Verrucoside, a New Cytotoxic Pregnane Glycoside from a Gorgonian Eunicella verrucosa

Y. Kashman, D. Green, C. Garcia, and D. Garcia Arevalos

J. Nat. Prod., **1991**, 54 (6), 1651-1655• DOI:
10.1021/np50078a026 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 6, pp. 1651-1655, Nov-Dec 1991

VERRUCOSIDE, A NEW CYTOTOXIC PREGNANE GLYCOSIDE FROM A GORGONIAN *EUNICELLA VERRUCOSA*

Y. KASHMAN,* D. GREEN,

School of Chemistry, Tel Aviv University, Ramat Aviv 69978, Israel

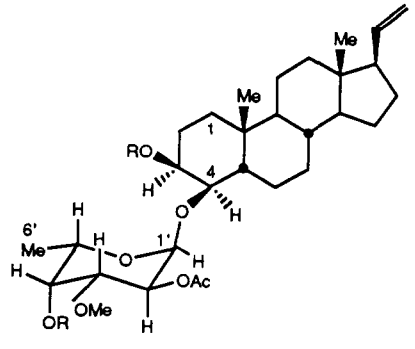
C. GARCIA, and D. GARCIA AREVALOS

PharmaMar SA 28760, Tres Cantos, Madrid, Spain

ABSTRACT.—The structure of verrucoside, a new pregnane glycoside 4β-*O*-[2-*O*-acetyl-α-L-digitalopyranosyl]-5β-pregn-20-en-3β-ol [**1**], isolated from the gorgonian *Eunicella verrucosa*, has been determined mainly by 2D nmr and mass spectra.

In our search for biologically active marine natural products (1) we have isolated from a gorgonian, *Eunicella verrucosa* Verrill (Gorgonaceae), collected near Cadiz, Spain, compound **1** which was responsible for the cytotoxic activity of the CH_2Cl_2/MeOH extract of this organism. The extract was partitioned between different solvents (see Experimental), and the CCl_4-soluble fraction was subjected to repeated Si gel cc to afford compound **1** (25 mg from 500 mg of extract).

Compound **1**, designated verrucoside, was obtained as an amorphous powder: $[\alpha]_D$ −30° (*c* = 2, $CHCl_3$); ir ν max ($CHCl_3$) 3450 (OH), 1740, 1270 (ester), 900 s (−HC=CH_2) cm^{-1}. The molecule was assigned the molecular formula $C_{30}H_{48}O_7$ by cims (*m/z* 521 $[MH]^+$) and elemental analysis. The ^{13}C-nmr spectrum [PND and DEPT (2) experiments] showed the 30 carbon atoms of the molecule to consist of one carbonyl, two quaternary (sp^3), 13 methine (one sp^2 and 12 sp^3), nine methylene (one sp^2 and eight sp^3), and five methyl groups (in total $C_{30}H_{46}$) (Table 1). Evident also from the nmr data were one methoxyl (δ_H 3.02 s), one acetate (δ_H 1.83 s), one vinyl [δ_H 6.82 ddd (*J* = 16, 11, 7), 5.09 d (*J* = 11), and 5.10 d (*J* = 16)], an anomeric carbon (δ_C 98.7), and two hydroxyl groups [δ_C 68.7, 68.6, and δ_H 4.19 brq (*J* = 3), 3.65 dd (*J* = 12, 3)]. The latter two OH groups (completing the 48 protons of the molecule) were confirmed by a micro acetylation of **1** (2 mg), to triacetate **2** (δ_H 2.05, 2.09, 2.14; three 3H singlets). Most significant for the structure elucidation of **1** was its ci mass spectrum; apart from the molecular ion, two prominent peaks at *m/z* 203 (100%) $[220-17]^+$ and 301 $[521-220]^+$ (5%) suggested the molecule of **1** to be a glycoside consisting of a $C_{21}H_{34}O_2$ (*m/z* 318) aglycone and a $C_9H_{16}O_6$ (*m/z* 220) sugar unit. The spectral data of the latter C_9 unit, namely, the ^{13}C-nmr (one anomeric and four adjacent methinoxy carbons) and the ^{1}H-nmr (a chain of five CH-O-groups, C-1′ to 5′, with a methyl terminus, C-6′) spectra (Table 1), clearly proposed for this moiety an *O*-methyl-*O*-acetyl-6-deoxy hexose structure. The connectivities between the protons of this hexose were determined by a COSY (3) experiment, and the carbon and the CH-correlation assignments by HMQC (4) and HMBC (5) experiments. The stereochemistry of the sugar (equatorial H-1′ and -4′ and



