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TWO ICHTHYOTOXIC DIACYLGLYCEROLS FROM THE OPISTHOBRANCH MOLLUSC UMBRACULUM MEDITERRANEUM

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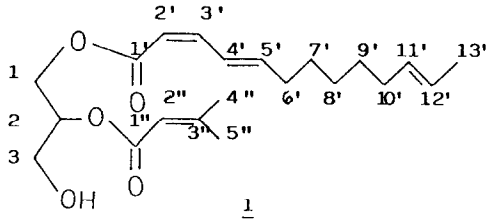
Abstract: Two ichthyotoxic diacylglycerols, umbraculumin-A (1) and -C (2), have been isolated from the skin of the opisthobranch mollusc Umbraculum mediterraneum and their structures determined by spectroscopic means.

In our continuing research on the defense allomones of nudibranchs[1] and other opisthobranch molluscs, we have found that the skin extracts of the rare opisthobranch Umbraculum mediterraneum Lamarck show marked ichthyotoxic activity in the mosquito fish bioassay[2]. Fractionation of the extracts has resulted in the isolation of two major bioactive compounds, umbraculumin-A and -C, whose structures are reported here.

A large specimen of U. mediterraneum (ca. 20 cm. in diameter) was deprived of the shell and dissected for removing the digestive gland and the skin was extracted with acetone. Removal of the solvent and extraction of the residual water with diethyl ether afforded a residue (700 mg) which was flash-chromatographed on a SiO_2 column (benzene-diethyl ether, 9:1) to give three fractions of medium polarity (A-C). Fraction A (72 mg) contained umbraculumin-A (1) and sterols which were precipitated by addition of CH_3CN; the solubles were purified by preparative TLC (n-hexane-ethyl acetate, 7:3) to afford 10 mg of umbraculumin-A (1) which proved very toxic[3] to the mosquito fish Gambusia affinis at 10 μg/ml.

Umbraculumin-A (1), $[\alpha]_D$ -24.3 (c 0.8, $CHCl_3$), has a molecular formula of $C_{21}H_{32}O_5$ which was established by HREIMS (found M^+ m/z 364.2233, calculated 364.2241). The presence of α,β-unsaturated ester moieties in 1 could be readily deduced from the IR spectrum (1710 cm^{-1}) coupled with the presence of two carbonyl carbons in the ^{13}C-NMR spectrum at δ 166.0 and 166.5. Examination of ^1H- and ^{13}C-NMR data (Table 1) and decoupling experiments pointed to a glycerol residue in which only one primary alcoholic function was free, the other two being esterified by α,β -unsaturated acyl residues.

The structure of the acyl residues was deduced by interpretation of a ^1H-^1H COSY spectrum in conjunction with decoupling experiments, leading to assignments of all proton signals as shown in Table 1. To start with, the isolated vinyl proton at δ 5.72 was long range coupled



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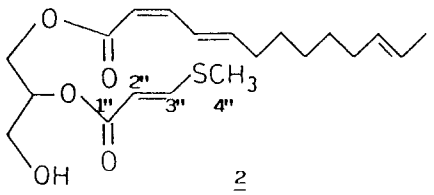
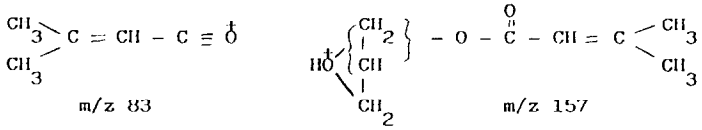


2

to both the methyl signals at δ 2.17 and 1.90, suggesting that one of the glycerol alcholic functions is esterified by 3,3 -dimethylacrylic acid (senecioic acid). The chemical shift values of the vinyl methyls were assigned on the observation that the δ 5.72 proton shows a positive enhancement in NOE difference experiments when the δ 1.90 methyl is irradiated and were found in agreement with the expected values[4]. The two most prominent fragment ions in the mass spectrum of 1 occurred at m/z 83 and 157 and were interpreted as reported below.



