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NEW STERPURANE SESQUITERPENOID FROM THE MEDITERRANEAN *ALCYONUM ACAULE*: STRUCTURE OF 3-ACETOXY-STERPURENE

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Abstract.- A new sesquiterpenoid, 3-acetoxy-sterpurene (1), has been isolated from the alcyonacean (Octocorallia) *Alcyonum acaule* and characterized mainly by spectral analysis. 1 is the first sterpurane sesquiterpenoid from marine organisms.

Tropical alcyonaceans have been object of numerous studies aimed to establish their structure[1], to know their ecological role[2] and to value their pharmacological potentialities[1]. On contrary, the studies on Mediterranean species are until now limited to *Alcyonum palmatum*[3] and to *Alcyonum coralloides*[4-6]. This is due to the fact that the distribution of alcyonaceans is primarly in tropical seas while in more temperate water they are uncommon.

In this paper we report the results obtained studying the Mediterranean *Alcyonum acaule* (Kükental).

Alcyonum acaule was collected in May 1986 near Alghero (Sardinia, Italy) at a depth of 40 meters. The alcyonarians were frozen at -20°C and subsequently soaked in acetone. The diethyl ether soluble fraction from the acetone extract was fractionated by a series of chromatographic steps which led to 3-acetoxy-sterpurene (1): colourless oil; $[\alpha]_D$ = +128.6° (c = 2.2, $CHCl_3$); molecular formula $C_{17}H_{26}O_2$ (based on high resolution mass measurement of the parent ion).

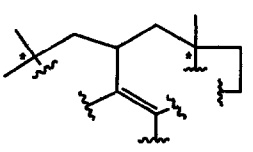
The elemental composition along with other spectral evidence suggested for 1 a tricyclic sesquiterpenoid skeleton containing a tetrasubstituted double bond (^{13}C-NMR, δ 123.55 and 140.75) and exhibiting an acetoxy substituent (ν_{max} 1740 cm^{-1}; ^{1}H-NMR, δ 2.00, 3H s; ^{13}C-NMR δ 169.58 and 21.07). The analysis of the ^{1}H-NMR spectrum (Table 1) placed immediately on the double bond three of the four substituents: a vinylic methyl (δ 1.46), an isolated methylene (δ 2.12), a methine (δ 2.62) linked to two methylenes (δ 1.65 and 1.24; δ 1.48 and 1.36). The ^{1}H-NMR spectrum was completed by an isolated $-CH_2-CH_2-$ system (δ 2.37, 1.94, 1.65 and 1.29) and by three methyl singlets (δ 1.06, 1.08 and 1.18). Further data were obtained by the analysis of the 2D-experiments (Table 1). In particular, the ^{1}H-^{13}C long range correlation (J =6Hz) led to the partial structure **a**.

Bearing in mind that we know only three of the four substituents on the double bond, the fourth one has to be the quaternary carbon (^{13}C-NMR, δ 81.14) linked to the acetoxy group. This assignment was confirmed

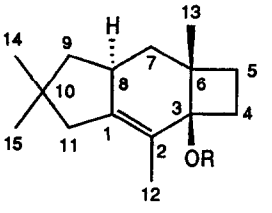
TABLE 1 - ^{1}H and ^{13}C-NMR data for 3-acetoxy-sterpurene (1)[a]

C	δ ^{13}C	m[b]	δ ^{1}H at C[c] m (J in Hz)	δ ^{1}H correlated to C[d]
1	123.55	s	-	1.48 (H-7)
2	140.75	s	-	-
3	81.14	s	-	-
4	31.86	t	2.37 ddd (10.7, 10.0,10.0) 1.94 ddd (10.7, 10.0, 2.8)	-
5	22.41	t	1.65 ddd (10.0, 10.0, 10.0) 1.29 ddd (10.0, 10.0, 2.8)	-
6	44.35	s	-	1.18 (H-13's), 1.36 (H-7) 1.65 (H-5), 1.94 (H-4)
7	34.69	t	1.36 dd (11.7, 11.7) 1.48 dd (6.3, 11.7)	1.18 (H-13's), 1.65 (H-9) and/or (H-5)
8	37.49	d	2.62 br m	1.24 (H-9), 1.36 (H-7) 1.48 (H-7), 2.12 (H-11)
9	47.77	t	1.24 dd (12.7, 12.7) 1.65 dd (12.7, 8.4)	1.06 (H-14's),1.08 (H-15's)
10	36.84	s	-	1.06 (H-14's),1.08 (H-15's)
11	44.64	t	2.12 AB q (14.3)	-
12	12.82	q	1.46 br s	-
13	23.47	q	1.18 s	1.29 (H-5), 1.65 (H-5)
14	29.51[e]	q	1.06[f] s	-
15	30.11[e]	q	1.08[f] s	-
$\underline{C}OCH_3$	169.58	s	-	2.00 ($COC\underline{H}_3$)
$CO\underline{C}H_3$	21.07	q	2.00 s	-