1 R = H
2 R = Ac

TABLE 1. ^{1}H- and ^{13}C-nmr Data of Verrucoside [**1**] (500 MHz, for the protons, in C_6D_6).

Position	δ_C	δ_H		^{1}H-^{13}C *connectivities (HMBC)*		^{1}H-^{1}H *connectivities (COSY)*	
		α	β	^2J	$^3J(^4J)$	from α-H to:	from β-H to:
1	25.6 (t)	1.68 eq	1.29 ax			1β, 2β	2α
2	29.3 (t)	1.34 ax	1.66 ax	H-3	(H-19)	3α	3α
3	68.7 (d)	4.19 eq	brq $J=3$		H-2α		4α
4	79.3 (d)	3.65 ax	dd $J=12,3$		H-1′	5β	
5	43.0 (d)	1.90 ddd (12, 3, 2)		H-6β, H-4α	(H-2α)		6β, 6α
6	20.5 (t)	2.00 eq	1.60 ax	H-7β		6β, 7α, 7β	7α
7	26.7 (t)	0.87 ax	1.34 eq	H-6α		7β	8β
8	36.0 (d)		1.30 ax	H-14α	(H-12β)		14α, 9α
9	41.9 (d)	0.95 ax			H-12β, Me-19	11β, 11α	
10	37.2 (s)			H-1α, Me-19			
11	20.9 (t)	1.26 eq	1.10 ax			11β, 12β	12β
12	37.8 (t)	0.96 ax	1.68 eq			12β	
13	43.9 (s)			H-17α	H-16α		
				H-14α, H-12α			
14	55.4 (d)	0.80 ax ddd (13, 11, 7)				15α, 15β	
15	24.9 (t)	1.53	1.07	H-14α, H-16α		15β, 16β	16β
16	27.6 (t)	1.54	1.80	H-20		16β, 17α	17α
17	55.8 (d)	2.00 ax		H-20	H-21	16β, 17α, 20	
18	13.1 (q)		0.55 s	H-17α	H-14α		
19	24.0 (q)		0.93 s				
20	140.6 (d)	5.82 ddd (16, 11, 7)		H-17α, H-21		21	
21	115.2 (t)	5.09 d (16) 5.10 d (11)		H-17α			
1′	98.7 (d)	5.31 eq d (4)		H-2′α	H-4, H-5′		2′α
2′	77.3 (d)	5.41 ax dd (10, 4)		H-1′β	3′-OMe	3′β	
3′	70.8 (d)	3.46 ax dd (10, 3)		H-4′			
4′	68.8 (d)	3.48 eq dd (3, 1)		H-5′			
5′	66.7 (d)	3.91 dq (1,6)			H-1′β		6′α
6′	16.6 (q)	1.36 eq d (6)		H-5′β			
2′-OAc	20.7 (q)	1.83 s					
	170.0 (s)						
3′-OMe	57.5 (s)	3.02 (s)					

axial H-2′, -3′, and -5′), deduced from the coupling constants of H-1′–H-5′ and confirmed by decoupling experiments, led to the 2-*O*-acetyldigitalose (2-*O*-acetyl-3-*O*-methyl-fucose) structure. The equatorial methyl (C-6′) was ascertained from the absence of an nOe between this methyl group and the axial H-3′, while an enhancement of H-4′, while irradiating Me-6′, was observed (4.8%). Also in agreement with the digitalose moiety in **1** was the observed ^{1}H-nmr shift of H-4′ in triacetate **2**.

