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CEMBRENOLIDES OF A SOFT CORAL Lobophytum sp.
(COELENTERATA, OCTOCORALLIA, ALCYONACEA)

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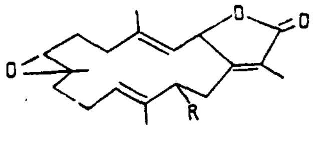
The known cembrenolide sarcophin and its new acetoxy derivative – 13-acetoxy-7,8-epoxycembra-1(15),3,11-trien-2,16-olide – have been isolated from the soft coral Lobophytum sp.. The compounds isolated are inhibitors of the activity of Na^+K^+-ATPase and are membranotropic agents. The structures of the compounds have been shown on the basis of the results of NMR spectroscopy.

Alcyonaria, or soft corals, are sources of terpenoids exhibiting various activities [1]. The presence of cembrene diterpenes is characteristic for three genera of Alcyonaria – Sarcophyton, Lobophytum, and Sinularia [1-3].

We have studied the composition of the diterpene fraction of the soft coral Lobophytum sp.. The animals were collected in the offshore waters of the republic of Madagascar during tropical expeditions of the Scientific-Research Vessel Professor Bogarov. In preliminary investigations, on aqueous alcoholic extract of the coral showed cytotoxic activity in relation to Ehrlich tumor cells. From a hexane extract of the lyophilized coral we isolated two compounds which, from the results of mass, IR, and ^1H and ^{13}C NMR spectroscopies were assigned to the cembrenolides.



I. R = H_2
II. R = H, OAc

The first compound (yield 0.01% on the weight of the freeze-dried coral) corresponded in its physicochemical characteristics to the known cembrenolide sarcophin (I) which has been isolated previously from the Red Sea coral Sarcophyton glaucum [2].

The second substance, more polar than sarcophin (yield 0.015% on the weight of the lyophilized animal) contained an acetoxy group, the presence of which was confirmed by a three-

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TABLE 1. Chemical Shifts and Spin-Spin Coupling Constants (Hz, shown in parentheses) for the Signals in the PMR Spectra of Compounds (I) and (II)*

Compound	CH_3-17	CH_3-18	CH_3-19	CH_3-20	H-2	H-3	H-7	H-11	H-13	H-14	CH_3COO
I†	1,86 3H d (1,3)	1,90 3H d (1,3)	1,29 3H s	1,62 3H s	5,58 1H (dq 10; 1,3)	5,04 1H dq (10; 1,3)	2,08 1H t (4)	5,14 1H m			
II	1,78 3H t (1,5)	1,94 3H d (1,2)	1,23 3H s	1,60 3H br.s.	5,72 1H dq (10; 1,5)	4,97 1H dq (10; 1,2)	2,46 1H t (3,8)	5,49 1H ddq (10,6; 5,2; 1,5)	5,24 1H ddq (10.2; 1,3)	3,01 1H dd (10,2; 15)	1,95 3H s

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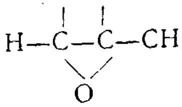
*The assignments were made on the basis of a homonuclear double resonance experiment (abbreviations: s) singlet; d) doublet; t) triplet; m) multiplet; dq) doublet of quartets; ddq) doublet of double of quartets; br.s) broad singlet.

†The assignments were made on the basis of a comparison with literature information [2].

proton singlet at 1.95 ppm in the ^1H spectrum. The ^{13}C NMR spectrum of the substance contained the signals of an acetoxy grouping at 20.8 and 169.9 ppm. The mass spectrum of the compound obtained also showed the presence of an acetoxy fragment in the molecule. This diterpenoid contained the functional groups characteristic for sarcophin:

1) An epoxy ring (signals of carbon at 61.4 and 59.3 ppm in the ^{13}C NMR spectrum; methyl singlet shifted downfield 1.23 ppm) and the triplet of the epoxide proton in a fragment at 2.46 ppm in the ^1H NMR spectrum; and



$H{-}C{-}C{-}CH_3$ (epoxide, O bridging the two C)

2) An α-methyl-β-butenolide grouping (signals of quaternary carbons at 173.5 and 134.1 ppm; quartets of the methyl group of a lactone at 8.75 ppm, and a doublet of a carbon present in the α-position to oxygen at 78.9 ppm in the ^{13}C spectrum).

Other indices of the ^1H and ^{13}C NMR spectra, which are given in Table 1, showed that (II) was an acetoxy derivative of sarcophin.

