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A NEW SPERMATOSTATIC GLYCOSIDE FROM THE SOFT CORAL *SINULARIA CRISPA*

L.M.V. TILLEKERATNE,* G.K. LIYANAGE,

Department of Chemistry

W.D. RATNASOORIYA,

Department of Zoology, University of Colombo, Colombo 3, Sri Lanka

MOHAMAD B. KSEBATI, and FRANCIS J. SCHMITZ*

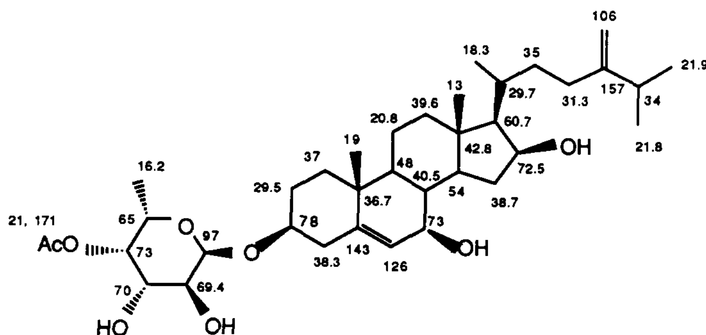
Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019

ABSTRACT.—The steroid glycoside **1** has been isolated from the Sri Lankan soft coral *Sinularia crispa*. The structure has been determined by spectroscopic (^{1}H and ^{13}C nmr) techniques. Glycoside **1** showed spermatostatic activity on rat cauda epididymal spermatozoa.

In our search for antifertility agents from marine organisms we have screened the CH_2Cl_2-MeOH (1:1) extracts of a number of alcyonacean soft corals for spermatostatic activity on rat cauda epididymal spermatozoa (1). The crude extract of one of these soft corals, *Sinularia crispa* Tixier-Durivault (Alcyonaceae) showed potent spermatostatic effects at 0.5 mg/ml on rat cauda epididymal spermatozoa. In this paper we report the structure of a new spermatostatic steroid glycoside **1** isolated from this source as one of the compounds responsible for spermatostatic effects.

The crude CH_2Cl_2/MeOH extract was dissolved in aqueous MeOH and partitioned separately against hexane, $CHCl_3$, and EtOAc. The $CHCl_3$ and EtOAc solubles were combined and fractionated by gel permeation chromatography and reversed-phase hplc to give pure **1** plus other compounds.

The molecular formula of glycoside **1** was determined by high resolution fabms to be $C_{36}H_{58}O_8$ ([M + Na]$^+$ 618.4080 ± 7.9 ppm), corresponding to eight degrees of unsaturation. ^{13}C-nmr data supported this formula and also revealed that there were two double bonds [one trisubstituted, 143 (s), 126 (d), and one exocyclic methylene, 157 (s), 106 (t)], one ester carbonyl [171 (s)], one acetal [97 ppm (d)], and seven other oxygenated carbons. The acetal carbon was confirmed by decoupling (see below) to be part of a pyranose sugar; the carbonyl resonance was due to an acetate group (methyl proton signals at 2.18 ppm). Hence the aglycone was confirmed to be tetracyclic. A steroid skeleton was indicated by ^{1}H-nmr signals for two quaternary methyl singlets at δ 0.9 and 1.06, a methyl doublet at δ 1.01, a six-proton doublet at δ 1.03 for the methyls of an isopropyl group and a



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multiplet at 3.5 ppm typical of the steroidal H-3α. The ^{13}C-nmr data also were in general indicative of a steroid (2).

The anomeric proton of the sugar unit was identified by its correlation with the ^{13}C-nmr signal of the anomeric carbon, and the corresponding one bond ^{1}H/^{13}C coupling (172 Hz) confirmed that the glycosidic linkage was α-oriented (3). Using the anomeric proton as a starting point, difference double resonance (DDR) experiments and information from both COSY and RCT spectra (4) established the H-1′ to H-6′ sequence in the sugar unit. The low field position of H-4′, 5.20 ppm, required that this hydroxyl group be acetylated. The relative stereochemistry of this sugar unit followed from the vicinal coupling constants (see Experimental) and independent confirmation that the anomeric oxygen is α-oriented (see above). The observed coupling constants are in good agreement with those reported for 1-methoxy α-fucose (5). The presence of an acetylated moiety as determined by nmr spectroscopy was also consistent with the base peak observed in the low resolution ei mass spectrum, *m/z* 412, which corresponds to the loss of this acetylated sugar plus a hydrogen from the molecular ion.

The 3.5 ppm multiplet attributed to the H-3α of a steroid correlated with a carbon doublet resonance at 78 ppm. DDR and COSY data revealed the proton sequence from H-3 to H-8. Difference decoupling (irr 5.29 ppm) established allylic coupling between H-6 and both C-4 protons and also coupling between H-6 and H-7, thus fixing the location of the trisubstituted double bond. The large coupling between H-7 and H-8 (8 Hz) was consistent with a diaxial relationship and hence a 7β-OH configuration. Two additional 11.3 Hz couplings in the H-8 signal are consistent with the H-8β orientation (axial) characteristic of a conventional steroid with 9α,14α neighboring protons.

The remaining hydroxyl group could be assigned to either position 15 or 16 based on the multiplicity of the corresponding carbinol proton and its vicinal neighbors (determined by difference decoupling spectra) and the chemical shifts of carbons 14–17. However, the chemical shift of C-20 (29.7 ppm) [firmly established by H/C correlation after identifying H-20 by decoupling (irr H-21 doublet)], which is 5–6 ppm upfield from that in conventional sterols (ca. 36 ppm) (6), can only be accounted for by the γ-gauche effect of a 16β-OH substituent (6,7). The chemical shifts for C-17 and C-14 are then assigned as 60.7 and 54 ppm, respectively, which is in the correct relative order for the β and γ substituent effects of the 16β and 7β hydroxyl groups on these carbon resonances.

