Journal of Natural Products
Vol. 52, No. 2, pp. 391-394, Mar-Apr 1989

ISOLATION AND IDENTIFICATION OF A NEW PREGNENE GLYCOSIDE FROM THE GORGONIAN *PSEUDOPLEXAURA WAGENAARI*

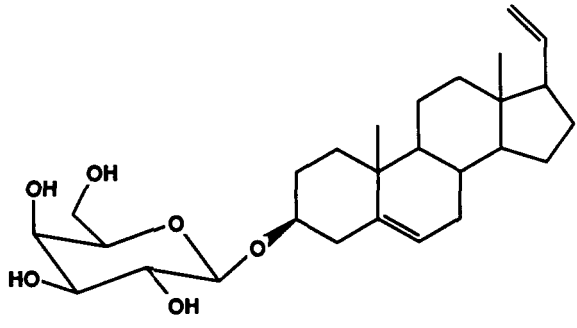
JOHN M. WASYLYK, GARY E. MARTIN, ALFRED J. WEINHEIMER, and MAKTOOB ALAM*

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Houston, Houston, Texas 77204-5515

ABSTRACT.—A new pregnene glycoside was isolated from the gorgonian *Pseudoplexaura wagenaari* and its structure determined by two-dimensional nmr spectroscopy.

Extracts from four gorgonians of the genus *Pseudoplexaura*—*Pseudoplexaura porosa, Pseudoplexaura flagelosa, Pseudoplexaura crucis,* and *Pseudoplexaura wagenaari*—have been shown to contain several novel sterols and terpenes (1–4). In this communication we report the isolation and identification of a new pregnene-type steroidal glycoside [**1**] from *P. wagenaari* Stiasny (Plexauridae). The structure of **1** was established by spectroscopic methods and was finally confirmed by single crystal X-ray crystallography (5).

Repeated chromatography over Si gel of the polar fraction (CH_2Cl_2, from solvent partitioning) of the iPrOH extract followed by crystallization afforded needles, mp 268–270°. The ^{1}H-nmr spectrum in C_5D_5N showed the presence of two methyl singlets (δ 0.54 and 0.87), a complicated methylene envelope (between δ 0.8 and 2.0), a methine proton (δ 3.98), and a vinylic proton multiplet (δ 5.38) suggesting the possibility of a steroidal moiety in **1**. Additionally, four broad singlets at δ 6.38, 6.60, 6.81, and 7.05 along with resonances at δ 4.11 (1H, t, J = 6.0 Hz), 4.23 (1H, dd, J = 9.3, 2.9 Hz), 4.41 (2H, m), 4.60 (1H, m), and 4.92 (2H, m) implied that **1** is a steroidal glycoside. The sugar moiety in **1** was also supported by the presence of four acetoxy methyl resonances (δ 2.01, 2.03, 2.09, and 2.12) in the ^{1}H-nmr spectrum of the acetylated product. The ^{13}C-nmr spectrum showed the presence of a total of 27 carbons (3 × C, 12 × CH, 10 × CH_2, and 2 × Me), six of which (δ 103.3, 78.6, 76.4, 75.4, 72.8, and 70.3) could be assigned to a sugar residue; the remaining 21 carbons could thus belong to a C-21 pregnane-type aglycone. The ^{13}C resonances at δ 141.4 (s), 140.1 (d), 121.8 (d), and 114.9 (t) along with the mass spectral fragment at *m/z* 299 (100%), suggested that the aglycone was a pregnadienol (mol wt



