by us to the vinyl proton and that of 5·55 to the lactonic one on the basis of comparisons with available closely related compounds.[4] The low field position of the latter proton also comes from the fact of its being double allylic and its multiplicity is due to the formation of a suitable geometry[5] with the αβ-unsaturated lactone α-Me group, thereby enabling their mutual homoallylic coupling. The remaining absorptions in the NMR spectrum are two allylic protons at δ2·37 and four methylenic ones which appear under the Me absorptions.

The unsaturation of the various moieties shown in Chart 1 accounts for six out of the seven unsaturations in sarcophine, the remaining one (in the absence of further Me groups), must be a macrocyclic ring which should connect the different functional sites.

The existence of three double bonds was confirmed by hydrogenation. Moreover this reduction could be carried out in a stepwise fashion thus leading to partially hydrogenated compounds each further contributing to the structure elucidation. After hydrogenation for 45 min over $Pd/CaCO_3$, a dihydro derivative (2) m.p. 164°–166°, $\alpha_D^{25°}$ + 113·5° (c, 0·6 MeOH), M. wt. 318. NMR: (δ)0·95 d (J = 7 Hz, 3H); 1·25 s (3H); 1·81 brs (6H) and a clear AB quartet 5·02 dq (J = 10; 1·5 Hz) and 5·50 dq (J = 10; 1·5 Hz) was obtained. The NMR spectrum clearly shows the selective reduction of the trisubstituted double bond which gave rise to the signals at δ1·63 and 5·15 in 1.

Hydrogenation for 6-8 h led to the uptake of 2 mol of hydrogen resulting in saturation of both trisubstituted double bonds. While the hydrogenation of the first one was stereoselective, yielding mainly one isomer, this was not the case with the second double bond. Nevertheless one of the tetrahydro isomers (3) could be crystallized out in a pure state m.p. 98–99° (light petroleum), $\alpha_D^{25°}$ + 99° (c, 0·4 MeOH), NMR (δ): 0·95 d (J = 7 Hz, 3H), 1·02 d



1: Sarcophine
2: 10,11-dihydrosarcophine
3: 2,3,10,11-tetrahydrosarcophine
4: 10,11-epoxysarcophine

(J = 7 Hz, 3H), 1·20 s (3H), 1·75 brs (3H) 2·65 m (1H) and 4·78 m (1H)—next to the lactonic O atom. Full hydrogenation was complete after 48 h and led to a mixture of hexahydro stereoisomers. Several structures could be proposed for 1 according to the biosynthetic principles; attempts were made to use the mass spectrum of compounds 1–4 for structure elucidation. The problem with the mass spectrum of 1 was the large number of possible fragmentations due to the various functional groups, and indeed the overall picture of the mass spectrum resembles those of aliphatic hydrocarbons and only two outstanding peaks were noted at m/e 164 (28%) and 151 (25%) the parent peak being m/e 81. Even after full structure determination by X-ray analysis *vide infra* it was hard to propose unequivocal explanations for these peaks, e.g. the generation of the base peak at m/e 81 ($C_6H_9^{\oplus}$) can be explained in the same manner as described for methyl 10,11-epoxyfarnesoate,[6] which contains three of the sarcophine's functional sites in the same sequence, and thus it should contain atoms 8, 9, 10, 11, 12 and 20 (for numbering see Fig 2). A second possibility

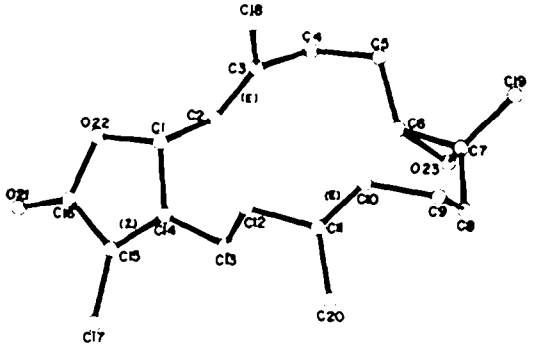
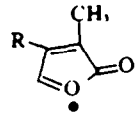