[a] Measured in $CDCl_3$ solution with TMS as internal standard.
[b] By DEPT sequence.
[c] The assignments were aided by homonuclear and heteronuclear 2D experiments.
[d] By long-range HETCOR experiment (J=6Hz)
[e,f] Values with the same superscript could be inverted.



a



1 R = $COCH_3$
2 R = H

TABLE 2 - ^{1}H and ^{13}C-NMR data for 3-hydroxy-sterpurene (2)[a]

C	$\delta\,^{13}C$	m	$\delta\,^{1}H$ at C m (J in Hz)	$\Delta\delta\,^{1}H$[b]
1	126.48	s	-	
2	141.04	s	-	
3	73.50	s	-	
4	35.53	t	2.15 ddd (11.1, 11.1, 11.1) 2.00 ddd (11.1, 10.9, 2.2)	0.73 0.63
5	22.15	t	1.55 ddd (11.1, 10.9, 11.1) 1.20 ddd (11.1, 11.1, 2.2)	
6	43.99	s	-	
7	34.58	t	0.85 dd (11.3, 12.9) 1.55 dd (12.9, 6.3)	0.70
8	37.45	d	2.54 m	0.30
9	48.50	t	1.65 dd (11.6, 6.8) 1.10 dd (11.6, 11.6)	
10	36.77	s	-	
11	45.00	t	2.12 br s	0.23
12	12.66	q	1.63 br s	0.63
13	23.46	q	1.20 s	0.67
14	29.60[c]	q	1.06[d] s	0.14
15	30.45[c]	q	1.08[d] s	0.12

[a] Measured in $CDCl_3$ solution with TMS as internal standard.
[b] After addition of 0.1 moles of $Eu(fod)_3$ per mole of 2.
[c,d] Values with the same superscript could be inverted.

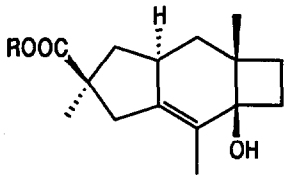
by the obtainment, by thermic treatment of 1 adsorbed on silica gel powder, of an unstable conjugated diene derivative (UV λ_{max} (CH_3OH) 259 nm; ^{1}H-NMR δ 5.23, 1H). On the basis of the reported evidence many possible structures could be proposed, one of these obtained linking wrongly the two starred carbons of a has been previously tentatively suggested [7].

However, the analysis of the NMR spectra (table 2) of the alcohol 2, obtained by treatment of 1 with $LiAlH_4$, allowed to link immediately C-3 to C-4. In fact, the ^{13}C-NMR value of C-4 was significantly downshifted after deacetylation ($\Delta\delta$ = +3.67). Furthermore, the ^{1}H-NMR spectra of 2, in presence of the deuterated lanthanide shift reagent $Eu(fod)_3$ (Table 2), revealed strong induced shifts for the protons of both the methyl at C-6 and the vinylic methyl, allowing to place the quaternary C-3 between C-2 and C-6 and the vinylic methyl at C-2. A *cis*-relationship of the C-3 OH to the C-6 methyl was also evident.

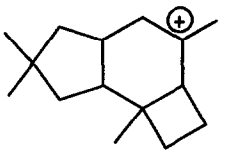
The structure of 1, completed linking C-11 to C-10, revealed a sterpurane skeleton.

Comparison with the spectral data[8] of sterpuric acid (3) and its methyl derivative (4) confirmed the suggested structure. In fact, the typical mass fragment due to the loss of ethylene by cleavage of the

cyclobutane ring was relevant also in the mass spectra of **1** and **2**, while the ^{1}H-NMR resonance values of **3** and **4** were highly similar to those of **2**. In particular, chemical shift and coupling pattern of the axial H-8 in **2** ($\delta \approx 2.54$) allowed to assign to this proton the same relative stereochemistry exhibited in **3**. This datum was confirmed by the absence of NOE between CH_3-6 and H-8 (NOESY and NOE difference experiments).



