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LIPID CONSTITUENTS OF THE RED ALGA *ACANTOPHORA SPICIFERA*

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Key Word Index—*Ancantophora spicifera*; Rhodomelaceae; Cholest-4-ene-3α, 6β-diol; cholest-4-en-3-one; *o*-pthalic acid bis-(2 ethyl nonyl)-ester; lauric acid.

Abstract—A new steroid cholest-4-ene-3α, 6β-diol together with the known cholest-4-ene-3-one, lauric acid and *o*-phthalic acid bis-(2-ethyl nonyl)-ester were isolated from the red alga *Acantophora spicifera*. The structures of these compounds were established on the basis of their spectral data.

INTRODUCTION

Acantophora spicifera is a red alga belonging to family Rhodomelaceae. The methanolic extract of this seaweed exhibited antifertility activity which on fractionation located the activity in the petroleum ether and chloroform soluble fractions. The crude extract also exhibited antiviral activity against SFV (*Semliki Forest Virus*) [1].

This red alga is also reported to possess *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Candida albicans* and *Mycobacterium smegmatis* [2].

In our earlier communication we have reported the isolation of a novel steroid 11α-hydroxycholestan-3,6 dione [3] and the natural occurrence of 5α-cholestane 3,6 dione [4] besides known steroids and fatty acids [5]. A new dipeptide [6] diaurantiamide acetate has also been reported from this source. Herein we wish to report another *novel* steroid cholest-4-ene-3α, 6β-diol (**4**), cholest-4-ene-3-one (**1**), lauric acid or dodecanoic acid (**3**) and *o*-phthalic acid bis-(2 ethyl nonyl)-ester (**2**).

RESULTS AND DISCUSSIONS

Compound **1** had ^{1}H NMR ($CDCl_3$) spectrum characteristic of steroids. Its mass spectrum with M^+ at *m/z* 384 and prominent peaks at *m/z* 124 (B-ring cleavage), *m/z* 342 (M^+-42) loss of ketene and *m/z* 229 (M^+-42-SC) indicated the presence of Δ^4-3-ketone moiety. This was further supported by the presence of signals at δ 198(C-3), 170(C-5) and 123.64(C-4) in its ^{13}C NMR spectrum as well as absorption at 1728(C=O), 1620 and 1273(=CH−)cm^{-1} in its IR spectrum. This established the structure of **1** as cholest-4-en-3-one.

Compound **2** obtained as a viscous oil exhibited a ^{1}H NMR spectrum typical of a phthalate ester with a signal for four aromatic protons at δ 7.6, the signal for four methyl groups appeared at δ 0.9 as a triplet. The existence of an aliphatic side chain was evident from the absorption in the region δ 1.0–1.8 which integrated for 30 protons. The ester methylene protons (4H) appeared at δ 4.16 as a doublet (J=6 Hz). Its mass spectrum exhibited a molecular ion peak at M^+ 474 besides the characteristic base peak at *m/z* 149 and the fragment at *m/z* 167 typical of phthalate ion [7] further confirming it to be a phthalate ester. It was identified as *o*-phthalic acid bis-(2-ethyl nonyl)-ester.

Compound **3** was obtained as a colourless solid, m.p. 46°C and the formula of $C_{12}H_{24}O_2$ was assigned on the basis of its mass spectrum which showed peaks at *m/z* 183 (M^+-17) and *m/z* 155 (M^+-45) with a weak molecular ion peak at *m/z* 200. The ^{1}H NMR spectrum 200 MHz ($CDCl_3$) showed a triplet at δ 0.9 (3H, $-CH_3$ group of aliphatic chain), a multiplet centered at δ 1.3 (20H, 10 $-CH_2$ groups of the aliphatic chain), a quintet centered at δ 1.62 (2H, protons β to the acid group) and a triplet at δ 2.35 was assigned to the two protons α to the carboxyl group. The presence of a carboxyl group was further evidenced by the presence of a signal δ at 179.7 in its ^{13}C NMR spectrum. The signals for methyl and methylene groups were observed at δ 14.06, 21.7, 22.6, 24.7, 29.00, 29.24, 29.35, 29.42, 29.66, 31.93 and 34.03, respectively. On the basis of the above spectral data the compound was identified as dodecanoic acid or lauric acid.

Compound **4** was isolated as colourless crystals

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Table 1. ^{13}C NMR spectral data of compound **4** (50 MHz, $CDCl_3$, TMS)

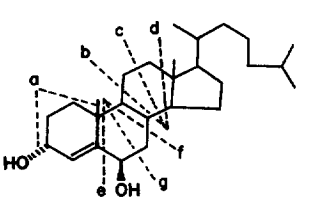
C	δ	C	δ
1	38.53	15	22.94
2	27.72	16	39.16
3	66.5	17	55.75
4	127.63	18	11.9
5	147.6	19	21.13
6	73.12	20	35.36
7	27.26	21	17.42
8	35.01	22	36.24
9	53.84	23	23.24
10	36.9	24	38.68
11	21.36	25	27.1
12	29.56	26	21.13
13	42.4	27	21.36
14	55.55		

Table 2. ^{1}H NMR spectral data of compound **4** (200 MHz, $CDCl_3$, TMS)