The structure of the aglycone of **1** was established upon comprehensive nmr studies of this part of the molecule. Among others, COSY, double quantum COSY (7), HMQC, and HMBC experiments (in C_6H_6-d_6, which was found to be the most suitable solvent) have been evaluated. All correlations observed in these experiments are presented in Table 1. Assisted by the HMQC experiment, which has established all eight sp^3 geminal methylene pairs of protons, the DQ-COSY experiment determined all the vicinal H-H connectivities within the aglycone except for those next to the angular methyl groups (Me-18, -19) (Table 1). On the basis of the latter data alone, a Δ^{20}-pregnane skeleton could have been suggested. However, this structure was unequivocally confirmed by the HMBC experiment (see $^2J_{CH}$ to $^4J_{CH}$, Table 1). Comparison of the carbon chemical shifts of **1** and its aglycone (see below) with those of pregnedioside A (4α-*O*-β-D-arabinopyranosyloxy-5α-pregn-20-en-3β-ol) (10), 5β-pregnane, and 5β-cholestan-3β-ol (11) (in $CDCl_3$ or pyridine-d_5) (Table 2) was in full agreement with the aglycone being 5β-

TABLE 2. ^{13}C-nmr Data for Verrucoside [**1**], the Aglycone of Verrucoside, Pregnedioside A, 5β-Pregnane, and 5β-Cholestan-3β-ol.

Carbon	Compound						
	1[a]	**1**[b]	**1**[c]	aglycone of **1**[a]	pregnedioside A	5β-pregnane[d]	5β-cholestan-3β-ol[d]
C-1	26.8	25.6	25.2	27.0	36.5	37.7	30.0
C-2	29.6	29.3	28.8	30.5	29.1	21.4	27.9
C-3	68.8	68.7	68.5	68.8	76.4	(27.1)	67.1
C-4	79.4	79.3	78.9	70.5	88.5	27.3	33.6
C-5	43.0	43.0	42.4	44.8	50.4	43.9	36.6
C-6	19.9	20.5	19.9	25.4	23.6	(27.6)	26.3
C-7	27.2	26.7	26.4	27.2	32.2	26.8	26.7
C-8	36.0	36.0	35.6	36.5	35.4	36.0	35.7
C-9	41.8	41.9	41.6	42.5	55.0	41.1	39.8
C-10	37.3	37.2	36.9	37.5	38.0	35.5	35.1
C-11	20.5	20.9	20.5	21.6	20.9	20.6	21.2
C-12	37.8	37.8	37.5	38.4	37.8	38.5	40.3
C-13	43.9	43.9	43.5	44.8	43.7	42.3	42.7
C-14	55.4	55.4	55.2	56.2	55.7	56.2	56.7
C-15	24.9	24.9	24.5	21.6	24.9	24.6	24.2
C-16	27.4	27.6	27.1	27.9	27.5	28.3	28.3
C-17	55.6	55.8	55.5	56.2	55.6	53.2	56.4
C-18	13.1	13.1	12.7	13.5	13.1	12.5	12.1
C-19	24.4	24.0	23.6	24.5	13.8	24.3	23.9
C-20	140.2	140.6	139.7	140.6	140.1	23.1	35.8
C-21	114.8	115.2	114.5	115.2	114.7	13.3	18.7

[a]In pyridine-d_5.
[b]In C_6D_6.
[c]In $CDCl_3$.
[d]In $CDCl_3$; data for these compounds is from Blunt and Stothers (11).

pregn-20-ene-3β,4β-diol. The 3β,4β configuration of the two alcohols was clear from the coupling constants of the corresponding protons (Table 1). Most indicative for the AB-cis ring junction were the chemical shifts in **1** of C-9 (a 13 ppm upfield shift, in comparison to the 5α-series, by two γ-effects of C-2 and -4) and C-19 (a 10 ppm downfield shift due to the removal of two γ-effects by the same C-2 and -4 atoms). Whereas the δ_C values of rings B–D, except for C-6, which is strongly influenced by the 4β-hydroxyl (a 6.5 ppm upfield γ-effect), are virtually the same as in the model compounds, changes were observed in the carbon atom chemical shifts of ring A. The latter changes can be best explained by the effects of the two hydroxyls at C-3 and -4. After establishing the pregnane and digitalose parts of **1**, the 4-*O*-glycoside linkage between the two was determined from the HMBC experiment in which connectivities between C-4 and H-1′ and between C-1′ and H-4 have been observed. The equatorial stereochemistry of the anomeric proton was clear from the 4 Hz coupling constant between H-1′ and H-2′ (12) (H-2′ and H-3′ have to be axial because of the 10 Hz coupling between them).

