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UMBRACULUMIN-B, AN UNUSUAL 3-HYDROXYBUTYRIC ACID ESTER FROM THE OPISTHOBRANCH MOLLUSC *UMBRACULUM MEDITERRANEUM*

G. Cimino°, A. Spinella°*, A. Scopa^ and G. Sodano^*

°Istituto per la Chimica di Molecole di Interesse Biologico,CNR,via Toiano 6, 80072 Arco Felice (NA), Italy

^Istituto di Chimica, Università della Basilicata, via N. Sauro 85, 85100 Potenza, Italy

Abstract: The structure and stereochemistry of Umbraculumin-B, a metabolite isolated from the skin extracts of the opisthobranch mollusc *Umbraculum mediterraneum*, have been established by spectroscopic and chemical methods.

We have recently reported[1] the isolation and structure of two ichthyotoxic diacylglycerols, umbraculumin-A and -C, which could account for the chemical defense of the opisthobranch mollusc *Umbraculum mediterraneum*. We whish to report now the structure of a third unrelated compound, named umbraculumin-B (**1**), accompanying the above compounds in the skin extracts of *U. mediterraneum* but inactive in the mosquito fish bioassay[2].

Fractions containing umbraculumin-B were obtained from the SiO_2 column chromatography also affording the other umbraculumins and were further purified by preparative TLC (benzene-diethyl ether; 7:3) where **1** is slightly more polar than umbraculumin-C; 4 mg of **1** were obtained from the skin extracts of the single specimen also used[1] for the isolation of umbraculumin-A and -C.

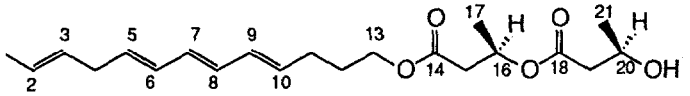
HREIMS showed that umbraculumin-B possesses the same molecular formula $C_{21}H_{32}O_5$ as umbraculumin-A (found M^+ m/z 364.2228, calculated 364.2241) and was accompanied by a small amount of a compound m/z 366, formulated as the 2,3-dihydroderivative of **1** by careful integration of the appropriate ^{1}H-NMR signal (δ 5.45). The presence of four double bonds in **1** was inferred by a combination of UV and NMR data. The UV spectrum (*n*-hexane) has the characteristic shape of a conjugated triene chromophore with maxima at 279 (ε 28,000), 268 (ε 36,000) and 259 (ε 26,000) nm. The NMR proton signals for the central part of the triene moiety (from C-6 to C-9) were overlapped, even at 500 MHz, giving rise to a complex signal between 6.03 and 6.12 δ, while H-5 and H-10 were sufficiently distinct (δ 5.65 and 5.60, respectively) to allow assignments by decoupling experiments on irradiation of the appropriate adiacent methylene protons (see table). The large *J* values for the *H-5/H-6* and *H-10/H-9* couplings secured the *E* geometry for these double bonds. Although the ^{13}C-NMR spectrum did not allow the assignment of the individual resonances from C-6 to C-9, the chemical shift values of these carbons (130.9; 131.5) indicated that the 7,8 double bond should have the *E* geometry, since consistently lower values are expected in a triene in which the central double bond has a *Z* geometry[3].

H-2 and H-3 were also overlapped at δ 5.45 and coupled to the vinyl methyl at δ 1.65; this moiety was previously found also in umbraculumin-A and -C[1] and , similarly, the *E* geometry of the 2,3 double bond has been derived from the ^{13}C chemical shift of the vinyl methyl[4]. Since irradiation of the C-4 methylene affects both the C-1,C-5 and the C-2,C-3 protons, the proton sequence from C-1 to C-10 was fully established; additional decoupling experiments also gave the C-10/C-13 proton sequence. The ^{1}H-^{1}H COSY spectrum confirmed the above results.

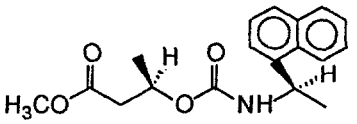
TABLE. NMR data of umbraculumin-B (1)[a].

Position	δ ^{1}H (multiplicity; J, Hz)	δ ^{13}C
1	1.65 (m)	18.0
2	5.45 (m)	126.4[b]
3		128.9[b]
4	2.78 (m)	35.8
5	5.65 (m;$J_{5\text{-}6}$ 14.2)	133.0
6		
7	6.03-6.12 (m)	131.5 (2C)
8		130.9 (2C)
9		
10	5.60 (m;$J_{10\text{-}9}$ 15.0)	132.4
11	2.15 (q; 7.4)	29.2
12	1.72 (m)	28.4
13	4.10 (t; 6.6)	64.4
14		171.8[c]
15	2.60 (ddd;15.3, 7.8, 4.7)	40.8
16	5.35 (m)	67.7
17	1.33 (d; 5.9)	20.0
18		170.5[c]
19	2.47 (ddd;16.5, 8.6, 2.9)	43.5
20	4.20 (m)	64.4
21	1.20 (d; 6.1)	22.5

[a]$CDCl_3$; Bruker WM 500. [b,c]Values with identical superscripts may be interchanged.



1



2

The ^{13}C-NMR spectrum showed the presence of two ester carbonyls at δ 170.5 and 171.8 and of three sp^3 carbons linked to oxygen, one CH_2 (δ 64.4) and two CH (δ 64.4 and 67.7) by DEPT sequence. These functions were readily accomodated in two β-hydroxybutyric acid residues linked to each other,one of them esterifying the tridecatetraenol residue, by observing the C-15/C-17 and C-19/C-21 proton sequences both by ^{1}H-^{1}H COSY and by proton decoupling experiments. In particular the C-15 and C-19 methylenes resonated in the ^{1}H-NMR spectrum as well resolved AB parts (8 lines each) of two distinct ABX systems, the X parts (H-16 and H-20) being coupled to the two methyl doublets at δ 1.33 and 1.20 respectively. A ^{1}H-^{13}C HETCOR spectrum allowed the assignment of the individual ^{13}C resonances as reported in the table.

The absolute configuration at C-20 was determined to be *R* as follows. Umbraculumin-B (3 mg) dissolved in toluene (0.5 ml) was reacted (80°C; 16h) with an excess (20 µl) of (*R*)(-)-1-(1-naphtyl)-ethylisocyanate. After elimination of the solvent the crude product was dissolved in 0.5 ml of 1N H_2SO_4/CH_3OH and allowed to react (r.t.; 4 days). The solution was neutralized with solid $NaHCO_3$ and the product purified by preparative TLC (CH_2Cl_2/EtOAc, 95:5) to afford the uretane **2**. By using the above chiral isocyanate the 1-(1-naphtyl)ethyl uretanes of (*R*,*S*)- and (*S*)-3-hydroxybutyric acid methyl ester were prepared by conventional procedures. Their ^{1}H-NMR spectra ($CDCl_3$) differ mainly in the chemical shift value of the carboxymethyl group which resonates at δ 3.70 in the *R*,*R* diastereomer and δ 3.58 in the *R*,*S* diastereomer. The uretane **2** derived from umbraculumin-B displays a δ 3.70 value for the carboxymethyl group and hence the *R* stereochemistry was inferred for the 3-hydroxybutyric acyl residue. The stereochemistry at C-16 is also tentatively designed as *R* since it is obvious to expect that the two 3-hydroxybutyric acyl residues may have the same absolute stereochemistry.

Whereas umbraculumin-A and -C are very toxic to fish at 10 and 0.1 µg/ml respectively[1], umbraculumin-B is inactive at 10 µg/ml.

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