A New Steroidal Glycoside from a Caribbean Gorgonian, *Eunicea* sp.[1]

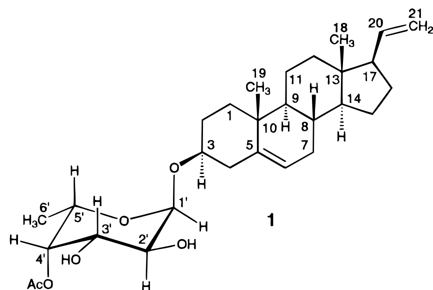
Oscar M. Cóbar,[2] Abimael D. Rodríguez,* and Omayra L. Padilla[3]

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, Río Piedras, Puerto Rico 00931-3346

Received July 2, 1997®

A new saponin possessing a pregnene-derived aglycon (**1**) has been isolated from the Caribbean gorgonian octocoral *Eunicea* sp. The structure of the new compound was assigned on the basis of chemical and spectral studies.

Marine octocorals of the order Gorgonacea produce an extensive range of structurally interesting terpenoid metabolites.[4,5] In the shallow-water habitats of the Caribbean Sea gorgonian soft corals of the genus *Eunicea* are among the most abundant octocorals.[6] In this paper, we report the structure of a new steroidal glycoside (**1**) isolated as a minor component from an undescribed gorgonian species of *Eunicea*. Only one gorgonian-derived steroidal glycoside has been reported from a Caribbean location prior to this investigation.[7]



The specimens of *Eunicea* sp. used in this chemical study were collected in March 1996, near Santa Marta, Colombia, in the eastern Caribbean Sea. Freshly collected animals were stored frozen and subsequently extracted with 1:1 $CHCl_3$−MeOH. Size-exclusion chromatography followed by Si gel chromatography of the hexane extracts gave (+)-α-muurolene as a major metabolite (0.7% based on dry wt).[8] ^{1}H- and ^{13}C-NMR analysis of the nonpolar chromatographic fractions showed the presence of minor quantities of several diterpene glycosides.[9] The lipid extract of *Eunicea* sp. was also found to contain minute amounts of a new saponin possessing a pregnene-derived aglycon. Subsequently, purification by Si gel chromatography yielded pure saponin **1**, which represented about 0.001% of the organic extract.

Compound **1** analyzed for $C_{29}H_{44}O_6$ by mass spectrometry in combination with interpretation of ^{13}C-NMR data. The eight degrees of unsaturation inherent in the molecular formula of **1** could be accounted for by only two carbon−carbon double bonds and one ester carbonyl group. Hence, **1** possessed five rings. A sharp resonance at 2.17 ppm (3H, s) showed that the carbonyl group was probably derived from an acetyl residue. Further, a predominant group of methine resonances between δ 3.6 and 5.2 (5H) and a conspicuous methyl signal at 1.13 ppm (3H, d, J = 6.5 Hz) in conjunction with the presence of an obvious ^{13}C-NMR acetal resonance (δ 97.2, d) suggested the presence of a cyclized, acetylated 6′-deoxyhexose unit. Comparison of the ^{13}C-NMR bands from typical 6′-deoxyhexose acetate models showed that **1** contained an acetylated 6′-deoxyhexose ring in the pyranose form.[10] The structural details of the tetracyclic portion of the molecule were readily determined by NMR and mass spectrometry analyses. The aglycon analyzed for $C_{21}H_{32}O$ by HRMS of the saponin and was confirmed as an alcohol by intense $M^+ - H_2O$ fragmentation. The ^{13}C- and ^{1}H-NMR spectra of **1** immediately suggested that the aglycon was the degraded sterol pregna-5,20-dien-3β-ol (pregnadienol), the same aglycon that had been previously isolated from the sponge *Damiriana hawaiiana*[11] and the distantly related octocorals *Gersemia rubiformis*,[12] *Muricea californica*,[13] *Muricea fruticosa*,[13] and *Pseudoplexaura wagenaari*.[7] By subtraction of the molecular formula of pregnadienol from the overall formula of **1**, the sugar component was shown to possess the composition $C_8H_{12}O_5$. Elimination of one acetyl residue from the formula left $C_6H_{10}O_4$, which is the formula of a typical deoxy-hexose.

