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VERRUCOSIN-A AND -B, ICHTHYOTOXIC DITERPENOIC ACID GLYCERIDES WITH A NEW CARBON SKELETON FROM THE DORID NUDIBRANCH *DORIS VERRUCOSA*

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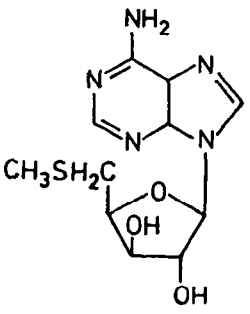
Abstract.- The dorid nudibranch *Doris verrucosa* contains in its skin extracts two glycerides (*2-3*) linked to diterpenoid acid residues. The terpenoids show a rearranged tricyclic skeleton. The structures, inferred from spectral and chemical evidence, were unambiguously determined by X-ray diffraction analysis of *3*. Both *2* and *3* are highly ichthyotoxic.

The chemical behaviour of naked molluscs has recently been object of many chemical investigations[2]. We have previously[3] reported the isolation of an analog (*1*) of methylthioadenosine (MTA) from the dorid nudibranch *Doris verrucosa* Cuvier. Now, in our continuing search[4,5] for potential allomones in the defensive secretions of opisthobranch molluscs, we have isolated two isomeric monoacetylated diterpenoic acid glycerides, verrucosin-A (*2*) and -B (*3*), from the skin extracts of *D. verrucosa*. The structures have been established by a combination of spectral analysis, chemical degradation and X-ray diffraction study.

The carbon skeleton of the diterpenoid part is unprecedented in nature, it could be generated from an *ent*-isocopalane (*4*) or isocopalane[6] precursor by a concerted rearrangement, involving the methyl at C-10, followed by expulsion of a proton to give the $\Delta^{5(6)}$ double bond. Rimuene (*5*) is an example of diterpene showing a rearranged rosane skeleton likely formed in similar way from a pimarane precursor[7]. It is noteworthy that glycerides of *ent*-isocopalane acid (*6-8*) have already been found in the skin extracts of the British Columbia dorid *Archidoris montereyensis* [8,9].

Specimens of *D. verrucosa* (100 individuals) were collected in the Bay of Naples during June-September 1985. The animals, stored for few days at -20°C, were carefully dissected and then the dorsum and the hepatopancreas were separately extracted. The diethyl ether soluble fraction (900 mg) from the acetone extract of the dorsum was chromatographed on silica gel column using various combinations of diethyl ether-petrol to give an unresolved mixture A (200 mg) of compounds slightly more polar than sterols. The mixture A was fractionated by HPLC on μ-Porasil column giving in order of increasing polarity verrucosin-A (*2*, 52 mg), a complex mixture B (48 mg) and verrucosin-B (*3*, 23 mg). *3* was obtained as white crystals from hexane-diethyl ether.

Verrucosin-A (*2*) was analyzed by HREIMS to have the same formula, $C_{25}H_{40}O_5$, as *7*. In addition, the mass spectrum of *2* revealed at higher masses a pattern of fragmentation very similar to that of *7*, including the base peak at m/z 286. The ^{1}H-NMR spectrum of *2* in $CDCl_3$ (Table 1), when compared with that of *7*, showed



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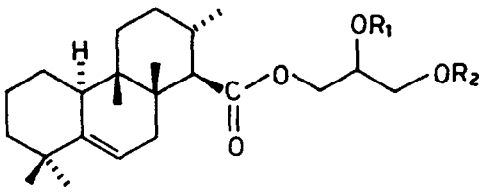
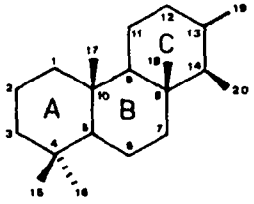


2 : $R_1 = H$; $R_2 = COCH_3$

3 : $R_1 = COCH_3$; $R_2 = H$

11 : $R_1 = R_2 = H$



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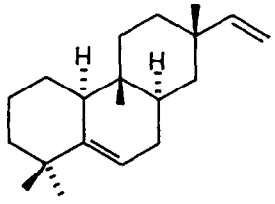

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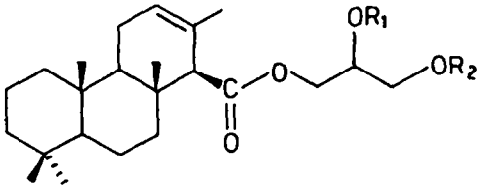