Fig 2. Sarcophine structure according to X-ray data.

which could not be excluded was that this fragment originates from C atoms 2, 3, 4, 5, 6 and 18 due to cleavage of the C_1–C_2 bond, followed by transannular cleavage of the epoxide together with hydrogen migration.[7] The fact that the second mechanism should also be taken into account is supported by the appearance of the m/e 81 (82%) peak even in the mass spectrum of the dihydro derivative 2 and the diepoxide 4. Cleavage of the C_1–C_2 bond is assisted by the stability of ion a, a cleavage that can also be responsible for the m/e 164 ($C_{10}H_{12}O_2$) ion which is obtained by further cleavage of the easily breakable double allylic bond C_{12}–C_{13};[8] the latter ion appears to the extent of 70% in 2 (the base peak being m/e 318, $M^{\oplus}$). In the dihydro derivative two additional outstanding peaks appearing at m/e 112 (50%, $C_7H_{12}O$) and m/e 207 (33%, $C_{11}H_{19}O_2$) can be explained in several ways by cleavages originating from the epoxide,[7] the oxygen of which should thus be included in the first fragment.



ion a

The general picture of the mass spectrum of 3 differs completely from those of the two former ones (1 & 2) as only two functional groups remained, giving rise to several strong peaks: m/e 320 (60%, $M^{\oplus}$), 302 (11%, M-H_2O), 292 (10%, M-CO), 251 (100%), 207 (95%), 179 (85%), 151 (45%), 125 (55%), 124 (65%), 112 (87%).

However, mainly because of the large number of possible fragmentation mechanisms of epoxides, still more than one mechanism could be suggested for most of the peaks, e.g. the loss of a $C_3H_5^{\cdot}$ radical (m/e 69) to give the parent peak m/e 251 can be explained by at least four reasonable mechanisms: (a) rearrangement of the epoxide to a 6-methyl-7-keto derivative followed by C–C_8 and C_{12}–C_{13} cleavages. (b) The same epoxide rearrangement as in (a) followed by a McLafferty rearrangement to cleave the C_6–C_7 bond and then the allylic C_{12}–C_{13} one. (c) Again the same epoxide rearrangement followed this time by a McLafferty rearrangement in the other direction with cleavage of C_5–C_6 and then C_1–C_2 near the lactone. (d) Epoxide transannular cleavage followed by C_{10}–C_{11} splitting. The situation is similar with most of the other fragments. The high sensitivity of 1–4 towards acid and base, together with H–D exchange at allylic positions during deuteration of the double bonds prevents further study of the mass spectrum. Thus the only method which remained for unequivocal structure elucidation was X-ray analysis which indeed furnished the relative positions of the functional groups and the stereochemistry of the molecule.

X-ray study. Oscillation, Weissenberg and precession photographs showed the crystals of 1 to be orthorhombic and their space group was uniquely determined as $P2_12_12_1$. The unit cell dimensions, fitted to diffractometer measurements, are: a = 12·429 (3), b = 13·766 (3) and c = 10·750 (1). The density of the crystal, as measured by flotation, is d_m = 1·15 g/cm^3 while that calculated for 4 molecules in the unit cell is d_c = 1·141 g/cm^3.

Intensity data were collected on a Syntex $P\bar{1}$ diff-

ractometer, using graphite-monochromatized copper radiation with the monochromator set in parallel mode. 1895 independent reflections were measured, including 43 reflections with $1 \leqslant 2\sigma$ (I), comprising 45 percent of the copper sphere contents. 14 low angle reflections were too strong to be measured along with the rest, were remeasured with reduced current and were scaled up with the aid of the intensities of four standard reflections. The latter were recorded at regular (2 h) intervals, their averge deviation from the mean intensity values being about 2%.