H	δ (J/Hz)
4	5.5 (1H,*bs*)
18	0.73 (3H,*s*)
19	1.23 (3H,*s*)
21	0.92 (*d*,$J=6$ Hz)
26/27	0.85 (H,*d*,$J=6.6$ Hz)
26/27	0.87 (3H,*d*,$J=6.6$ Hz)

m.p. 248°C (reported 253–256°C) [8]. Its ^{1}H NMR (Table 2) spectrum displayed an ethylenic proton at δ 5.5 (*br s*, H-4) and an isopropyl group at δ0.85(3H,*d*,$J=6.6$Hz,$H_3^{-26/27}$) and δ0.87(3H,*d*,$J=6.6$Hz, $H_3^{-26/27}$). Also present were singlets at δ 0.73 and 1.23 corresponding to Me-18 and Me-19 respectively. A doublet at δ 0.92($J=6$ Hz) was assigned to Me-21 protons. A two proton signal at δ 4.18 (2H overlapping narrow multiplet) was attributed to methine protons at C-3 (3β-H) and C-6 (6α-H), in agreement with this C-3 and C-6 signals were observed at δ 66.5 and δ 73.12 respectively (Table 1). The nature of the signal at δ 4.18 suggested that the two protons are equatorial with the β-orientation for the hydroxyl group at C-6 and α-orientation for the hydroxyl group at C-3 in the new sterol. The downfield shift (δ 4.18) of carbinol protons suggested the presence of a double bond in the vicinity of carbons carrying hydroxyl groups. The presence of two —OH groups in the molecule was also evident from two successive losses of 18 mass units from the molecular ion peak at *m/z* 402 in its mass spectrum (fragments *m/z* 384 (base peak) and *m/z* 366). The location of one of the hydroxyl group at C-3 was evident from the presence in the mass spectrum of the fragment at *m/z* 369 which involves elimination of water subsequent to the loss of angular methyl group [9, 10]. The second hydroxyl group was placed at C-6 on the basis of a carbinol methine chemical shift and the deshielded Me-19 signal in the ^{1}H NMR spectrum as well as a ^{13}C NMR signal at δ 21.36. This was further reinforced by the isolation of 3,6 diones [3, 4] from the same source and hence the double bond was placed at C-4. On the basis of the above spectral evidence the structure of this compound **4** was established as cholest-4-ene-3α, 6β-diol, and the genesis of the various fragments in its mass spectrum could be explained as shown in Scheme 1. Cholest-4-ene-3α, 6β diol has been reported in the literature as a synthetic product [8] but it is being reported here for the first time as a natural compound.

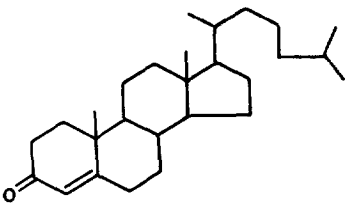
Pollution of seawater and marine animal tissues by



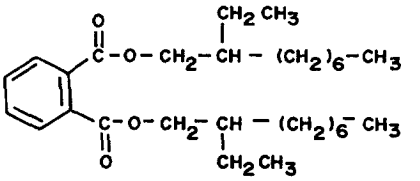
4

m/z	Probable Structure
402	M^+
384	$M^+ - H_2O$
369	a $[M^+ - (H_2O + CH_3)]$
366	$M^+ - 2H_2O$
351	$M^+ - (2H_2O + CH_3)$
287	$M^+ - (SC + 2H)$
271	$M^+ - (SC + H_2O)$
253	$M^+ - SC + 2H_2O$
246	$M^+ - [SC + CH_3 + C_2H_3(\text{ring D})]$
229	$247^+ - H_2O$
211	$229^+ - H_2O$
109	b
95	c
81	d
55	e
135	f
149	g

Scheme 1.



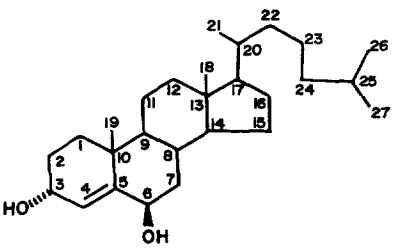
1



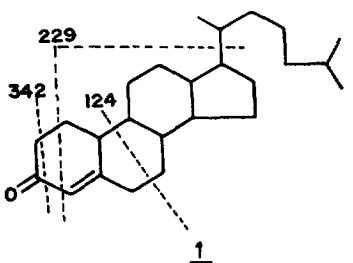
2

$H_3C - (CH_2)_{10} - COOH$

3



4



1

phthalate esters in the *ortho*-form is well known [11, 12] but there is a solitary reference on the pollution of marine alga *Stoechospermum marginatum* [13] by the esters. Ortho-phthalic esters are being reported here for the first time as pollutants of marine red alga *Acantophora spicifera*. The presence of *para*-esters, dimethyl terephthalate in marine red alga *Phyllophora nervosa*, *Acantophora delilei* and *Hypnea musciformis* has been reported by Kasim *et al* [14].

EXPERIMENTAL

Mps: uncorr; IR: KBr discs; ^1H NMR (200 MHz) and ^{13}C NMR (50 MHz) $CDCl_3$ with TMS as int. standard; EIMS: 70 ev; TLC: silica gel G, EtOAc-:Petrol(20:80) and spots visualized by exposure to I_2 vapours.

Collection, extraction and isolation

Acantophora spicifera (7 Kg) was collected at low tides during premonsoon periods, air-dried and extracted 3× with $CHCl_3$. The combined extracts were evaporated under reduced pressure to give a residue (130 g, 1.9% dry wt.) which was chromatographed over silica gel and eluted with mixtures of increasing polarity of petrol-EtOAc. Elution gave four compounds in the order designated as **1 2, 3** and **4**. All these compounds were purified by repeated chromatography over silica gel.

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