6 : $R_1 = R_2 = H$

7 : $R_1 = H$; $R_2 = COCH_3$

8 : $R_1 = COCH_3$; $R_2 = H$

strong analogies. In particular, it revealed that both, *2* and *7*, possess a $CH_3COOCH_2CH(OH)CH_2O$- residue (complex five protons multiplets between δ 4.08 and 4.25 and three proton singlet at δ 2.10) linked to a diterpenoic acid (four tertiary and one secondary methyl signals at δ 1.08, 1.06, 1.01, 0.70 and at δ 0.79, respectively).

However, the ^{1}H-NMR spectrum of *2* differs from that of *7* from the absence of the signal at δ 1.60, assigned to the vinyl methyl at C-13, which is replaced by a doublet at δ 0.79 and, in addition, for the substitution pattern of the trisubstituted double bond. In fact, the olefinic signal at δ 5.38 is long-range coupled with a methine proton signal at δ 2.37 and directly coupled with two geminal protons resonating at δ 1.50 and 2.00 and assigned to an adjacent methylene group which from the simple coupling pattern was further linked to a quaternary carbon atom. Homoallylic couplings were also observed between the protons resonating at δ 2.37 and 2.00. Owing to this evidence and bearing in mind a tricyclic diterpenoid skeleton, the position of the double bond appears to be confined in the B ring between the carbons 5 and 6. However, the observed long-range couplings involving the methine signal at δ 2.37 require a hydrogen and

TABLE 1 - ^{1}H-(500 MHz) and ^{13}C-(62.9 MHz) NMR data for verrucosin-A (*2*)

C	δ C[a]	m[c]	δ H at C[a,d]	δ H at C[b,d]	multiplicity[b] (J,Hz)
1	28.4	t	1.05 1.70	1.02 1.55	dtd (3.7;11.9;13.0) m
2	22.8	t	1.62	1.48 1.52	m m
3	40.8	t	1.20 1.40	1.25 1.40	m m
4	38.0[f]	s			
5	144.9	s			
6	113.9	d	5.38	5.56	d (6.4)
7	35.0	t	1.50 2.00	1.71 2.06	dd (18.0; 6.4) bd (18.0)
8	37.3[f]	s			
9	36.3[f]	s			
10	36.1	d	2.37	2.40	bd (12.1)
11	29.7[e]	t	1.40	1.20 1.35	m m
12	29.7[e]	t	1.55	1.15 1.30	m m
13	29.0	d	1.98	2.05	m
14	51.7	d	2.35	2.48	d (12.0)
15	29.6	q	1.08	1.19	s
16	28.3	q	1.06	1.14	s
17	18.7	q	0.70	0.69	s
18	18.1	q	1.01	1.16	s
19	21.4	q	0.79	0.89	d (6.3)
20	170.9	s			
21	64.3	t	4.09	4.06 3.92	dd (11.5; 5.8) dd (11.5; 4.6)
22	68.4	d	4.05	3.77	m
23	65.3	t	4.15	4.01	m
CH_3-CO	175.2	s			
CH_3-CO	20.7	q	2.09	1.59	s

[a] $CDCl_3$. [b] C_6D_6. [c] By DEPT sequence. [d] Assignments made by 2D homo-(COSY 45) and hetero-correlations (HETCOR) and by ^{1}H-^{1}H decoupling experiments. [e] Overlapping signals. [f] Assignments may be interchanged.

not the customary methyl group at C-10. The ^{13}C-NMR spectrum of *2* (Table 1)[10] confirmed the observed structural analogies with *7* in particular for the resonances due to the diacetylated glycerol residue while relevant chemical shift differences were recorded for almost all the carbons of the terpenoid part. However, all the data were consistent with a rearranged isocopalane skeleton containing a $\Delta^{5(6)}$ double bond and with the methyl group at C-10 shifted to C-9 as suggested in *2*. According with this hypothesis, the high-field signal at δ 0.70 was assigned, by comparison with model compounds, to the axial methyl at C-9. In fact, a significative shielding effect due to the presence of the $\Delta^{5(6)}$ double bond has been observed for the axial C-9 methyl group in rimuene (*5*)[11,12] and, more recently, in the olefin *9*[13].

