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ISOLATION AND STRUCTURE OF ISOPROPYL-α-D-GLUCOSIDE FROM THE CORAL *SCLEROPHYTUM CAPITALIS*

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ABSTRACT.—Marine soft coral *Sclerophytum capitalis* was found to contain an unusually high concentration of isopropyl-α-D-glucopyranoside, which was isolated and identified by spectroscopic methods.

Recently we reported (1,2) the isolation and structure of a number of diterpenes from the $CHCl_3$ extract of the soft coral *Sclerophytum capitalis* (Pratt). An investigation of the polar fraction of this coral has resulted in the isolation of two glycosides, which were identified by spectroscopic methods as isopropyl-α-D-glucopyranoside and sitosterol-β-D-glucopyranoside.

The MeOH extract of *S. capitalis* upon evaporation and lyophilization gave a residue which was defatted with hexane, followed by trituration with $CHCl_3$. The $CHCl_3$-insoluble residue was extracted with MeOH, and the extract was evaporated to dryness. The residue from the MeOH extract upon repeated Si gel chromatography followed by either preparative tlc or hplc gave two glycosides.

The ^1H nmr spectrum of the first compound (0.4% yield) in D_2O showed the presence of an isopropyl residue [δ 0.97 (3H, d, $J = 6.7$ Hz), 1.02 (3H, d, $J = 6.7$ Hz), 3.70 (1H, dq, $J = 6.7$ Hz)] along with seven oxymethine protons [δ 3.18 (1H, dd, $J = 9.0$, 5.9 Hz), 3.32 (1H, dd, $J = 9.7$, 3.8 Hz), 3.47 (1H, d, $J = 9.2$ Hz), 3.51–3.68 (3H, m), 4.82 (1H, d, $J = 3.7$ Hz)] suggesting that this compound could be isopropylhexose. The ^1H nmr spectrum of the acetylation product also supported this inference. The ^1H-^1H correlation [COSY] (3) and ^1H-^{13}C correlation [HC-COSY] (4) spectral data were consistent with the proposed structure. The ^{13}C chemical shifts of C-2, C-3, C-4, C-5, and C-6 in this compound and those of the corresponding carbons in methyl-α-D-glucopyranoside matched well when the two spectra were run under identical conditions, indicating that the isopropyl group was attached to C-1 of the glucose residue. The configuration of the glycosidic linkage was determined to be α on the basis of the coupling constant [$J_{H\text{-}1,H\text{-}2} = 3.7$ Hz] (5). The position of the isopropyl group and configuration of the glycosidic bond was further confirmed by enzymatic hydrolysis with α-glucosidase. A sample of the hydrolytic product was directly analyzed by gc, resulting in the identification of iPrOH. The reaction mixture on dialysis against H_2O followed by lyophilization of the dialysate gave a residue that was identified as α-D-glucose by gc of the silyl derivative. On the basis of spectral data this compound was identified as isopropyl-α-D-glucopyranoside.

The other compound (0.1% yield) was isolated as colorless needles, mp 300–303°, and was identified as sitosterol-β-D-glucopyranoside on the basis of spectroscopic data of the parent compound and its acid hydrolytic products.

Although sugars constitute the primary metabolites of living organisms, and have been found as components of glycosides (terpenoidal, steroidal, etc.) as well as complex carbohydrates, occurrence of simple sugars in marine invertebrates is not well documented. In fact,

the occurrence of isopropyl glucoside in such a high concentration (0.4% of the total MeOH extract) in a marine organism is unprecedented. Because PrOH was not used at any phase of storage, extraction, and/or purification it would be safe to assume that isopropyl-α-D-glucopyranoside is not an artifact produced during this investigation. However, the production of isopropyl-α-D-glucopyranoside by symbiotic algae, such as zooxanthallae, cannot be ruled out at this point.

EXPERIMENTAL

INSTRUMENTATION.—Mp's were determined on a Fisher-Johns melting point apparatus. The spectra were recorded on the following instruments: nmr, Nicolet NT-300 and Bruker AM-400 (WB); ms, Finnigan Model 1020, equipped with an Incos data system; hplc, Waters Associates Model LC 1200, equipped with a 10μ silica cartridge in a Radial Compression Module (Model RCM100) and Model 401 differential refractometer; gc, HP model 5730A gas chromatograph equipped with a Model 3385 data system and a 0.25″ × 6′ glass column packed with either 2.5% SE-30 on gas chrom Q (for sugar and aglycone) or Porapak Q (for alcohol). α-Glucosidase was purchased from Sigma Chemical Co., St. Louis, MO.