The complete assignment of the deoxy-hexose unit was accomplished by spin-decoupling, coupling constant analysis, and NOE experiments. Through these experiments, the sugar unit in **1** was identified as a 4′-*O*-acetylfucose in a α-pyranoside configuration. The C-4′ proton was apparent as the lowest sugar methine proton (δ 5.20), indicating that the acetate ester was at the sugar C-4′ position. The anomeric proton at C-1′ was the second lowest field sugar methine proton (δ 5.04). This proton was a doublet with J = 4.0 Hz, which confirmed it as an equatorial proton, thus showing the hemiacetal linkage as axial or α. Because H-3′ was split into a doublet of doublets (J = 9.7 and 3.4 Hz), it was clearly an axial proton. From this we concluded that H-2′ was *trans axial* to H-3′ and cis to H-1′; therefore, the C-2′ hydroxyl must be equatorial. Furthermore, H-4′ (a doublet with J = 3.4 Hz) had to be equatorial, cis to both H-3′ and H-5′. A NOESY correlation from H-3′ to H-5′ confirmed the 1,3-diaxial relationship of the latter protons. Because the anomeric oxygen is trans to CH_3-6′, the monosaccharide belongs to the α-series. The stereochemistry of the 6′-deoxy-monosaccharide unit was determined by chiral GC-MS analysis of the acid hydrolysate.[14] Thus, the sugar component in the marine-derived saponin **1** was concluded to be 4′-*O*-acetyl-α-L-fucopyranose.

An HMBC correlation between the anomeric proton at 5.04 ppm (C-1′) to a carbon at 78.2 ppm (C-3)

* To whom correspondence should be addressed. Phone: (787) 764-0000, ext-4799. FAX: (787) 751-0625. E-mail: abrodrig@upracd.upr.clu.edu.

® Abstract published in *Advance ACS Abstracts,* November 1, 1997.

S0163-3864(97)00321-2 CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognosy

connected the monosaccharide to the A ring of pregnadienol and yielded the final structure, **1**. Attempts to confirm the identity and absolute configuration of the pregnadiene aglycon by comparison of the hydrolysis product of **1** with the authentic natural product were precluded by the limited amount of sample available (1.8 mg).[13,15] However, by use of Hudson's rules of isorotation, the negative molecular rotation difference between glycoside **1** and L-fucose suggested that the pregnadiene aglycon in **1** must possess the same absolute configuration as found in *Muricea californica* and *Muricea fruticosa*.[16]

Experimental Section

General Experimental Procedures. Experimental details have been reported.[17] The enantiomeric sugars and *N*-heptafluorobutyrylimidazole were purchased from Sigma.

Animal Material. *Eunicea* sp. is an undescribed species belonging to the subgenus *Euniceopsis* with a characteristic strongly armed anthocodial crown and reduced club sclerites in the external layer. The external appearance of *Eunicea* sp. resembles *E. fusca* Duchassaing & Michelotti, while their sclerites and anthocodial crown resemble *Eunicea calyculata* (Ellis & Solander) forma *coronata* Bayer. *Eunicea* sp., however, showed notable differences in its calyx architecture and arrangement besides the presence of a characteristic colorless and coarse, capstan sclerites uncommon in other *Eunicea* species. Also, this species has been observed in simpatry at several Colombian Caribbean reefs. *Eunicea* sp. forms individual colonies of up to 1 m in height and profusely branched, while *E. fusca* is generally below 0.4 m in height, forming a dense bed of asexual fragments. On the other hand, a phylogenetic cladistic analysis revealed an ample dichotomous separation consistent with other species and several autoapomorphies, in both external and spicular characters, clear evidence of a valid species. Specimens of *Eunicea* sp., under the name Sánchez USNM 97733, are on deposit in the octocoral collection, Smithsonian Institution, Washington, DC, under the curatorship of Dr. Frederick M. Bayer. Additional voucher specimens (ICN-MHN−CO-098) are on deposit at the Instituto de Ciencias Naturales−Museo de Historia Natural, Universidad Nacional de Colombia.

Collection and Extraction. Minced and freeze-dried specimens of *Eunicea* sp. (2.1 kg) collected near Santa Marta Bay, Colombia, were extracted with $CHCl_3$−MeOH (1:1) (8 × 1 L). After filtration the crude extract was evaporated to yield a residue (170.5 g) that was suspended in H_2O and then partitioned between *n*-hexane (6 × 2.5 L), $CHCl_3$ (8 × 4 L), and *n*-BuOH (5 × 1.5 L). The hexane extract was concentrated to yield 72.0 g of a green oil, which, after filtration in toluene solution, was fractionated by size-exclusion chromatography on a Bio-Beads SX-3 (toluene). The combined portions were concentrated to obtain three main fractions, the last one of which consisted of (+)-α-muurolene (14.5 g, 0.7% based on dry wt).[8] The remaining two fractions [fractions A (31.7 g) and B (24.3 g)] were concentrated and evaluated by NMR. A portion of fraction B (ca. 7.0 g) was chromatographed over Si gel (750 g) with 20% hexane−EtOAc and fractionated roughly into subfractions 1 through 16 on the basis of TLC analyses. Subfraction 15 (65.6 mg) was purified by column chromatography on Si gel using 4:1 hexane−Me_2CO to afford the C_{21} pregnene-glycoside **1** (1.8 mg; 0.0025% of the hexane extract).