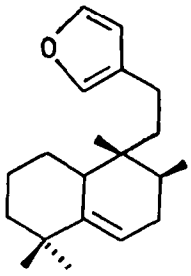
TABLE 2 - ^{1}H-(500 MHz) and ^{13}C-(62.9 MHz) NMR data for compound *10*[a]

C	δC	m[b]	δH at C[c]	multiplicity(J,Hz)	Δδ H[d]
1	28.46	t	1.06 1.63	m m	
2	23.22	t	1.50 1.55	m m	
3	41.34	t	1.27 1.37	m m	
4	38.01[e]	s			
5	145.61	s			
6	114.69	d	5.43	d (6.5)	0.84
7	33.65	t	1.95 2.09	bd(18.2) dd(18.2; 6.5)	0.82 1.70
8	38.36[e]	s			
9	36.40[e]	s			
10	36.52	d	2.50	bd(12.7)	1.05
11	30.39[f]	t	1.40	m	
12	31.32[f]	t	1.37	m	
13	29.66	d	1.60	m	3.70
14	46.56	d	1.25	m	3.60
15	29.75	q	1.10	s	0.31
16	28.59	q	1.04	s	0.65
17	19.17	q	0.73	s	0.50
18	18.87	q	0.87	s	1.26
19	21.80	q	0.98	d (6.3)	2.00
20	62.35	t	3.58 3.43	dd(11.3; 3.6) dd(11.3; 2.8)	4.70

[a] C_6D_6. [b] By DEPT sequence. [c] Assignments made by 2D homo-(COSY 45) and hetero-correlations (HETCOR) and by ^{1}H-^{1}H decoupling experiments. [d] After addition of 0.4 moles of Eu(fod)$_3$ per mole of *10*. [e,f] Assignments with identical superscripts may be interchanged.

The proposed structure was confirmed by a series of mono- and two-dimensional NMR experiments. All the ^{1}H and ^{13}C chemical shifts of *2* were assigned (Table 1) on the basis of the data observed by homo-nuclear decoupling experiments, DEPT sequence, ^{1}H-^{1}H and ^{1}H-^{13}C correlations. Unambiguous ^{1}H and ^{13}C assignments for all methyls were also provided by the analysis of the below reported NMR data of the alcohol *10*, obtained by reduction of *2* with $LiAlH_4$. The NMR experiments have been recorded in $CDCl_3$ for sake of comparison with model compounds[8,9,11,12,13] and in C_6D_6 for obtaining better resolved spectra. About the stereochemistry of *2*, a *trans* diaxial orientation for the C-14 and C-13 protons was immediately deduced from their coupling constant (J_{14-13} = 12.0 Hz). The remaining stereochemical assignments were ascertained by the analysis of the NMR data collected for the alcohol *10* (Table 2). Comparison of the ^{1}H-NMR spectrum of *10* with that of *2* obviously revealed the loss of the signals due to the glycerol residue and the appearence of an ABX system with resonances at δ 3.58, 3.43 and 1.25, assigned to the methylene protons of the hydroxymethyl group and to H-14. In addition, the analysis of the spectrum allowed correct assignments for all the methyl resonances. The 3H doublet at δ 0.98 and the 3H singlet at δ 0.73 were easily assigned to the methyls at C-13 and C-9, respectively. A substantial upfield shift observed for the methyl at δ 0.87 (δ 1.16 in *2*) allowed to localize the methyl at C-8. The signal at δ 1.10 was assigned to the equatorial methyl at C-4 on the basis of a positive n.O.e. with the olefinic proton at δ 5.38. Finally, the remaining signal at δ 1.04 was

ascribed to the axial methyl group at C-4. ^{1}H-NMR data in C_6D_6 in the presence of the shift reagent Eu(fod)$_3$ (Table 2) proved the relative configurations of *10* and, in particular, the *cis* junction between the rings B and C. In fact, strong downfield shifts were observed for the methyl protons at C-8 (Δδ 1.26) and at C-13 (Δδ 2.0); while within the less downshifted methyls, the C-4 axial methyl group exhibited a more consistent shift (Δδ 0.65) than the C-9 (Δδ 0.50) and the C-4 equatorial (Δδ 0.31) methyls. These observed shifts were consistent with the distances measured on Dreiding models only with a B-C *cis* ring junction, which was further confirmed by the consistent induced-shift of the axial H-10 (Δδ 1.05).