EXTRACTION AND ISOLATION.—*S. capitalis* collected from the waters of Enewetak, Micronesia was immersed in MeOH immediately after collection. The coral was collected and identified by Dr. R.E. Schrouder. A voucher specimen is deposited in the Department of Medicinal Chemistry, U. of Houston collection. The MeOH extract was decanted, concentrated, and lyophilized to give 41.5 g of a residue which was defatted with hexane, followed by trituration with $CHCl_3$ and finally with MeOH. The residue from the MeOH-soluble fraction (21 g) was subjected to cc over Si gel (500 g, 70–230 mesh, E. Merck). The column was eluted with a step-wise gradient of MeOH (5–25%, 500 ml each) in EtOAc. Fractions 14–19 (100 ml each) showed the presence of two major compounds on tlc (Si gel, solvent EtOAc), and were combined. Repeated chromatography of the residue from fractions 14–19 resulted in the separation of partially purified isopropyl-α-D-glucopyranoside and sitosterol-β-D-glucopyranoside.

Isopropyl-α-D-glucopyranoside.—Partially purified isopropyl-α-D-glucopyranoside (450 mg) was purified by preparative tlc using EtOAc-MeOH (8:2) as solvent to give 166 mg of isopropyl-α-D-glucopyranoside as a hygroscopic powder: mp 32°, $[\alpha]_D$ 7.5 ($c = 0.0275$, MeOH); ^{13}C-nmr (D_2O) δ 21.2 (C-1 or 3), 23.0 (C-3 or 1), 61.5 (C-6′), 70.6 (C-4′), 71.2 (C-2), 72.0 (C-5′), 72.4 (C-2′), 73.4 (C-3′) and 96.9 (C-1′); ^{1}H nmr (D_2O) δ 0.97 (3H, d, =6.7 Hz), 1.02 (3H, d, $J = 6.7$ Hz) 3.18 (1H, dd, $J = 9.0$, 5.9 Hz), 3.32 (1H, dd, $J = 9.7$, 3.8 Hz), 3.51–3.68 (3H, m), 3.47 (1H, d, $J = 9.2$ Hz), 3.70 (1H, dq, $J =$ 6.7 Hz, H-2), 4.82 (d, $J = 3.7$ Hz, H-1′).

Acetylation.—Isopropyl-α-D-glucopyranoside (87 mg) was dissolved in 2 ml of pyridine-Ac_2O (1:1). The solution was allowed to stand overnight, after which the reaction mixture was poured over crushed ice, mixed, and filtered. The residue was purified by hplc on a Si gel cartridge with EtOAc-hexane (1:1) as the solvent: ^{1}H-nmr ($CDCl_3$) δ 1.08 (3H, d, $J = 6.2$ Hz, H-1 or 3), 1.20 (3H, d, $J = 6.2$ Hz, H-3 or 1), 1.97, 1.99, 2.01, and 2.10 (3H each, s, CO*Me*), 3.82 (1H, dq, $J = 6.2$, Hz, H-2), 4.06 (1H, m, H-5), 4.10 (1H, m, H-6α), 4.22 (1H, dd, $J = 10.2$, 4.6 Hz, H-6β), 4.76 (1H, dd, $J = 10.2$, 3.7 Hz, H-2′), 5.01 (1H, t, $J = 9.7$ Hz, H-4′), 5.15 (1H, d, $J = 3.7$ Hz, H-1′), 5.45 (1H, t, $J = 9.7$ Hz, H-3′).

Enzymatic hydrolysis.—Isopropyl-α-D-glucopyranoside (2.3 mg) was dissolved in a solution of 2.5 mg of α-glucosidase in 1.5 ml of H_2O (pH 6.3). The mixture was allowed to stand overnight. A 2-μl sample was directly injected on a Porapak Q column (column temperature 80°). The reaction mixture (1.2 ml) was dialyzed (2×), at room temperature against distilled H_2O (50 ml). The dialysate was lyophilized and analyzed for sugars, as silyl derivative (tri Sil Z), by gc (2.5% SE-30 on gas chrom Q).

Sitosterol-β-D-glucopyranoside.—Sitosterol-β-D-glucopyranoside was purified by hplc utilizing two columns (RP-18) connected in series, with MeOH-H_2O (92:8) as solvent system. Crystallization from MeOH gave needles: mp 300–303°; $[\alpha] -35°$; ^{1}H-, ^{13}C-nmr, and ms data matched well with the literature (6,7).

Acid hydrolysis.—Sitosterol-β-D-glucopyranoside (40 mg) was refluxed in 5 ml of 1 N aqueous H_2SO_4-MeOH (1:1), for 18 h, after which the reaction mixture was concentrated and extracted with EtOAc. The residue from the EtOAc was purified by hplc, on a Si gel cartridge, to yield needles, mp 137–138°, and identified as sitosterol by direct comparison with an authentic sample using accepted techniques (mmp, ^{1}H nmr, ^{13}C nmr, ms). The aqueous layer was neutralized with $Ba(OH)_2$ and filtered. The filtrate was lyophilized and the sugar was analyzed, as silyl derivative (using Tri-sil Z, Pierce), by gc.

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