Acid hydrolysis (8) of **1** afforded the aglycone and a mixture of the two possible ethyldigitalosides. The latter two ethyl glycosides were directly hydrolyzed and the acetates of the sugar removed by NH_3 to afford L-(−)-digitalose, $[\alpha]_D$ −70° (c = 0.1, H_2O). This compound is the enantiomer of the 6-deoxyhexose isolated from terrestrial sources (9). 3-*O*-methylfucose has been reported from another marine source, namely, from a shellfish; however, as the identifi-

cation was done by gc-ms, no chirality was determined (6).

Verrucoside has been found to have the following IC_{50} values: P-388 IC_{50} = 5.9 μg/ml; A-549 (human lung carcinoma) IC_{50} = 7.2 μg/ml; and HT-29 (human colon carcinoma) IC_{50} = 6.3 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1 dm microcell. Low-resolution mass spectra were recorded on a Finnigan-4021 mass spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-360 spectrometer, equipped with an Aspect 3000 computer and operated at 360 MHz and 90.5 MHz for ^{1}H and ^{13}C, respectively, and on an AM-500 spectrometer. All chemical shifts are reported with respect to TMS (δ = 0).

ISOLATION OF VERRUCOSIDE 4β-*O*-[2-*O*-ACETYL-α-L-DIGITALOPYRANOSYL]-5β-PREGN-20-EN-3β-OL [**1**].—A sample of the gorgonian *E. verrucosa* (PharmaMar 28.06.89.1.0.26), collected near Cadiz, Spain (36° 33.15′ N, 6° 18.72′ W at a depth of 11 m) in June 1989 and deep frozen immediately after collection, was lyophilized to give 250 g of dry material. Extraction of this with CH_2Cl_2-MeOH (1:1) afforded 500 mg of crude material. The CCl_4-soluble fraction of the latter extract (partitioned between 70% aqueous MeOH and CCl_4) was flash chromatographed through a Si gel H (Merck) column eluted with EtOAc/hexane. Verrucoside [**1**] (25 mg) was eluted with EtOAc-hexane (1:1) as an amorphous material: [α]D −30° (*c* = 2.0, CH_2Cl_2). Found C 69.48, H 9.18; $C_{30}H_{48}O_7$ requires C 69.20, H 9.29. Ir ($CHCl_3$) 3450, 1740, 1270, 900 cm^{-1}; cims (CH_4) *m/z* (rel. int.) $[MH]^+$ 521 (1.5), $[MH - HOAc]^+$ 461 (0.8), 329 (5.5), $[aglycone - OH]^+$ 301 (4), $[aglycone - 2H_2O]^+$ 283 (4), $[digitalose]^+$ 219 (1), $[C_9H_{15}O_5]^+$ 203 (100); ^{1}H and ^{13}C nmr see Table 1.

VERRUCOSIDE DIACETATE [**2**].—Verrucoside (2 mg) was left overnight at room temperature in Ac_2O-pyridine (1:1) (0.5 ml). Evaporation under reduced pressure of the reaction mixture afforded compound **2**: an oil; ^{1}H nmr ($CDCl_3$) δ 5.73 ddd (*J* = 16, 11, 7, H-20), 5.33 dd (*J* = 3, 1, H-4′), 5.19 brd (H-3), 5.08 d (*J* = 4, H-1′), 4.95 m (H-21, -21′, and -2′, 3H), 4.02 brq (*J* = 6, H-6′), 3.80 dd (*J* = 12, 3, H-4), 3.67 dd (*J* = 10, 3, H-3′), 2.05 s, 2.09 s, 2.14 s (3H each, the triacetates).