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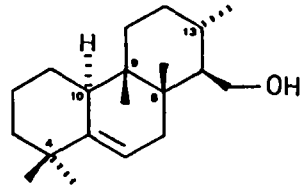

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The spectroscopic data of verrucosin-B (*3*) showed strong similarities with those of *2* suggesting that *3* is a positional isomer of *2* in which the 2-hydroxy group of the glycerol residue is acetylated (H-22, δ 5.01; C-22, δ 72.6). *3*, analogously to *2*, yielded the alcohol *10* by treatment with $LiAlH_4$ and the glyceride *11* by alkaline hydrolysis. We have observed that *3* slowly rearranges to *2* in $CDCl_3$ (NMR tube) probably because of the presence of traces of acid. It is likely that there has been a partial interconversion within *3* and *2* during the extractive and chromatographic work. In fact, it is well known that acyl groups in acylglycerols easily migrate from the oxygen to which they are attached to an adjacent free hydroxyl group by a transesterification process catalyzed by acid, base or heat.

However, crystallization of *3* from n-hexane-diethyl ether gave crystals suitable for a conclusive X-ray crystallographic analysis which led without ambiguities to determine structure and stereochemistry of verrucosin-A and -B.

The structure of *3* (verrucosin-B) was solved using direct methods and refined to a crystallographic R factor of 0.042. In the absence of atoms with strong anomalous scattering, the absolute configuration could not be determined and the chosen configuration corresponds to that of C(8) in the *ent* isocopalane skeleton[6]. A view of the final crystallographic model is shown in Fig. 1 together with the atom labelling scheme used in the X-ray work.

The diterpenoid moiety exhibits a *cis B/C* junction with the C(14) atom *trans* to the methyl C(17). The ring *C* presents the C(18) methyl group in axial position, whereas the two methyl substituents at C(9) and C(13) are equatorial. The substituent group at C(14) is also equatorial and C(20) is gauche to C(18) and C(19). The two cyclohexane rings (*A* and *C*) display a chair conformation as evidenced by the intracycle torsional angles all close to ± 60° and the Cremer and Pople[14] puckering parameters which are $Q = 0.541$, $q_3 = 0.534$, $q_2 = 0.084$ Å, $\theta = 8.96°$, $\phi = -20.8°$ and $Q = 0.578$, $q_3 = 0.567$, $q_2 = 0.112$ Å, $\theta = 11.21°$, $\phi = -6.2°$ for the two rings respectively. The distorsion from an ideal chair conformation in the ring *A* is toward a small flattening at C(4), which optimizes the intramolecular contacts between the equatorial methyl C(15) and the C(6)-H group. The ciclohexene ring *B*,

TABLE 3 - Crystal data

Formula	$C_{25}H_{40}O_5$
Formula weight	420.6
Space group	$P2_12_12_1$
a, Å	11.175(1)
b, Å	11.863(2)
c, Å	18.200(2)
V, Å^3	2412.7(9)
Z	4
D_x, g/cm^3	1.158
λ CuKα, Å	1.54178
θ_{max} (°)	75
No indep. refl.	2802
No refl. above 3σ(I)	2632
R	0.0416
R_w	0.0633

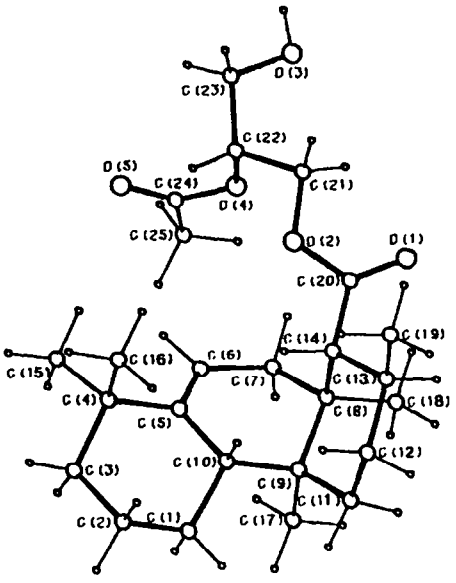


Fig.1 - Perspective drawing of verrucosin-B (compound *3*) together with the atom labelling scheme used in the X-ray work.