Structure amplitudes were obtained by applying Lorentz and modified (for parallel monochromator mode) polarization corrections.[8]

No corrections were made for absorption or extinction.

The structure determination proceeded by direct methods.

Approximate scale and temperature factors were used in the calculation of normalized structure factors. An application of program MULTAN[9] in an automatic phase determination process yielded 4 sets of phases,* with high figures of merit, which were consistent with the indications of the Σ_1 formula.[10] An E map, based on the best of these sets, readily led to the recognition of 20 out of the 23 heavy atoms and the missing 3 atoms were subsequently located in a different map.

The heavy atom structure was then refined by a block diagonal least-squares method, using the local version[11] of program ORFLS.[12] The refinement was initially overall isotropic and later individual isotropic temperature factors were assigned to all the heavy atoms which resulted in an agreement factor R = 0·14. The calculation was concluded with a few cycles of anisotropic refinement during which the remeasured reflections were discarded because of probable extinction and inaccurate scaling. The final R value, R = 0·105, is based on 1850 observed reflections, with intensities greater than 2σ (I).

Atomic scattering factors for C and O were taken from Hanson *et al.*[13]

The final atomic positional and thermal parameters along with their standard deviations are given in Table 1. Fig 2, illustrating the molecular structure, and the molecular geometry presented and discussed below are based on these parameter values.

The structure of 1 is seen to consist of a 14-membered cembrane-type, skeleton, which carries the above mentioned $\alpha\beta$-unsaturated methyl γ-lactone, the epoxide group and the three Me substituents. The bond distances, bond angles and some dihedral angles are shown in Tables 2, 3 and 4 respectively.

The lactone group is fused to the macrocycle *via* C(1) and C(14) while the oxygen bridges across the C(6)–C(7) bond, the configuration of which is transoid (Table 4). There are two double bonds in the macrocycle, C(2)–C(3) = 1·332(10) Å, and C(10)–C(11) = 1·314(11) Å, both being slightly twisted out of the trans configuration (Table 4). The only short transannular contact is C(6)–C(10) = 3·21 Å, the next shortest being C(12)–C(2) = 3·70 Å. A comparison of the macrocyclic ring in 1 with its closest published model, cembrene,[14] shows a similarity in overall shape but a detailed correlation is precluded by the different structures of the macrocycles in the two compounds.

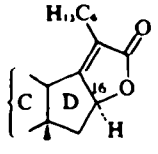
Calculation of best planes shows that the lactone group, including its Me substituent, is very nearly planar as shown in Table 5. It should also be pointed out that the angle formed by the best planes of the lactone and the C(1)–C(2)–C(3)–C(4)–C(18) group is about 90·5°. The dimensions of the lactone and the epoxide groups agree well with those published in the literature.[15,16] There are some discrepancies in C–C distances as compared to published values, which appear to be 0·02–0·04 Å too long in the sarcophine macrocycle. This may be due, in part, to the absence of hydrogens from the present structure.

Intermolecular contacts, shorter than 3·6 Å, are C(17)...C(2) = 3·47 Å and C(18)...C(13) = 3·35 Å.

CD and conformational study. With the knowledge of the configuration of 1 the question arose as to whether it is possible to determine the absolute configuration using CD data.

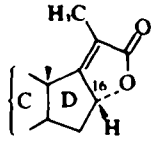
The $n \rightarrow \pi^*$ CD band of $\alpha\beta$-unsaturated γ (and δ) lactones, in the 240–260 nm region, is well known.[17,18] A sign-chirality relationship suggested by Beecham[18] attributed the Cotton effect sign to the chirality of C=C–C=O in the γ (and δ) lacetone, likewise in $\alpha\beta$-unsaturated ketones.[19] Although the data concerning γ-lactones are mainly that of α-methylene γ-lactones,[17] a few examples of endoun-

*Which were essentially the same.