m/z 83 m/z 157

A maximum at 214 nm (ε 14,680) in the UV spectrum (hexane) of 1 was consistent[5] with the presence of the senecioic acid residue. The other maximum present in the UV spectrum occurred at 263 nm (ε 21,250) and was assigned[5] to an ester having two double bonds in conjugation. The ^{1}H-^{1}H COSY spectrum and decoupling experiments indicated the sequence of the four vinyl protons from C-2' to C-5' and the presence of five consecutive methylene units, the first one at δ 2.20 being linked to C-5' while the last one at δ 1.95 (C-10') being linked to a double bond in which both protons resonate at the same chemical shift value (δ 5.40). A methyl group (δ 1.64) was also linked to this double bond, showing couplings with the δ 5.40 protons and homoallylic coupling with the C-10' methylene. From these data it was inferred that the other acyl group had the gross structure of the hitherto unknown trideca-2,4,11-trienoic acid. The stereochemistry of the double bonds was deduced as follows. The E geometry of the 11',12' double bond was established in consideration of the ^{13}C chemical shift values of C-10' and C-13'[6]. The E geometry of the 4',5' double bond was established in consideration of the H-4'/H-5' J value (15.0 Hz) and observing a positive enhancement of the C-4' proton in a NOE difference spectrum when the C-6' methylene was irradiated. On the other hand, the H-2'/H-3' J value (11.3 Hz) suggested a Z geometry for the 2',3' double bond.

The position at which each acyl group was linked on the glycerol unit was established as follows. A DEPT sequence and a ^{1}H-^{13}C heterocorrelation allowed the assignment of the ^{13}C chemical shift values (Table 1) with the exception of the two carbonyl carbons. ^{1}H-^{13}C long range heterocorrelation spectra (Table 1) allowed the assignments to these carbons by the observation that the carbonyl carbon resonating at δ 166.5 was correlated with the vinyl proton at δ 6.58 and thus belongs to the tridecanoic acyl residue, while the carbonyl carbon at δ 166.0 correlated with the C-4" methyl protons at δ 2.17 disclosing its belongings to the senecioic acyl residue. In addition, the δ 166.5 carbonyl carbon showed a long range correla-

TABLE 1. NMR Data for Umbraculumin-A and -C (1 and 2)[a]

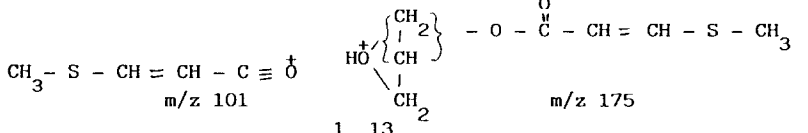
1				2		
δ ^{1}H (multiplicity, J Hz)	δ ^{13}C	δ ^{1}H correlated long range	C	δ ^{1}H (multiplicity, J Hz)	δ ^{13}C	δ ^{1}H correlated long range
4.34 (d, 5.0)	61.8 t[b]		1	4.36 (d, 5.0)	61.6 t[b]	
5.12 (p, 5.0)	71.7 d	4.34[d]	2	5.15 (p, 5.0)	72.4 d	
3.75 (m)	61.8 t		3	3.78 (m)	61.6 t	
	166.5 s	6.58[c,d], 4.34[c,d]	1'		166.5 s	6.59[c,d]
5.57 (d, 11.3)	114.6 d		2'	5.57 (d, 11.3)	114.4 d	
6.58 (t, 11.3)	146.4 d		3'	6.59 (t, 11.3)	146.9 d	
7.32 (dd, 15.0, 12.1)	127.0 d	5.57[d]	4'	7.32 (dd, 15.0, 12.1)	126.9 d	5.57[d]
6.09 (dt, 15.0, 7.0)	146.4 d	2.20[c]	5'	6.10 (dt, 15.0, 7.0)	146.8 d	
2.20 (m)	33.0 t		6'	2.20 (m)	33.0 t	
1.42 (m)	28.7 t		7'	1.45 (m)	28.6 t	
1.33 (m)	28.7 t[e]		8'	1.32 (m)	28.7 t[e]	
1.33 (m)	29.4 t[e]		9'	1.32 (m)	29.3 t[e]	
1.95 (m)	32.4 t		10'	1.96 (m)	32.4 t	
5.40 (m)	131.4 d	1.64[d]	11'	5.40 (m)	131.5 d	1.65[d]
5.40 (m)	124.7 d	1.64[d]	12'	5.40 (m)	124.8 d	
1.64 (m)	17.8 q		13'	1.65 (m)	17.8 q	
	166.0 s	2.17[c]	1"		164.8 s	7.81[c]
5.72 (m)	115.6 d	2.17[c,d], 1.90[c,d]	2"	5.69 (d, 14.9)	112.3 d	
	158.1 s	2.17[c,d], 1.90[c,d]	3"	7.81 (d, 14.9)	148.8 d	2.34[c]
1.90 (d, 1.2)	27.4 q		4"	2.34 (s)	14.3 q	
2.17 (d, 0.9)	20.3 q		5"			
2.34 (bs)			-OH	2.34		