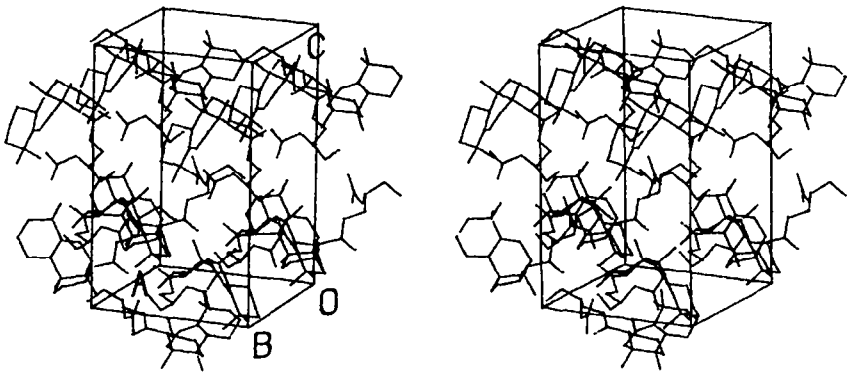


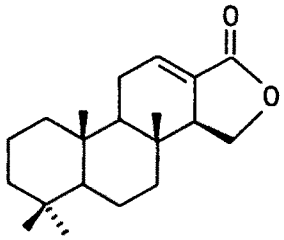
Fig.2 - Stereoview of the molecular packing of *3*, H-atoms and hydrogen bond have been omitted.

which presents the double bond between C(5) and C(6), adopts the expected half-chair conformation slightly distorted in the direction of an half-boat, with C(8) 0.543 Å below and C(9)0.217 Å above the best plane defined by C(5), C(6), C(7) and C(10) atoms. All bond lengths and bond angles are in good agreement with the expected values. In the C_{20} diterpenoid moiety the C(sp^3)-C(sp^3) bond lengths range from 1.502(2)Å to 1.569(2)Å with a mean value of 1.534Å, the longest values are associated to the four-substituted carbon atoms. The corresponding valency angles of the tetrahedral carbon atom range from 107.4(2)° to 115.4(1)° with a mean value of 110°. The double bond in ring *B* is 1.330(2)Å.

The molecular arrangement in the crystal is shown in Fig. 2. The molecules, related by the screw axis along x, are held together in an infinite chain by hydrogen bonds between the hydroxylic O(3) and the carbonylic O(5) oxygens [O(3)-H-----O(5)=2.784(2)Å].

The carbon skeleton of verrucosins should derive from isocopalane diterpenes which formally are also considered the biogenetic precursors of the tetracyclic spongiane diterpenes. However, it is noteworthy that after the earlier isolation[15] of isoagatholactone (*12*) a growing number of spongiane diterpenes has been isolated from sponges, while isocopalane diterpenes have rarely been reported. Until now tetracyclic diterpenes deriving from precursors with the carbon skeleton of verrucosins have not been encountered in nature. Likely, the $\Delta^{12(13)}$ double bond is essential for the additional cyclization.

Diterpenoid and farnesic acid glycerides (*13-15*) are also present in the British Columbia dorid nudibranch *Archidoris odhneri*[16]. One of these farnesic acid glycerides (*14*) has been also found in *D. verrucosa* as minor component of the fraction B. The widespread presence and the localization on the skin strongly suggest an ecological role for these glycerides. The glycerides *2* and *3* are higly toxic to the mosquito fish *Gambusia affinis* at the 1.0 and 0.1 µg/ml levels, respectively, while the farnesic acid glyceride *14* is non toxic at 10 µg/ml level. The production of toxic substances by *D. verrucosa* would appear to contribute greatly to the survival of this nudibranch in the predator-rich areas in which it lives.



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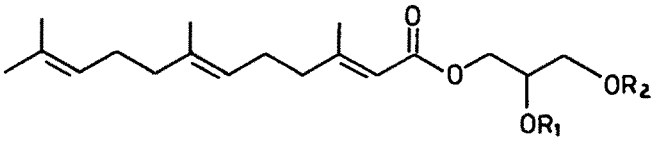