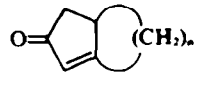
I

$\Delta\epsilon$ (250 nm) = −9
max



III

$\Delta\epsilon$ (250 nm) = +8
max



II

n = 4 a = −2900
n = 5 a = +1000

Table 1. Fractional coordinates and thermal parameters ($\times 10^4$Å^2)*

Atom	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{23}	U_{13}
C1	0·3149 (6)	0·3277 (5)	0·3049 (8)	376 (39)	343 (35)	468 (45)	28 (34)	24 (36)	48 (38)
C2	0·3794 (6)	0·4088 (5)	0·3617 (7)	296 (34)	416 (36)	384 (37)	12 (31)	− 6 (32)	− 4 (33)
C3	0·4247 (6)	0·4834 (5)	0·3027 (7)	335 (36)	471 (41)	406 (41)	48 (34)	27 (35)	1 (35)
C4	0·4842 (6)	0·5620 (6)	0·3717 (8)	340 (39)	478 (43)	523 (46)	− 73 (36)	− 8 (39)	84 (39)
C5	0·4344 (7)	0·6626 (6)	0·3542 (8)	403 (41)	474 (43)	530 (47)	− 94 (37)	6 (41)	− 33 (41)
C6	0·3213 (7)	0·6723 (5)	0·4090 (7)	504 (45)	419 (37)	357 (38)	− 31 (38)	− 19 (35)	46 (37)
C7	0·2573 (7)	0·7604 (5)	0·4040 (7)	677 (53)	381 (38)	388 (40)	− 91 (41)	25 (35)	29 (43)
C8	0·1344 (8)	0·7547 (6)	0·4055 (9)	651 (54)	398 (39)	680 (58)	155 (42)	− 55 (43)	− 158 (52)
C9	0·0848 (8)	0·7284 (6)	0·2751 (10)	582 (53)	353 (38)	819 (65)	47 (40)	48 (45)	88 (54)
C10	0·1152 (7)	0·6241 (6)	0·2396 (8)	475 (43)	404 (41)	516 (48)	61 (37)	− 57 (39)	114 (42)
C11	0·0557 (6)	0·5464 (6)	0·2566 (8)	469 (43)	412 (38)	452 (43)	116 (36)	− 38 (38)	112 (38)
C12	0·1063 (6)	0·4461 (5)	0·2339 (8)	472 (42)	316 (34)	437 (44)	89 (33)	18 (34)	62 (38)
C13	0·1276 (6)	0·4003 (5)	0·3668 (7)	413 (39)	360 (34)	394 (38)	82 (33)	40 (32)	15 (37)
C14	0·2062 (6)	0·3172 (5)	0·3634 (7)	340 (36)	378 (33)	316 (35)	3 (32)	12 (32)	36 (32)
C15	0·1980 (6)	0·2288 (5)	0·4199 (7)	435 (40)	344 (35)	414 (39)	9 (35)	− 28 (34)	1 (37)
C16	0·3012 (7)	0·1788 (6)	0·4038 (9)	498 (46)	436 (44)	635 (53)	95 (43)	− 94 (46)	− 66 (45)
C17	0·1059 (7)	0·1852 (7)	0·4934 (9)	528 (50)	610 (54)	612 (56)	152 (45)	118 (50)	60 (45)
C18	0·4224 (8)	0·4948 (7)	0·1606 (8)	716 (59)	678 (54)	291 (40)	− 62 (49)	54 (41)	104 (44)
C19	0·3022 (9)	0·8549 (6)	0·3446 (11)	882 (69)	373 (42)	906 (73)	− 104 (48)	154 (51)	5 (68)
C20	− 0·0589 (8)	0·5464 (7)	0·3084 (10)	494 (52)	602 (52)	707 (61)	61 (46)	67 (51)	58 (50)
O21	0·3276 (6)	0·1006 (5)	0·4417 (8)	823 (50)	438 (34)	1144 (65)	148 (36)	203 (42)	46 (51)
O22	0·3697 (4)	0·2352 (4)	0·3356 (6)	428 (28)	414 (27)	626 (34)	79 (25)	5 (28)	100 (29)
O23	0·3086 (6)	0·7316 (5)	0·5188 (6)	810 (42)	641 (37)	451 (33)	76 (37)	84 (31)	62 (35)