[a]Spectra were recorded on Bruker WM-500 and WM-300 spectrometers in $CDCl_3$ solutions. Assignements were made by ^{1}H-^{1}H and ^{1}H-^{13}C COSY. [b]Multiplicity by DEPT sequence. [c]Correlations observed using a J value of 6 Hz in an experiment of ^{1}H-^{13}C COSY via long range couplings. [d]Correlations observed using a J value of 12 Hz. [e]May be interchanged.

tion with the glycerol's methylene resonating at δ 4.34, indicating that the tridecanoic acyl residue was linked to C-1 (no sterechemical numbering) of the glycerol and, by inference, that the senecioic acyl residue was linked at C-2.

Fraction B was inactive in the mosquito fish bioassay andwas set aside. Fraction C (13 mg) was further purified by preparative TLC (benzene-diethyl ether, 7:3) affording 8 mg of umbraculumin-C (2), $[\alpha]_D$ +7.0 (c 0.5, $CHCl_3$) which proved much more active than umbraculumin-A, being very toxic[3] to Gambusia affinis at 0.1 μg/ml.

The molecular formula of umbraculumin-C (2) was determined as $C_{20}H_{30}O_5S$ by HREIMS (found M^+ m/z 382.1774, calculated 382.1813). The NMR data (^1H-^1H COSY, DEPT, ^1H-^{13}C heterocorrelation; Table 1) readily established the presence in umbraculumin-C too of the cis-2,trans-4,trans-11--tridecatrienoic acyl residue linked to a glycerol unit. The remaining signals readily accomodated for the presence in 2 of a trans-3-(methylthio)-acrylic acyl residue. The ^1H- and ^{13}C-NMR data closely match those reported for other derivatives of trans-β-methylthioacrylic acid[7,8]. The UV spectrum displayed a single absorption at 267 nm (ε 35,470), due to the chromophores of both acyl residues, and finally the mass spectrum displayed the most prominent peaks at m/z 101 and 175 interpreted as reported below.



Unfortunately in the case of umbraculumin-C (2) ^1H-^{13}C long range heterocorrelation spectra failed in giving correlations between the carbonyl carbons and the protons of the glycerol unit. However the substitution pattern was deduced to be as depicted in 2 taking into account that in the ^{13}C-NMR spectrum of 2 the carbonyl carbon of the tridecanoic acyl residue exhibits exactly the same chemical shift value as in 1. Since it is known[9] that substitution at the primary vs. secondary alcoholic function of the glycerol moiety in glycerides results in a difference lying in about 0.4 ppm of the carbonyl carbon chemical shift value for a given acyl residue, it was deduced that the tridecanoic acyl residue is linked in umbraculumin-C too at the primary alcoholic function.

The umbraculumin-A and -C being toxic to fishes and being located on the skin should represent the deterrent of U. mediterrancum against predators. It is noteworthy that we have recently isolated diglycerides of different structure and very toxic to fish from the skin of the nudibranch mollusc Doris verrucosa[10].

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