13 : $R_1 = R_2 = H$

14 : $R_1 = H$; $R_2 = COCH_3$

15 : $R_1 = Ac$; $R_2 = H$

TABLE 4 - Positional parameters and equivalent isotropic temperature factors (Å^2) with esd in parentheses for compound *3*

$$B_{eq} = 4/3 \, \Sigma_i \, \Sigma_j \, b_{ij} \, \vec{a}_i \, \vec{a}_j$$

	x	*y*	*z*	B_{eq}		*x*	*y*	*z*	B_{eq}
O(1)	0.4734(2)	0.6568(2)	0.27978(10)	5.66(4)	C(11)	0.8726(3)	0.7942(3)	0.2017(2)	5.50(6)
O(2)	0.5677(1)	0.6040(1)	0.38215(8)	3.74(3)	C(12)	0.7884(3)	0.8715(2)	0.2441(2)	5.27(6)
O(3)	0.3051(2)	0.3749(2)	0.44898(11)	5.67(4)	C(13)	0.6666(3)	0.8215(2)	0.2622(1)	4.46(5)
O(4)	0.5508(2)	0.3679(1)	0.40263(8)	3.95(3)	C(14)	0.6833(2)	0.7026(2)	0.2952(1)	3.48(4)
O(5)	0.6704(2)	0.2993(2)	0.49049(10)	5.97(4)	C(15)	0.9820(4)	0.4792(3)	0.4797(2)	6.41(7)
C(1)	1.0834(3)	0.7050(3)	0.3048(2)	5.67(6)	C(16)	0.9186(4)	0.6804(3)	0.4847(2)	6.89(8)
C(2)	1.1389(3)	0.7265(3)	0.3797(2)	6.79(8)	C(17)	0.9599(3)	0.6013(3)	0.1819(2)	6.15(7)
C(3)	1.1185(3)	0.6251(3)	0.4300(2)	6.29(7)	C(18)	0.6901(3)	0.6072(3)	0.1700(1)	5.01(6)
C(4)	0.9858(3)	0.5915(2)	0.4381(1)	4.88(5)	C(19)	0.5988(4)	0.8987(2)	0.3150(2)	6.84(8)
C(5)	0.9250(2)	0.5853(2)	0.3630(1)	3.88(4)	C(20)	0.5632(2)	0.6536(2)	0.3159(1)	3.60(4)
C(6)	0.8466(2)	0.5049(2)	0.3463(1)	4.06(5)	C(21)	0.4607(2)	0.5470(2)	0.4073(1)	3.93(4)
C(7)	0.7695(2)	0.5054(2)	0.2787(1)	4.03(5)	C(22)	0.5015(2)	0.4496(2)	0.4542(1)	3.62(4)
C(8)	0.7562(2)	0.6227(2)	0.2438(1)	3.78(4)	C(23)	0.4004(3)	0.3979(2)	0.4971(1)	4.46(5)
C(9)	0.8843(2)	0.6750(2)	0.2348(1)	4.26(5)	C(24)	0.6338(2)	0.2970(2)	0.4276(1)	4.05(5)
C(10)	0.9482(2)	0.6829(2)	0.3112(1)	4.06(5)	C(25)	0.6748(3)	0.2170(3)	0.3698(2)	5.50(6)

EXPERIMENTAL

NMR spectra were recorded on Bruker WM 500, WM 270 and WM 250 spectrometers (δ ppm/TMS). The 2D NMR spectra were obtained using Bruker's microprograms. Mass spectra were taken on AEI MS-30 and Kratos MS-50 instruments. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Silica gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Preparative HPLC purifications were carried out on a Waters apparatus equipped with μ-Porasil column and with an R. I. detector.

Isolation procedure.

100 specimens (average length 3 cm) of *D. verrucosa*, collected in the Bay of Naples during June-September 1985, were dissected and then the digestive glands and the mantles were separately extracted with acetone at room temperature for one day. Both extracts were evaporated at reduced pressure and the residual water was extracted sequentially with diethyl ether and n-butanol. The ethereal extract of the mantles was concentrated to give 900 mg of crude material, that was chromatographed on a SiO_2 column(light petrol-diethyl ether, 8:2). The fraction (200 mg) slightly more polar than sterols (Rf 0.3 on TLC, n-hexane-ether, 8:2) was subjected to HPLC [μ-Porasil, 7,8 mm (ID) x 30 cm; n-hexane-ethyl acetate, 85:15; flow rate 4 ml/min] yielding *2* (52 mg, Rt 0.47), *3* (23 mg, Rt 0.67), and an unresolved mixture (48 mg, Rt 0.53) which, after a further purification by analitical HPLC [μ-Porasil, 3.2 mm (ID) x 30 cm ; n-hexane-ethyl acetate, 85:15; flow rate 2 ml/min], gave *14* (10 mg, Rt 0.62) .