Pregnene glycoside 1: saponin isolated as a colorless oil; $[\alpha]^{24}{}_D$ −150° (*c* 0.3, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε) 216 (3.34); IR (neat) 3410, 2917, 2867, 2848, 1740, 1451, 1437, 1374, 1259, 1237, 1162, 1131, 1080, 1039, 972, 909, 820, 799 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (1H, dd, *J* = 4.2, 15.2 Hz, H-1a), 1.87 (1H, dd, *J* = 5.4, 15.2 Hz, H-1b), 1.58 (1H, m, H-2a), 1.86 (1H, m, H-2b), 3.49 (1H, m, H-3), 2.25 (1H, m, H-4a), 2.35 (1H, ddd, *J* = 1.9, 3.1, 12.1 Hz, H-4b), 5.36 (1H, br d, *J* = 5.2 Hz, H-6), 1.48 (1H, m, H-7a), 2.02 (1H, m, H-7b), 1.70 (1H, m, H-8), 0.96 (1H, m, H-9), 1.40 (1H, m, H-11a), 1.53 (1H, m, H-11b), 1.25 (2H, m, H-12a,b), 1.02 (1H, m, H-14), 1.18 (1H, m, H-15a), 1.68 (1H, m, H-15b), 1.54 (1H, m, H-16a), 1.79 (1H, m, H-16b), 1.97 (1H, m, H-17), 0.61 (3H, s, Me-18), 1.02 (3H, s, Me-19), 5.76 (1H, ddd, *J* = 7.8, 10.8, 16.5 Hz, H-20), 4.96 (1H, d, *J* = 10.8 Hz, H-21a), 4.98 (1H, d, *J* = 16.5 Hz, H-21b), 5.04 (1H, d, *J* = 4.0 Hz, H-1′), 3.79 (value reported in C_6D_6 solution: 1H, dd, *J* = 4.0, 9.7 Hz, H-2′), 3.92 (1H, dd, *J* = 3.4, 9.7 Hz, H-3′), 5.20 (1H, d, *J* = 3.4 Hz, H-4′), 4.12 (1H, br q, *J* = 6.5 Hz, H-5′), 1.13 (3H, d, *J* = 6.5 Hz, H-6′), 2.17 (3H, s, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 37.3 (t, C-1), 29.7 (t, C-2), 78.2 (d, C-3), 38.8 (t, C-4), 140.1 (s, C-5), 122.2 (d, C-6), 32.0 (t, C-7), 37.4 (d, C-8), 50.4 (d, C-9), 36.8 (s, C-10), 20.7 (t, C-11), 29.6 (t, C-12), 43.4 (s, C-13), 55.9 (d, C-14), 24.9 (t, C-15), 27.2 (t, C-16), 55.3 (d, C-17), 12.7 (q, C-18), 19.4 (q, C-19), 139.8 (d, C-20), 114.5 (t, C-21), 97.2 (d, C-1′), 69.4 (d, C-2′), 70.1 (d, C-3′), 73.0 (d, C-4′), 65.2 (d, C-5′), 16.2 (q, C-6′), 20.8 (q, OCO*C*H_3), 171.3 (s, O*C*OCH_3); EIMS *m*/*z* (rel int) 300 (2), 284 (18), 283 (86), 282 (100), 267 (11), 213 (8), 189 (12), 171 (26), 161 (13), 159 (11), 149 (19), 147 (13), 145 (12), 135 (13), 129 (29), 121 (19), 119 (15), 111 (22), 109 (24), 107 (21), 105 (21), 97 (37), 95 (38), 93 (28), 91 (23), 85 (36), 83 (48), 79 (26), 71 (59), 69 (82), 67 (39), 57 (80), 55 (82); HRFABMS *m*/*z* 511.30161 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_6Na$, 511.30356); HREIMS *m*/*z* 300.24540 $[M - C_8H_{12}O_5]^+$ (calcd for $C_{21}H_{32}O$, 300.24546).