*The anisotropic temperature factor is given by

$$\exp[-2\pi^2(h^2a^{*2}U_{11} + 2hka^*b^*U_{12} + \ldots)]$$

where h, k, l and a*, b*, c* are reflection indices and reciprocal unit cell edges respectively.

Table 2. Bond distances and estimated standard deviations (Å)

Bond	Length	Bond	Length
C1–C2	1·504 (10)	C1–C14	1·498 (10)
C2–C3	1·332 (10)	C14–C15	1·364 (10)
C3–C4	1·506 (11)	C15–C16	1·466 (12)
C4–C5	1·529 (11)	C15–C17	1·516 (12)
C5–C6	1·530 (12)	C3–C18	1·535 (11)
C6–C7	1·452 (11)	C7–C19	1·554 (12)
C7–C8	1·530 (13)	C11–C20	1·530 (13)
C8–C9	1·573 (14)	C1–O22	1·481 (9)
C9–C10	1·533 (11)	C16–O22	1·365 (10)
C10–C11	1·314 (11)	C16–O21	1·197 (11)
C11–C12	1·536 (11)	C6–O23	1·444 (10)
C12–C13	1·584 (11)	C7–O23	1·444 (10)
C13–C14	1·505 (10)		

saturated lactones are available.[18,20,15a–c] Among them are the fusidic acid derivative **l** and the steroid lactone **m**, with right and left handed chirality respectively, for which the expected opposite signed Cotton effects were found.[18]

The influence of the C-16 configuration in **l** and **m** (the ethereal lactone-bearing C atom) on the Cotton effect appears clearly, and for a moment it looks as if, using this data the absolute configuration at C-1 of **1** could be determined. However, as will be explained in the following discussion such a comparison is wrong as the type of the C=C–C=O chromophore is different. Turning from **l** & **m** to loliolide[20] in which the endo unsaturated γ-lactone is fused to a 6-membered ring instead of the 5-membered one in **l** and **m**, the intensity of the Cotton effect is considerably reduced due to diminished C=C–C=O chirality which is imposed by the ring fusion. Similar behaviour is known for endo-αβ-unsaturated ketones (**n**).[21] When the fused ring is further expanded, the co-planar lactone, as in the non fused butenolides,[15] is at last obtained. Thus the C=C–C=O chromophore is not chiral any more as was the case in **l** and **m**. This seems to be the case with sarcophine, and in this case, only the second sphere would determine the Cotton effect sign. Apparently a chiral center on the ring itself as in **1** (C-1) should then determine the Cotton effect sign which, indeed, is expected to be small. In the event, compounds **1** and **2** show quite remarkable negative Cotton effects, in contrast to **3** (Fig 3). This difference in intensity must be due to the C_7–C_8 double bond. The influence of a substituent (or ring segment)—double bond on the Cotton effect of

Table 4. Some dihedral angles (°)