1

300). A 17-vinyl substituent was indicated by the resonances in the ^{1}H-nmr spectrum at δ 5.73 (1H, m), 4.98 (1H, d, J = 11.7 Hz), and 4.97 (1H, d, J = 15.7 Hz), which correspond well with the literature values for vinyl pregnenes (6). The ^{1}H-^{1}H correlation spectrum (COSY) (7) clearly showed the connectivities between resonances at δ 5.73 and 4.98, as well as connectivities between H-20 (δ 5.73) and H-17 (δ 2.01, ddd, J = 15.4, 8.7, 1.35 Hz). The scalar (J) coupling pathways leading from H-3α to H-4α and H-4β, and to H-2α and H-2β, and finally to H-1α and H-1β, were also elucidated from the COSY spectrum of **1**. The connectivities between the sugar protons were easily discerned from the COSY spectrum, which showed coupling proceeding from H-1′ (δ 4.92) to H-2′ (δ 4.45) to H-3′ (δ 4.21) to H-4′ (δ 4.6), and from H-5′ (δ 4.11) to H-6′ (δ 4.45). Coupling between H-4′ and H-5′ was observed only when slices representing the proton resonating at δ 4.11 were individually printed. The stereochemistry of the glycosidic bond was determined as β on the basis of $J_{H\text{-}1'H\text{-}2'}$ values [8.2 Hz (8)] and the absence of coupling between the protons H-1′ and H-3α (9).

A ^{1}H-^{13}C correlation (HC-COSY) spectrum (10) was used to identify protons bonded to individual carbons while the application of a relayed coherence transfer experiment (RCT2D) (11) allowed the observation of proton connectivities over three adjacent carbons. Although the HC-COSY experiment provides direct ^{1}H-^{13}C correlations, the RCT2D spectrum contains both the direct ^{1}H-^{13}C responses and relayed responses which arise from ^{1}H-^{1}H vicinal couplings. Thus, the RCT2D experiment allows the proton-proton and carbon-carbon connectivity network to be deduced irrespective of congestion in the proton spectrum if the carbon spectrum can be resolved. Using a RCT2D spectrum the proton and carbon resonances linking C-2 to C-3 and C-4 were identified, as well as the five-carbon (proton-bearing) segment from C-6 to C-11. Connectivities between the H-11 and C-12 and likewise H-12 and C-11 could not be established due to overlap of the heteronuclear correlation and relay responses. Similar overlaps prevented the establishment of the network between either H-15 and C-16 or C-15 and H-16. For the remainder of the aglycone, the connectivities between carbons 14 and 15, 16 and 17, and 20 and 21 were noted in the spectrum. Recently Hughes has applied RCT2D nmr spectroscopy to the establishment of proton chemical shifts in steroids (12).

In order to confirm the presence of a pregnene-type aglycone, **1** was hydrolyzed with 4.5 N H_2SO_4 in dioxane. The aglycone was extracted with EtOAc, which upon evaporation gave needles, mp 139°, eims *m/z* 300, 285, 282, and 267. The ^{1}H-nmr ($CDCl_3$) spectrum of the aglycone showed the presence of two methyls resonating at δ 1.02 (3H, s) and 0.61 (3H, s) along with four vinylic protons [δ 5.73 (1H, m), 5.36 (1H, m), 4.97 (1H, d, J = 11 Hz), and 4.96 (1H, d, J = 15 Hz)] and a carbinylic proton resonating at δ 3.52. The seven-line pattern of the proton at δ 3.52 was typical of 3β substituted steroids (13). The COSY spectrum of the aglycone showed the connectivities between protons resonating at δ 5.73, 4.98, 4.96, and 1.98 (H-17α). The sugar was identified as galactose by gc as the silyl derivative. The structure of **1** was finally confirmed by a single-crystal X-ray diffraction method (5) to be 3β-pregna-5,20-dienyl-β-D-galactopyranoside.