ACID HYDROLYSIS OF COMPOUND **1** TO GIVE 5β-PREGN-20-ENE-3β,4β-DIOL (AGLYCONE) AND L-(−)-DIGITALOSE.—Compound **1** (10 mg) was treated with concentrated HCl-C_6H_6-EtOH (1:1:48) (2 ml) at 65° for 3 h. After neutralization of the acid with Ag_2CO_3 (120 mg), the slurry was filtered and the eluent evaporated under vacuum to afford a residue (11 mg) which was applied to a Sephadex *LH-20* column. The fast-moving fractions contained the aglycone and the slow-moving fractions the substituted digitalose. Compound **3**: mp 125° (Me_2CO/hexane); eims *m/z* $[C_{21}H_{34}O_2 - H_2O]^+$ 300 (0.5%); [α]D +1.5° (*c* = 1.5, $CHCl_3$); ^{1}H nmr ($CDCl_3$, 360 MHz) 5.73 ddd (*J* = 16, 11, 7, H-20), 4.95 d (*J* = 11, H-21), 4.92 d (*J* = 16, H-21), 4.00 brq (*J* = 3, H-3), 3.87 dd (*J* = 12, 3, H-4), 0.99 s (Me-19), 0.58 s (Me-18); ^{13}C nmr see Table 2. The substituted digitalose was directly hydrolyzed with HOAc-THF-H_2O (3:1:1) at 65° for 1 h. The residue after evaporation was treated with 25% aqueous NH_3 for 1 h at 65°, and evaporation under vacuum of the reaction solution gave L-(−)-digitalose (3 mg) (9). Acetylation of the ethylglycoside with Ac_2O/pyridine (0.5 ml) at room temperature overnight gave 2,4-di-*O*-acetyl-1-β-*O*-ethyl-3-*O*-methylfucose as the main anomer. The β configuration was deducted from the coupling constant (8 Hz) between H-1′ and H-2′ (12). δ_H ($CDCl_3$) 5.20 dd (*J* = 7, 1, H-4′), 4.93 dd (*J* = 10, 8, H-2′), 4.27 d (*J* = 8, H-1′, axial), 3.79 dd (*J* = 10, 7, H-3′), 3.58 brq (*J* = 7, H-5′), 1.29 d (*J* = 7, Me-6′); 2.17 and 2.09 (two singlets each of 3H for the two acetates); [α]D −33 (*c* = 0.1, $CHCl_3$)

ACKNOWLEDGMENTS

We are very grateful to Dr. J.M. Tur for the identification of the gorgonian.

LITERATURE CITED

1. Y. Kashman, A. Rudi, S. Hirsch, S. Isaacs, D. Green, D. Blasberger, and S. Carmely, *New J. Chem.*, **14**, 729 (1990).
2. D.J. Pegg, D.M. Doddrell, and M.R. Bendall, *J. Chem. Phys.*, **77**, 2745 (1982).
3. A. Bax and R. Freeman, *J. Magn. Reson.*, **44**, 542 (1981).
4. L. Muller, *J. Am. Chem. Soc.*, **101**, 4481 (1979).
5. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
6. O. Itasaka, *J. Biochem.*, **60**, 435 (1966).
7. T.H. Mareci and R.J. Freeman, *J. Magn. Reson.*, **51**, 531 (1983).
8. S. Carmely, M. Roll, Y. Loya, and Y. Kashman, *J. Nat. Prod.*, **52**, 167 (1989).

9. T. Yamanchi and F. Abe, *Chem. Pharm. Bull.*, **38**, 669 (1990).
10. M. Kobayashi, Y. Kiyota, S. Orito, Y. Kyogoku, and I. Kitagawa, *Tetrahedron Lett.*, **25**, 3731 (1984).
11. J.W. Blunt and J.B. Stothers, *Org. Magn. Reson.*, **9**, 439 (1977).
12. P.J. Garegg and M. Haraldsson, *Tetrahedron*, **46**, 13 (1990).

Received 1 March 1991