3 R = H
4 R = CH_3



5

Sesquiterpenoid with sterpurane skeleton have been found only recently[8-10]. Their presence in nature is limited to the fungus *Stereum purpureum* and they are considered the causative agents of the so called silver leaf disease, common to plum, apple and other fruit trees. Sterpurane sesquiterpenoid, have been the subject of many biosynthetic[10-12] and synthetic[13-17] studies. The relevant interest for this class of natural products is due to its unusual biosynthesis from farnesyl pyrophosphate via humulene and protoilludyl cation (**5**).

It is relevant to underline that this is the first report of a sterpurane-sesquiterpenoid from a marine organism.

EXPERIMENTAL

UV spectra were obtained on a Varian DMS 90 spectrophotometer. IR spectra were recorded on a Perkin-Elmer Model 257 Infracord spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter, using a 10 cm microcell. Low-resolution and high-resolution mass spectra were recorded on an AEI MS-30 and on an AEI MS-50 spectrometers, respectively. ^{1}H and ^{13}C-NMR spectra were recorded at 500 and 125 MHz respectively on a Bruker WM 500 instrument, under Aspect-2000 control. The 2D-NMR experiments were obtained using Bruker's microprograms. Silica gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. HPLC purifications were carried out on a Water apparatus equipped with μ-Porasil column (7.8 mm i. d. x 30 cm) and with UV and RI detectors.

Extraction of *A. acaule* and isolation of 3-acetoxy-sterpurene (1)

The *A. acaule* (Kükental) (860 g, dry weight after extraction), collected in May 1986 at Alghero (Sardinia) at a depth of 40 meters, was frozen at -20°C until extraction with acetone. After elimination of the solvent *in vacuo*, the aqueous residue was extracted with diethyl ether. The ether extract was evaporated *in vacuo* to obtain an oil (8 g), which was fractionated on Si gel column by step gradient elution from light petroleum 40-70° to diethyl ether. The fractions, showing an Ehrlich positive compound (Rf 0.6 on TLC,

light petroleum : diethyl ether, 95:5) were subjected to preparative HPLC (light petroleum : diethyl ether, 96:4; flow rate 4 ml/min) yielding 1 (70 mg).

3-acetoxy-sterpurene (1)

$[\alpha]_D$ = +128.6° (c = 2.2, $CHCl_3$); IR ν_{max} (liquid film) 1740, 1370, 1250, 1230 cm^{-1}; EIMS m/z (%): 262.1939 (12) [M^+ calc. for $C_{17}H_{26}O_2$, 262.1933], 234 (30), 220 (95), 205 (15), 192 (100), 177 (26); ^1H and ^{13}C NMR (Table 1).

Thermolysis of 1

Compound 1 (10 mg) was dissolved in diethyl ether and adsorbed on silica gel (20 mg). The mixture was heated at 100° C for 3 min; after cooling, the silica gel was eluted with n-hexane to give an unstable conjugated derivative (2 mg). UV λ_{max} (CH_3OH) 259 nm; ^1H-NMR δ ($CDCl_3$): 5.23 (1H, br s), 2.86 (1H, m), 2,50 (1H, m), 1.64 (3H, d, J = 1.3), 1.28 (3H, s), 1.11 (3H, s), 1.03 (3H, s).

Reduction of 1 with $LiAlH_4$

3-acetoxy-sterpurene (1) (20 mg) was treated with $LiAlH_4$ (30 mg) in anhydrous Et_2O (5 ml) the mixture was stirred at room temperature for 30 min, then 10 ml of H_2O were added. The aqueous solution was extracted with Et_2O. The ether extract was purified on silica-gel column equilibrated with light petroleum: diethyl ether (4:1) yielding 2 (18 mg): $[\alpha]_D$ = +61.0° (c = 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3300 cm^{-1}; EIMS m/z (%): 220.1819 (30) [M^+, calc. for $C_{15}H_{24}O$, 220.1827], 205 (15), 192 (100), 177 (25); ^1H and ^{13}C NMR (Table 2).

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