The aglycone had been previously isolated from a sponge, *Gersemia* sp. or sea raspberry, found in the Atlantic Ocean near Newfoundland (14). The same sterol has also been identified in extracts from the sponge *Damiriana hawaiiana* (15) collected in Hawaii, and recently Bandurraga and Fenical (6) reported the isolation of four new esterfied amino-

galactose saponins from the Pacific gorgonian *Murucea californica* which contain the steroid nucleus. Our nmr data agrees with that previously reported (6), and by employing the HC-COSY spectrum we were able to assign all carbon resonances unambiguously, including revisions of those previously reported for C-3, C-7, C-8, and C-12.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Fisher-Johns apparatus and are uncorrected. All nmr experiments were carried out on a Nicolet NT-300 wide bore spectrometer operating at 300.068 and 75.459 MHz for ^1H and ^{13}C observations, respectively. The instrument was equipped with a Model 293-C pulse programmer and a 5-mm $^1H/^{13}C$ dual tuned probe. The COSY spectrum (7) was acquired on a sample prepared by dissolving 18 mg of **1** in 0.4 ml of deuterated pyridine. The HC-COSY spectrum was acquired by using the pulse sequence of Freeman and Morris (10) with phase cycling of Bax and Morris (16) to allow quadrature detection in both frequency domains. The ^{13}C multiplicities were determined using the APT experiment as reported by Patt and Shoolery (17). The gc was performed on a Hewlett-Packard 5730A gas chromatograph equipped with a 3385A Automation System and an OV-17 column.

P. wagenaari was collected from Key Biscayne, Florida, in 1979, and stored in iPrOH. A voucher specimen is deposited in the Department of Medicinal Chemistry, University of Houston. After evaporation to dryness, a total of 780 g of extract was partitioned between aqueous MeOH and hexane, CCl_4, and CH_2Cl_2. The latter of two fractions was chromatographed on Si gel (60–200 mesh) in step gradient fashion using 100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2. Fractions from 2 to 6% MeOH in CH_2Cl_2 were combined and rechromatographed on Si gel as above. Evaporation of the combined fractions from 5, 6, and 7% MeOH in CH_2Cl_2 gave a white compound which was recrystallized with CH_2Cl_2/MeOH to give needles, mp 268–270°: ^1H nmr (C_5D_5N) δ 7.05 (1H, bs), 6.81 (1H, bs), 6.60 (1H, bs), 6.38 (1H, bs), 5.73 (1H, m), 5.38 (1H, bd m), 4.98 (1H, d, J = 11.7 Hz), 4.97 (1H, d, J = 15.7 Hz), 4.92 (1H, d, J = 8.2 Hz), 4.60 (1H, m), 4.41 (2H, m), 4.23 (1H, dd, J = 9.3, 2.9 Hz), 4.11 (1H, t, J = 6.0 Hz), 3.98 (1H, m), 2.70 (1H, m), 2.44 (1H, m), 2.12 (2H, m), 2.01 (1H, ddd, J = 15.5, 8.7, 1.35 Hz), 1.89 (1H, m), 0.87 (3H, s), 0.54 (3H, s); ^{13}C nmr (C_5D_5N) δ 141.4 (s, C-5), 140.1 (d, C-20), 121.8 (d, C-6), 114.9 (t, C-21), 103.3 (d, C-1′), 78.4 (d, C-3), 76.8 (d, C-5′), 75.4 (d, C-3′), 72.8 (d, C-2′), 70.3 (d, C-4′), 62.4 (t, C-6′), 56.2 (d, C-14), 55.7 (d, C-17), 50.9 (d, C-9), 43.7 (s, C-13), 39.5 (t, C-4), 37.7 (t, C-12), 37.7 (t, C-1), 37.2 (s, C-10), 34.4 (t, C-7), 32.4 (d, C-8), 30.3 (t, C-2), 27.2 (t, C-16), 25.2 (t, C-15), 21.1 (t, C-11), 19.4 (q, C-19), 12.9 (q, C-18).