The results obtained did not, however, permit a determination of the positions of the functional groups in the diterpene under study. Establishment of the complete structure without a determination of the absolute configuration of the molecule.of the substance was made on the basis of the results of homonuclear double resonance. The spin-spin coupling between the protons resonating at 5.24 and 3.01 ppm (J = 10.2 Hz) revealed by these experiments permitted the conclusion that the signal of one of them related to a proton in the geminal position to an acetoxy group. The value of the spin-spin coupling constants (SSCC) is characteristic for the trans arrangement of the protons under discussion.

In homonuclear double resonance experiments with irradiation of the proton resonating at 3.10 ppm and the protons of the C-17 methyl group (1.78 ppm) a spin-spin connection of these protons with the proton having a signal at 2.12 ppm was detected. The value of one of the SSCCs (15 Hz) was characteristic for the geminal constant of the protons of a methylene group in the alllyl position. The existence of a spin-spin link with the C-17 methyl group determined the position of the above-mentioned methylene group and also the site of the acetoxy group at C-13.

Thus, the structure of the cembrenolide isolated can be illustrated as (I) (13-acetoxysarcophin).

It is known that sarcophin inhibits the activity of acetylcholinesterase and is ichthyotoxic in relation to the fish Gambusia affinis [2]. We have shown that sarcophin and acetyoxysarcophin in a concentration of 10^{-4} M causes 100% inhibition of the activity of Na^+K^+-ATPase isolated from rat brain, and in their action on rabbit erythrocytes both (I) and (II) cause partial hemolysis and induce the outflow of K^+ ions.

EXPERIMENTAL

The melting points were determined on a Boëtius stage. ^1H and ^{13}C NMR spectra were taken on Bruker HX-90E (90 MHz for ^{13}C) and Bruker 250-WH (250 MHz for ^1H) instruments for solutions in deuterochloroform (TMS, δ scale). IR spectra were obtained for solutions in chloroform on a Specord IR-75 instrument. Specific optical rotations were measured on a Perkin-Elmer 141 polarimeter. Mass spectra were obtained on a LKB-9000 chromato-mass spectrometer using direct introduction and an ionizing energy of 70 eV.

For chromatography we used type L silica gel (Czechoslovakia) with a grain size of 0.200-0.270 mm.

Isolation of the Cembrenolides from Lobophytum sp.. The soft coral Lobophytum sp. was collected during the expedition of the Scientific-Research Vessel Professor Bogarov in March, 1986, in Madagascar and was lyophilized there. The lyophilisate (0.55 kg) was extracted with hexane (3 × 0.5 liter) in a Soxhlet apparatus. The hexane extract (6 g) was chromatographed on silica gel in the hexane–methyl acetate (6:1) system. The fractions containing sarcophin (I) and compound (II) were rechromatographed in the same solvent system. As a result, sarcophin (I) (0.055 g; 0.01% on the weight of the lyophizate) was obtained in the form of acicular crystals; mp 133-133.5°C $[\alpha]_D^{20}$ + 93° (c 0.05; chloroform); according to the literature: mp 133-134°C; $[\alpha]_D$ + 92° [2]. Mass spectrum, m/z: 316 (M^+). IR spectrum: $\lambda_{max}^{CHCl_3}$ 1746 cm^{-1} (C=O). For the ^1H NMR spectrum ($CDCl_3$; 250 MHz), see Table 1.

The second compound, (II) (0.08 g; 0.014% yield on the weight of the dry raw material), was obtained in the form of tabular crystals with mp 131.5-132°C, $[\alpha]_D^{20}$ + 58° (c 0.1; chloroform). Mass spectrum, m/z: 374 (M^+), 331 ($M^+ - CH_3COO$). IR spectrum: $\lambda_{max}^{CHCl_3}$ 1740 cm^{-1} (C=O). For the ^{1}H NMR spectrum, see Table 1. ^{13}C NMR spectrum ($CDCl_3$; 90 MHz; abbreviations: s — singlet; d — doublet; t — triplet; q — quartet): 158.3 s (C-1); 78.9 d (C-2); 128.9 d (C-3); 144.8 s (C-4); 37.6 t (C-5); 25.2 t (C-6); 61.4 d (C-7); 59.3 s (C-8); 39.4 t (C-9); 23.5 t (C-10); 120.9 d (C-11); 124.9 s (C-12); 76.9 d (C-13); 33.5 t (C-14); 134.1 s (C-15); 173.5 s (C-16); 87.5 q (C-17), 16.2 q (C-18); 10.1 q (C-19); 16.7 q (C-20); 20.8 q and 169.9 s (CH_3COO—). The assignments were made by comparing the spectrum of the compound with information in the literature.

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