Most of the side chain structure was also verified by nmr analysis. Thus the methyls of the isopropyl group and the exocyclic methylene protons were shown to be coupled to H-25, and coupling between H-23 and H_2-28 was also confirmed by COSY data. Likewise the H-21 to H-20 coupling was confirmed. The chemical shift of H-9 was assigned from the H/C correlation data after all the other methine proton signals had been identified by decoupling or COSY information.

The location of the acetylated fucose unit at C-3 was established by an INAPT experiment (8), which showed coupling between H-3 and the anomeric carbon.

Pure **1** exhibited spermatostatic activity on rat cauda epididymal spermatozoa at 0.5 mg/ml.

EXPERIMENTAL

GENERAL.—^{1}H-nmr spectra were recorded at 300 MHz and ^{13}C spectra at 75.4 MHz on a Varian XL-300 spectrometer; chemical shifts are reported in ppm (δ) downfield from internal TMS. Ir spectra were measured on a Perkin-Elmer Model 298 spectrometer. High-resolution fab mass spectra were recorded on a VG ZAB-E mass spectrometer. An Altex 5 μm × 9.6 mm × 29.9

cm semipreparative Adsorbosphere reversed-phase C_{18} column was used for separation.

EXTRACTION AND ISOLATION OF STEROID GLYCOSIDE **1**.—Fresh specimens of *S. crispa* collected at Kalkudah, Batticaloa on the east coast of Sri Lanka were cut into small pieces and soaked in CH_2Cl_2-MeOH (1:1) at room temperature for 2 weeks. A voucher specimen is on deposit at the Museum of the Department of Zoology, University of Colombo, Sri Lanka (Registration No. D.Z.M. C 126). The organic layer was recovered by filtration and concentrated under reduced pressure at 28°. The resultant crude extract (71.4 g) was dissolved in 300 ml of MeOH-H_2O (9:1). The hexane solubles were extracted by partitioning twice with equal volumes of hexane. The aqueous phase was then diluted to give an MeOH-H_2O (3:2) solution, and this was partitioned twice against 300-ml portions of $CHCl_3$. The aqueous phase was concentrated under reduced pressure at 28° to remove MeOH, and the aqueous residue was extracted with an equal volume of EtOAc. The $CHCl_3$ and EtOAc solubles (12.7 and 0.23 g, respectively) were combined, concentrated at reduced pressure, and chromatographed over Sephadex LH-20, collecting 25-ml fractions.

Fractions were pooled based on Si gel tlc analysis [5% MeOH/$CHCl_3$ eluent, vanillin/H_2SO_4 spray] to give three major fractions, F-1, F-2, and F-3. Fraction F-2 was chromatographed again on Sephadex LH-20 using $CHCl_3$-hexane-EtOH (10:10:1), and 10-ml fractions were collected. Seven major fractions were obtained after combination based on tlc analysis. Fraction 6 of this group (0.23 g) showed two bright blue spots on tlc analysis conducted as described above. Resolution of this fraction by hplc using a C_{18} reversed-phase column with H_2O-MeOH (1:3) as eluent yielded three compounds, the major one being glycoside **1**, recrystallized from aqueous MeOH to obtain white needles: mp 172.5–173°; $[\alpha]^{28}D$ $-6.3°$ (c = 5.26, $CHCl_3$); ir ν max ($CHCl_3$) 3600–3200, 1720 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.29 (1H, br s, H-6), 5.20 (1H, br d, 3.5, H-4′), 5.03 (1H, d, 4, H-1′), 4.73 and 4.70 (each 1H, br s, H_2-28), 4.39 (1H, ddd, 8.5, 7.5, 5.3, H-16), 4.12 (1H, br q, 7, H-5′), 3.92 (1H, dd, 10, 3.5, H-3′), 3.73 (1H, dd, 10, 4, H-2′), 3.79 (1H, br d, 8, H-7), 3.50 (1H, m, H-3), 2.48 (1H, dt, 13, 7.5, 7.5, H-15β), 2.38 (1H, br dd, 14, 5, H-4α), 2.24 (1H, br dd, 13, 14, H-4β), 2.24 (1H, heptet, H-25), 2.16 and 1.88 (each 1H, m, H_2-23), 2.18 (3H, s, OAc), 1.92 and 1.65 (each 1H, m, H_2-2), 1.84 (1H, m, H-20), 1.52 (1H, dt, 5.3, 13, 13, H-15α), 1.47 (1H, dt, 8, 11.3, 11.3, H-8), 1.12 (3H, d, 7, H-6′), 1.06 (3H, s, H-19), 1.03 (6H, d, 7, H-26, -27), 1.01 (3H, d, 7, H-21), 1.02 (1H, m, H-9), 1.00 (1H, dd, 8.5, 11.3, H-17), 1.01 (1H, m, H-14), 0.90 (3H, s, H-18); ^{13}C nmr see structure **1**; hrms (12 eV) $[M - C_8H_{13}O_5]^+$ 429 (18), [M − H − fucosyl 4-acetate] 412 (100), 328 (14), 269 (25), 189 (32), 124 (93).

SPERMATOSTATIC ACTIVITY.—Cauda epididymal spermatozoa were obtained from anesthetized rats as described in detail previously (1). Only samples having sperm counts >70 × 10^6 sperm/ml, motility >80%, and morphology >95% normal forms were used. The glycoside **1** was dissolved in Krebs solution (9) containing 1% DMSO and 1% pentamethylene glycol, and antimotility effects were investigated for a period of 30 min using a microscopic method (1).

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