Peracetylation of **1**.—Compound **1** (5 mg) was acetylated with 50 μl Ac_2O and 50 μl pyridine at room temperature for 12 h, followed by a standard workup. ^1H nmr ($CDCl_3$) δ 5.73 (1H, m), 5.37 (1H, bd m), 5.18 (1H, dd, J = 10.5, 7.9 Hz), 5.02 (1H, dd, J = 10.5, 3.5 Hz), 4.98 (1H, d, J = 11.5 Hz), 4.95 (1H, d, J = 15.7 Hz), 4.55 (1H, d, J = 7.9 Hz), 4.15 (1H, dd, J = 24.3, 11.2 Hz), 4.14 (1H, dd, J = 17.9, 11.2 Hz), 3.88 (1H, dt, J = 13.6, 1.0 Hz), 3.74 (1H, bd m), 3.49 (1H, bd m), 2.15 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 1.98 (3H, s), 0.99 (3H, s), 0.60 (3H, s).

Hydrolysis of **1**.—Compound **1** (5 mg) was hydrolyzed in 4.5 N H_2SO_4 [9.0 N H_2SO_4-dioxane (1:1)] at room temperature for 24 h. The reaction mixture was diluted and extracted with CH_2Cl_2. The organic layer was washed with a saturated solution of $NaHCO_3$, concentrated, and dried over $MgSO_4$ to yield 3.2 mg of the aglycone, mp 139°: ^1H nmr ($CDCl_3$) δ 5.76 (1H, m), 5.36 (1H, bd m), 4.97 (1H, d, J = 11 Hz), 4.96 (1H, dd, J = 15 Hz), 3.52 (1H, m, J = 11.9, 11, 4.2 Hz), 1.02 (3H, s), and 0.61 (3H, s). The aqueous fraction was neutralized with $Ba(OH)_2$ solution, filtered, and freeze-dried. The sugar was analyzed as a silyl derivative (TriSil Z) by gc. The retention time was compared with standard sugars after silylation.

ACKNOWLEDGMENTS

This work was supported in part by grants (E-792 to GEM and E-745 to MA) from the Robert A. Welch Foundation, Houston, Texas and the University of Houston–Coastal Center. *P. wagenaari* was collected and identified by Dr. R.E. Schroeder.

LITERATURE CITED

1. W. Fenical, in: "Marine Natural Products, Chemical and Biochemical Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, 1979, Vol II, pp. 174–245.
2. A.J. Weinheimer, C.W.J. Chang, and J.A. Matson, in: "Progress in Chemistry of Organic Natural Products." Ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, New York, 1979, pp. 285–387.
3. B. Tursch, J.C. Brackman, D. Daloze, and M. Kaisin, in: "Marine Natural Products, Chemical and Biological Perspectives." Academic Press, New York, 1978, Vol. II, pp. 247–296.

4. L.J. Goad, in: "Marine Natural Products, Chemical and Biochemical Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, 1978, Vol. II, pp. 89–93.
5. M.B. Hossain, D. van der Helm, J.M. Wasylyk, and M. Alam, *Acta Crystallogr., Sect. C*, **43**, 2424 (1987).
6. M.B. Bandurraga and W. Fenical, *Tetrahedron*, **41**, 1057 (1985).
7. A. Bax, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
8. M. Kaneda, K. Mizutani, Y. Takahashi, G. Kurono, and Y. Nishikawa, *Tetrahedron Lett.*, 3937 (1974).
9. G. Batta and A. Liptak, *J. Am. Chem. Soc.*, **106**, 248 (1984).
10. R. Freeman and G.A. Morris, *J. Chem. Soc., Chem. Commun.*, 684 (1978).
11. P.H. Bolton, *J. Magn. Reson.*, **48**, 336 (1982).
12. D.W. Hughes, *Magn. Reson. Chem.*, **26**, 214 (1988).
13. N.S. Bacca and D.H. Williams, "Application of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, 1964, pp. 77–81.
14. J.F. Kingston, B. Gregory, and A.G. Fallis, *J. Chem. Soc., Perkin Trans. 1*, 2064 (1979).
15. C. Delseth, R.M.K. Carlson, C. Djerassi, T.R. Erdman, and P.J. Scheuer, *Helv. Chim. Acta*, **61**, 1470 (1978).
16. A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
17. S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, **46**, 535 (1984).

Received 12 August 1988