Verrucosin-A (*2*).

$[\alpha]_D$ + 37.3 (c 1.1, $CHCl_3$); IR (liquid film): 1725 cm^{-1}; EIMS, m/z (%): 420 (M^+, 5.1), 377 (1.7), 347 (3.4), 286 (100), 271 (20.3), 258 (28.8), 243 (40.7). HRMS 286.2293;for $C_{20}H_{30}O$ (M^+-acylglycerol, calculated 286.2297).

^{1}H-NMR: Table 1.

^{13}C-NMR ($CDCl_3$): Table 1; ^{13}C-NMR (C_6D_6, 125.8 MHz) δ 174.8 (C-20), 170.2 ($CH_3\underline{C}O$), 144.8 (C-5), 114.7 (C-6), 68.4 (C-22), 65.4 (C-23), 64.4 (C-21), 51.9 (C-14), 41.1 (C-3), 38.2 (C-4)[a], 37.5 (C-8)[a], 36.5 (C-9)[a], 36.4 (C-10), 35.4 (C-7), 30.0 (C-11 and C-12), 29.9 (C-15), 29.4 (C-13), 28.8 (C-1), 28.4 (C-16), 23.3 (C-2), 21.7 (C-19), 20.2 ($\underline{C}H_3$-CO), 18.9 (C-17), 18.4 (C-18). [a] Assignments may be interchanged.

Verrucosin-B (*3*).

$[\alpha]_D$ + 19.2 (c 0.5, $CHCl_3$); mp 118-120° (n-hexane-diethyl ether); IR (liquid film): 1710 cm^{-1}; EIMS, m/z (%): 420 (M^+, 2.6), 347 (7.9), 286 (15.8), 271 (9.2), 259 (13.1), 243 (26.3), 119 (100). ^{1}H-NMR (C_6D_6, 500 MHz) δ 5.51 (H-6, 1H, bd J = 6.4 Hz), 5.01 (H-22, 1H, quintet J = 5.1 Hz), 4.17 (H-21, 2H, m), 3.44 (H-23, 2H, d J = 5.3 Hz), 2.47 (H-1H, 1H, d J = 11.9 Hz), 2.40 (H-10, 1H, bd J = 12.5 Hz), 2.05 (H-13, 1H, m), 2.04 (H-7, 1H, bd J = 18.1 Hz), 1.72 (H-7, 1H, dd J = 18.1 and 6.4 Hz), 1.70 ($\underline{C}H_3$-CO, 3H, s), 1.19 (H-15, 3H, s), 1.15 (H-18, 3H, s), 1.14 (H-16, 3H, s), 0.89 (H-19, 3H, d J = 6.3 Hz), 0.70 (H-17, 3H, s).

^{13}C-NMR ($CDCl_3$, 125.8 MHz) δ 175.1 (C-20), 170.5 ($CH_3\underline{C}O$), 144.6 (C-5), 113.9 (C-6), 72.6 (C-22), 61.8 (C-21)[a], 61.3 (C-23)[a], 51.6 (C-14), 40.7 (C-3),

37.8 (C-4)[b], 37.2 (C-8)[b], 36.3 (C-9)[b], 36.0 (C-10), 34.9 (C-7), 29.7 (C-11 and C-12), 29.6 (C-15), 29.0 (C-13), 28.4 (C-1), 28.1 (C-16), 22.9 (C-2), 21.4 (C-19), 20.9 (CO-$\underline{C}H_3$), 18.7 (C-17), 18.1 (C-18). [a,b] Assignments may be interchanged.

Compound *10*

2 (9 mg) in dry THF (3 ml) was stirred with an excess of $LiAlH_4$ at reflux for 2 hours. Usual work up gave, after purification on silica-gel TLC (light petroleum-diethyl ether, 8:2), the alcohol *10* (5 mg).

EIMS, m/z (%): 290 (M^+, 6.6), 275 (18.4), 257 (5.3), 154 (55.3), 123 (100).

^{1}H- and ^{13}C-NMR: Table 2.

Compound *11*

2 (9 mg) was dissolved in MeOH (1 ml) and stirred with 10% KOH methanolic solution (4 ml) at room temperature overnigth. Usual work up gave *11* (5 mg) which was not further purified.