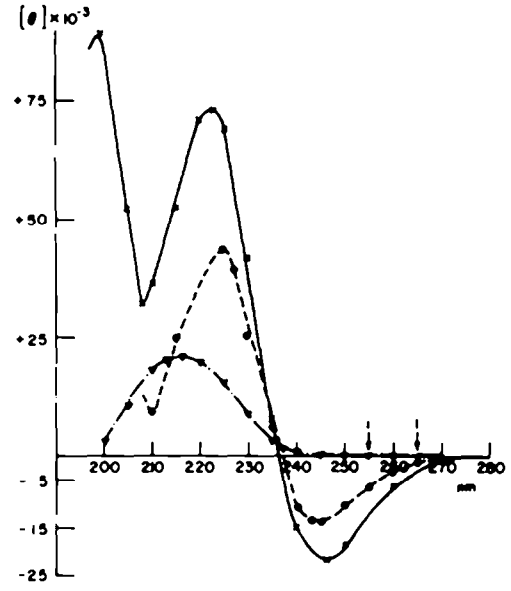
Dihedral angle	Degrees
C15–C16–O22–C1	1·2
O22–C16–C15–C14	1·6
C2–C1–C14–C13	57·1
O22–C1–C2–C3	121·1
C1–C2–C3–C4	177·9
C9–C10–C11–C12	170·6
C5–C6–C7–C8	152·5
C5–C6–C7–C19	2·1
C6–C7–C8–C9	79·6
C7–C8–C9–C10	67·3

Table 5. Best plane of the lactone group

Atom	C1	C14	C15	C16	C17	O21	O22
Deviation from lactone plane (× 10³ Å)	−6	−9	17	3	−4	−8	6

Table 3. Interbond angles and estimated standard deviations (°)

Angle	Degrees	Angle	Degrees
C1–C2–C3	127·2 (0·7)	O22–C1–C2	107·7 (0·6)
C2–C3–C4	121·8 (0·7)	C13–C14–C15	128·3 (0·7)
C3–C4–C5	113·1 (0·7)	C14–C15–C17	130·0 (0·7)
C4–C5–C6	113·8 (0·7)	C16–C15–C17	122·4 (0·7)
C5–C6–C7	124·2 (0·7)	C15–C16–C21	128·5 (0·8)
C6–C7–C8	120·2 (0·7)	O21–C16–O22	121·6 (0·8)
C7–C8–C9	113·2 (0·8)	C2–C3–C18	123·0 (0·7)
C8–C9–C10	110·0 (0·7)	C4–C3–C18	115·2 (0·7)
C9–C10–C11	126·1 (0·8)	C10–C11–C20	125·1 (0·8)
C10–C11–C12	118·6 (0·7)	C12–C11–C20	116·1 (0·7)
C11–C12–C13	106·4 (0·6)	C8–C7–C19	114·0 (0·7)
C12–C13–C14	112·9 (0·6)	C6–C7–C19	121·2 (0·8)
C13–C14–C1	121·6 (0·6)	C19–C7–O23	115·0 (0·7)
C14–C1–C2	112·4 (0·6)	C8–C7–O23	114·7 (0·7)
C1–C14–C15	109·9 (0·6)	C5–C6–O23	117·7 (0·7)
C14–C15–C16	107·6 (0·7)	C6–C7–O23	59·8 (0·5)
C15–C16–O22	110·0 (0·7)	C7–O23–C6	60·4 (0·5)
C16–O22–C1	108·8 (0·6)	O23–C6–C7	59·8 (0·5)
O22–C1–C14	103·8 (0·6)		



——CD of sarcophine. (1, C, 0·039 in MeOH)
- - - CD of dihydrosarcophine. (2, C, 0·247 in MeOH)
·—·CD of tetrahydrosarcophine. (3, C, 0·259 in MeOH)
|→ inflection, seen only at conc. × 10.

Fig 3. CD of compounds 1–3.

ketones, or even lactones, is known,[17,19] and is expected to give rise to a much stronger anti octant contribution when compared to the corresponding saturated substituent. Often even inversion of the sign is observed. Such behaviour in our case could explain the relatively strong effects in 1 and 2 whereas in the case of 3 only a very weak positive effect seems to be seen.* Returning now to the absolute configuration determination at C-1, it is clear that the rule for the chiral chromophores is no longer available and a suitable sector rule for *planar* $\alpha\beta$-unsaturated γ-lactones should be used. Such a rule should be based on a suitable lactone, containing a chiral center, (as in 1) with known absolute configuration; data which, to the best of our knowledge, are unpublished.