Chiral GC−MS Analysis of the Acid Hydrolysate of 1.[14] A 0.5-mg portion of glycoside **1** was dissolved in 1 N HCl (0.2 mL) and heated at 48−50 °C for 3 h. The reaction mixture was evaporated in a stream of nitrogen, stored under vacuum, suspended in *N*-heptafluorobutyrylimidazole (0.1 mL), heated for 30 min at 60 °C with occasional mixing, and evaporated in a stream of nitrogen. The residue was dissolved in CH_2Cl_2 (0.1 mL), and the resulting clear solution was chromatographed directly on a Chirasil-Val (Alltech) column. The oven temperature was maintained for 3 min at 60 °C and raised to 200 °C at 4 °C/min. Retention times for the authentic monosaccharide residues after simultaneous treatment with 1N HCl and worked up in the same manner (min): D-fucose (9.76), L-fucose (10.01). Retention time (min) of GC peak in the acid hydrolysate of pregnene glycoside **1**: 10.03 (L-fucose).

Acknowledgment. The assistance of J. A. Sánchez, F.M. Ablanque, and H. Castellanos from INVEMAR (Santa Marta, Colombia) in specimen collection is gratefully acknowledged. HRFABMS spectral determinations were performed by the Midwest Center for Mass Spectrometry at the University of Nebraska−Lincoln, a

National Science Foundation Regional Facility (Grant No. CHE8211164). HMBC and HREIMS determinations were performed by Ms. Catherine Ramírez and Mr. Rafael Almodóvar, respectively. We gratefully appreciate the taxonomic assignment provided by Dr. Frederick M. Bayer, Smithsonian Institution, Washington, DC. This study was supported by the NSF–EPSCoR (Grant No. R118610677), NIH–MBRS (Grant No. S06RR08102-17), NIH–MARC U*STAR (Grant No. 5T34GM07821-17), NSF–MRCE (Grant No. R11-8802961), and FIPI Programs of the University of Puerto Rico at Río Piedras.

References and Notes

(1) Taken in part from the Ph.D. Dissertation of O. M. Cóbar, University of Puerto Rico, 1996.
(2) Graduate student sponsored by the NSF–MRCE and UPR–FIPI programs.
(3) Undergraduate student supported by the NIH–MARC U*STAR program of the U. P. R.
(4) Coll, J. C. *Chem. Rev.* **1992**, *92*, 613–631.
(5) Rodríguez, A. D. *Tetrahedron* **1995**, *51*, 4571–4618.
(6) Bayer, F. M. *The Shallow-Water Octocorallia of the West Indian Region*, Martinus Nijhoff: The Hague, 1961.
(7) Wasylyk, J. M.; Martin, G. E.; Weinheimer, A. J.; Alam, M. *J. Nat. Prod.* **1989**, *52*, 391–394.
(8) (a) Ciereszko, L. S.; Karns, T. K. B. In *Biology and Geology of Coral Reef*; Jones, O. A.; Endean, R., Eds.; Academic Press: New York, 1973; pp 183–203. (b) Weinheimer, A. J.; Schmitz, F. J.; Ciereszko, L. S. In *Drugs from the Sea*; Freudenthal, H. D., Ed.; Marine Technology Society: Washington, D. C., 1968; pp 135–140.
(9) Three diterpenoid hexose glycosides possessing dilophol aglycons were also isolated from this specimen of *Eunicea* sp.; see Cóbar, O. M.; Rodríguez, A. D.; Padilla, O. L.; Sánchez, J. A. *J. Org. Chem.* **1997**, *62*, 7183–7188.
(10) Roussis, V.; Wu, Z.; Fenical, W.; Strobel, S. A.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 4916–4922.
(11) Delseth, C.; Carlson, R. M. K.; Djerassi, C.; Erdman, T. R.; Scheuer, P. J. *Helv. Chim. Acta* **1978**, *61*, 1470–1476.
(12) Kingston, J. F.; Gregory, B.; Fallis, A. G. *J. Chem. Soc.*, *Perkin Trans. 1* **1979**, 2064–2068.
(13) Bandurraga, M. M.; Fenical, W. *Tetrahedron* **1985**, *41*, 1057–1065.
(14) Leavitt, A. L.; Sherman, W. R. *Carbohydr. Res.* **1982**, *103*, 203–212.
(15) Julian, P. L.; Meyer, E. W.; Printy, H. C. *J. Am. Chem. Soc.* **1948**, *70*, 887–892.
(16) By use of Hudson's Rules of Isorotation, the molecular rotation [M] of **1** can be defined as sum of the $[M]_{pregnadienol} + [M]_{sugar}$. For references see: (a) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933–1944. (b) Ripperger, H.; Preiss, A.; Schmidt, J. *Phytochemistry* **1981**, *20*, 2434–2435.
(17) Rodríguez, A. D.; Soto, J. J. *J. Org. Chem.* **1996**, *61*, 4487–4490.

NP970321X