EIMS, m/z (%): 378 (M^+, 2.2), 363 (4.0), 286 (100), 271 (42.8), 259 (48.6), 258 (31.4), 243 (85.7).

^{1}H-NMR (C_6D_6, 270 MHz) δ 5.54 (H-6, 1H, d J = 6.4 Hz), 4.08 (H-21, 1H, dd J = 11.4 and 6.2 Hz), 3.94 (H-21, 1H, dd J = 11.4 and 4.6 Hz), 3.59 (H-22, 1H, m), 3.35 (H-23, 2H, m), 2.48 (H-14, 1H, d J = 11.9 Hz), 2.40 (H-10, 1H, bd J = 12.0 Hz), 2.06 (H-7 and H-13, overlapping signals), 1.72 (H-7, 1H, dd J = 19.8 and 6.4 Hz), 1.18 (H-15, 3H, s), 1.16 (H-18, 3H, s), 1.14 (H-16, 3H, s), 0.89 (H-19, 3H, d J = 6.3 Hz), 0.70 (H-17, 3H, s).

^{13}C-NMR (C_6D_6, 62.9 MHz) δ 144.8 (C-5), 114.8 (C-6), 70.8 (C-22), 65.0 (C-21)[a], 63.8 (C-23)[a], 52.1 (C-14), 41.1 (C-3), 38.3 (C-4)[b], 37.6 (C-8)[b], 36.5 (C-9)[b], 36.4 (C-10), 35.3 (C-7), 30.0 (C-11 and C-12), 29.8 (C-15), 29.4 (C-13), 28.7 (C-1), 28.3 (C-16), 23.2 (C-2), 21.6 (C-19), 18.8 (C-17), 18.4 (C-18). The signal due to the carbonyl carbon was not detected. [a,b] Assignments may be interchanged.

Crystal Structure Determination

Compound *3* crystallizes in the form of colourless prisms by slow concentration of a n-hexane and diethyl ether solution. A single crystal (0.6x0.5x0.4 mm) was selected for the crystallographic study.

Accurate cell parameters (see Table 3) were obtained by least-squares refinement of the setting angles of 24 reflections at medium θ ($25° \leq \theta \leq 32°$), using Ni-filtered CuKα radiation and Enraf-Nonius CAD-4F diffractometer on line with a PDP11/34 Digital computer 2802 independent reflections ($\theta_{max} = 75°$) were collected at room temperature, using ω scan mode. During the data collection the intensities of three standard reflections were monitored every 4 h (4% variation) in order to check the crystal and equipment stability. The intensities were corrected for Lorentz and polarization factors, but not for the absorption effect ($\mu = 5.96\ cm^{-1}$). The structure was solved by direct methods using MULTAN[17]. The refinement of the positional and anisotropic temperature parameters for non-hydrogen atoms was carried out by full-matrix (on F) least-squares cycles. The H atoms were generated at the expected positions taking into account the indications of the difference fourier map for the hydroxyl and methyl hydrogens. All the hydrogens were included in the last refinement as fixed atoms with the isotropic thermal parameters set equal to B_{eq} of the parent atoms. At convergence the discrepancy index $R = \Sigma\ ||F_o|-|F_c|| / \Sigma|F_o|$ was 0.0416 for the 2632 observed reflections ($I \geq 3\sigma(I)$, 101 and 012 excluded from final refinement for asymmetric background). R_w=

0.0633 with $w^{-1}=\sigma^2(F_o)$.

For the crystallographic work, the equipment of the "Centro di Metodologie Chimico-fisiche dell'Università di Napoli" and SDP package was used. Scattering factors were taken from Cromer and Waber[18].

In Table 3 are summarized some crystal data and the final atomic parameters with esd in parentheses are given in Table 4. Structure factors, hydrogen atoms parameters and anisotropic thermal parameters have been deposited together with a list of the geometrical internal parameters.

Ichthyotoxicity tests

Ichthyotoxicity assays were conducted using a mosquito fish, *Gambusia affinis* (Baird and Girard), according to ref. 19 and 20. In each test six fishes were placed in distilled water (70 ml) and acetone solutions (0.5 ml) of the test compound were added. 100% mortality of the test organisms was observed within 90 min using *3* at 0.1 μg/ml and within 45 min using *2* at 1.0 μg/ml concentration. Compound *14* was non toxic at 10.0 μg/ml concentration.

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