The phenomena of transannular interactions in medium ring macrocycles is well known[22] thus e.g. in germacranolides two conformers can exist at low or even room temperature.[23] Variable temperature NMR between 100° to − 120° did not show any similar change in conformation as far as could be concluded from the C-1-H, C-2-H AB-quartet and the chemical shifts of the various methyls. However, conformers of 1 can be suggested in which transannular interactions can be observed, e.g. a conformer similar to the one in which sarcophine exists in the solid state (1a). Observation of the Dreiding model, fixed according to the X-ray analysis parameters (1a), revelas several proton–proton distances ($d_{H\text{-}H}$) shorter than 3 Å, among which an NOE should be observed.[22,24] The NOE determination is of course limited by the chemical shifts of the protons under consideration. Actually, two simultaneous nuclear Overhauser effects, which could not be separated, were found between H_6 and H_{10} ($d_{H_6H_{10}} \simeq 2{\cdot}7$ Å in 1a) and between H_6 and H_7 ($d_{H_7H_6} \simeq 3$ Å in 1a); summing up to 18% while irradiating H_6 and observing H_7 and H_{10},† or 7% on the *vice versa* experiment. The existence of this NOE suggests a half chair conformation for the C_6–C_{10} segment as in 1a (Fig 2) thereby considerably reducing the freedom of mobility in the different possible conformers of the whole macrocycle. Further evidence for the above conclusion can be derived from the $J_{H_7H_8}$ and $\delta_{CH_3(18)}$ values. The large $J_{H_7H_8}$ value (10·5 Hz) is in agreement[25] with a dihedral angle of ~ 160° as in 1a or ~ 0° in a conformer in which the C_7–C_8 bond is rotated by 160° in comparison to 1a. The above mentioned deshielded vinyl Me group (at δ1·89) also agrees with either one of these conformers being paramagnetically shifted by the C-1 oxygen and the C_{10}–C_{11} double bond in 1a, or by the C-1 oxygen only in the second conformer. Altogether the above data suggest that at least those parts of the marcocycle about which information is received from the NMR (the lactone together with C-2, C-3, and the C_6–C_{11} segment) exist in solution in a fixed state. Such a conformation preference can explain the stereoselective hydrogenation and epoxidation. As mentioned above short hydrogenation only attacks the C_{10}–C_{11} double bond, which can be explained by the fact that the C_7–C_8 double bond suffers from steric hindrance from the C-1-oxygen. The situation is similar with epoxidation by m-chloro perbenzoic acid, to give 4, moreover, as expected for conformer 1a, the C_{10}–C_{11} bond is mainly attacked from one side (~ 80% in the case of 2 and ~ 80% in that of 4) most probably the direction being the same as the 18 Me in relation to the macrocycle (Fig 2). At last the high sensitivity of 1 towards acid may be explained on the basis fo the 1a conformer in which the epoxide can easily be attacked intramolecularly by the C_{10}–C_{11} π-bond, being oriented in suitable orientation for a transannular reaction.

The quantity of 1 found in *Sarcophytum glaucum* (Alcyonaria), collected at different places of the sea at various times of the year, changed remarkably. The sarcophine was found to be accompanied in every case by considerable amounts of closely related cembranes. The structure of the latter and the variation with time and place as well as the appearance of the cembranes in other related Alcyonaria

*No recamization is expected to occur during hydrogenation, as when D_2 is used instead of H_2 the C-1-H is still seen to full extent in the NMR spectrum.

†Measurement of the neighbouring H_1 integral which did not change served as proof of the authenticity of the experiment.

corals is being investigated. In this context it is worthy to mention the isolation of the cembranolide eunicin[26] from the gorgonian *Eunicea mammosa* which, like the Alcyonaria, belongs to the Octocorals.

EXPERIMENTAL

M.ps were taken on a Thomas Hoover capillary m.p. apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Infracord model 337 spectrophotometer. NMR were taken on a Varian HA-100 spectrometer using 5–10% soln. in $CDCl_3$, with TMS as an internal standard. Mass spectra were taken with an Hitachi Perkin-Elmer RMU 6 instrument. CD spectra were taken on a Cary 60 recording spectropolarimeter with the Model 6006 CD attachment.

Isolation of sarcophine 1. The fresh soft coral material (270 g, dry weight) was extracted during a period of 24 h with light petroleum in a soxhlet. The extract gave, after cooling, a crystalline ppt of 1 (8 g), m.p. 133°–134° (after recrystallization from acetone–light petroleum). α_D^{25} + 92° (*c*, 1·0, $CHCl_3$), (Found: C, 75·91; H, 8·79; O, 15·35. $C_{20}H_{28}O_3$ requires: C, 75·91; H, 8·92; O, 15·17%); ν_{max}^{KBr} 3000, 2950, 2940, 2920, 2850, 1750, 1675, 1450, 1390, 1270, 1180, 1160, 1100, 1060, 985, 935, 900, 860, 840 cm^{-1}; NMR (δ): 1·28 s (3H), 1·63 brs (3H), 1·85 t (3H), 1·89 d (3H), 2·68 t (1H), 5·05 and 5·55 AB quartet (2H) and 5·15 brt (1H).

Hydrogenation of 1 *to* 2. Compound 1 (100 mg) in EtOH (20 ml) was hydrogenated over 5% Pd on $CaCO_3$ at room temp and atm pressure for 45 min. The product which was obtained following work-up was crystallized from light petroleum–acetone m.p. 164°–166°, α_D^{25} + 113·5° (*c*, 0·6, MeOH), γ_{max}^{KBr} 2920, 2850, 1750, 1675, 1660, 1460, 1380, 1300, 1160, 1090, 1040, 980, 790, 760, 695 cm^{-1}; NMR (δ): 0·95 d (J = 7 Hz, 3H), 1·25 s (3H), 1·81 brs (6H) 5·02 and 5·50 AB quartet (J = 10·5 Hz and ~ 1·5 as in 1). Mass spectrum: *m/e* 318 (M^+), calcd. for $C_{20}H_{30}O_3$: 318.

Hydrogenation of 1 *to* 3. The hydrogenation was carried out as in the case of 2 but for 6–8 h m.p. 98°–99° (light petroleum–acetone), α_D^{25} + 99° (*c*, 0·4, MeOH), γ_{max}^{KBr} 2920, 2860, 1750, 1675 (w), 1460, 1440, 1090, 1040 cm^{-1}; NMR (δ): 1·02 d (J = 6·5 Hz, 3H); 0·95 d (J = 6·5 Hz, 3H), 1·20 s (3H), 1·75 brs (3H), 2·65 (1H), 4·78 m (1H). Mass spectrum: *m/e* 320 (M^+), cald. for $C_{20}H_{32}O_3$: 320.

Epoxidation of 1 *to* 4. Compound 1 (100 mg) in CH_2Cl_2 (5 ml) was left overnight at 5° with *m*-chloroperbenzoic acid (100 mg). The product which was obtained following work-up was crystallized twice from light petroleum, m.p. 137°–139°, α_D^{25} + 152° (*c*, 1·5 MeOH) ν_{max}^{KBr} 2900, 2840, 1750, 1675, 1450, 1390, 1300, 1275, 1245, 1090, 1055, 1005, 990, 830, 760 cm^{-1}; NMR (δ): 1·22 s (3H), 1·27 s (3H), 1·77 brs (3H), 1·84 brs (3H), 2·55 m (2H), 4·95 and 5·32 AB quartet (J = 10 Hz and ~ 1 Hz as in 1). The minor epoxide isomer gives rise to the singlet at δ1·15 (~ 20% in the crude product).

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