Journal of Natural Products
Vol. 50, No. 2, pp. 263-265, Mar-Apr 1987

A NEW SPIROSTANOL GLYCOSIDE FROM *AGAVE CANTALA*

O.P. SATI,* U. RANA, D.C. CHAUKIYAL,

Department of Chemistry, University of Garhwal, Srinagar (Garhwal), 246 174, India

and M. SHOLICHIN

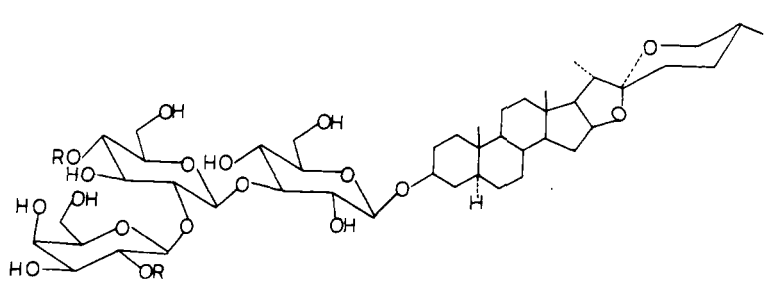
Natural Products Division, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, 57301, Japan

Agave plants have long enjoyed a reputation in the Indian system of medicine (1) and have recently been reported to exhibit anticancer activity (2). Different spirostanol glycosides have been reported (3-5) from the leaves and rhizomes, and a hecogenin-based triglucoside has been characterized (6) from the fruits of *Agave cantala* Roxb. (Agavaceae). Here, we report a new tigogenin based pentaglycoside from the fruits of this plant.

Repeated column chromatography of the complex saponin mixture obtained from the fruits of *A. cantala* afforded compound **1**, which was identified as 3-*O*-[β-D-xylopyranosyl(1→2)-β-D-galactopyranosyl (1→2)-β-D-xylopyranosyl(1→4) -β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl]-(25*R*)-5α-spirostan-3β-ol. It showed the typical ir spiroketal absorption of a 25 *R* spirostanol glycoside. Acid hydrolysis furnished tigogenin; and D-galactose, D-glucose, and D-xylose in the ratio of 1:2:2, as determined by photocolorimetry (7). A molecular weight of 1166 was established by the appearance of a molecular ion in its fabms and peaks at 1034[M-132(pentose-H_2O)], 902 [M-2×132], 872[1034-162(hexose-H_2O)], 740[M-(2×132)-162], 578 [M-2(132+162)] indicated the sequence of sugars.

Hydrolysis of the permethylate of **1** prepared by Hakomori's method (8), afforded 2,3,4-tri-*O*-methyl-D-xylose, 3,4,6-tri-*O*-methyl-D-galactose, 2,4,6,tri-*O*-methyl-D-glucose, and 3,6-di-*O*-methyl-D-glucose. 3,4,6-Tri-*O*-methyl-D-glucose and 3,6-di-*O*-methyl-D-glucose gave intense pink colors with Wallenfel's reagent (9), indicating monosaccharides unsubstituted in the 2-position.

Partial hydrolysis of **1** afforded tigogenin and four prosapogenins, namely PS_1, PS_2, PS_3, and PS_4. Interglycosidic linkages were determined by acid hydrolysis, and permethylation followed by acid hydrolysis of these units. The identification of the permethylated sugars was done by comparison with authentic samples (10,11), color reaction with Wallenfel's reagent, and $NaIO_4$ oxidation results. The types of linkages at the glycosidic positions and the positions of linkages were further confirmed by ^1H- and ^{13}C-nmr spectra. The assignment of signals in ^{13}C nmr was made by comparison with the reported data of tigogenin (12) and sugars (13). Insensitive



1 R=β-D-xylopyranosyl

Nuclei Enhancement by Polarization Transfer (INEPT) was particularly helpful for spectral editing.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were recorded with a 'Boetius' microscopic apparatus. Fabms was obtained on a JEOL JMS DX-300 instrument in the negative ion mode using triethanolamine as solvent and xenon as gas. ^{1}H-nmr spectra were recorded on a JEOL PS-100 (100 MHz) and ^{13}C nmr on a JEOL FX-100 Fourier-transform spectrometer (25 MHz) in C_5D_5N with TMS as an internal standard. Cc was performed on Si gel (BDH, 60-120 mesh) and tlc on Kieselgel 60 G (Merck). The spots on tlc were visualized by spraying with 10% alcoholic H_2SO_4 followed by heating. Pc was carried out on Whatman No. 1 paper using the descending mode and developed with aniline hydrogen phthalate. Colorimetric estimations (420 nm) were recorded on a Syntronic spectrophotometer type 103. The following chromatographic solvent systems were used: (A) $CHCl_3$-MeOH (9:1.5), (B) $CHCl_3$-MeOH-H_2O (13:7:2), (C) EtOAc-C_5D_5N-H_2O (10:4:3), (D) *n*-BuOH-EtOH-H_2O (5:1:4), (E) Light petroleum (60-80°)-EtOAc (1:1), C_6H_6-Me_2CO (8:2).

ISOLATION.—The fruits (2 kg) of wild *A. cantala* were collected from Srinagar, U.P., and specimens were identified by the Forest Research Institute, Dehradun, U.P. (a voucher specimen is available there). The plant material upon exhaustive extraction with MeOH afforded a complex mixture of saponins which yielded compound **1** (3 g) on repeated cc (solvent A).

COMPOUND **1**.—Colorless needles (350 mg) from MeOH, mp 301-304°; ir max cm^{-1} 3350 (OH), 985, 910, 895, 880 (intensity 895>910, 25 *R* spiroketal); fabms (*m/z*) 1166, 1065, 1034, 902, 872, 740, 578, 416, 139; ^{1}H nmr 4.86 (1H, d, J=7 Hz), 5.12 (3H, m), 5.56 (1H, d, J=6.2 Hz) [anomeric protons] ppm; ^{13}C nmr 79.6 (C-3 of tigogenin), 106.0, 103.7, 102.4, 104.7 (glycosylated carbons) ppm; INEPT 3/4J, irradicated ^{1}H, CH_2(out of phase): 21.2 (C-11), 28.9 (C-6), 29.1 (C-24), 29.9 (C-2), 31.8 (C-23), 32.1 (C-15), 32.3 (C-7), 37.2 (C-1), 40.2 (C-12), 60.7, 62.1, 66.9 [C-6 of hexose and C-5 of pentose units]. (Found: C, 56.14; H, 7.36. $C_{55}H_{90}O_{26}$ requires C, 56.68; H, 7.80).

ACIDIC HYDROLYSIS OF **1**.—Compound **1** (50 mg) was refluxed with 2N HCl-EtOH (1:1, 10 ml) on a boiling water bath for 3.5 h to afford the aglycone (tigogenin) colorless needles, mp 203-205° [lit. (10) mp 202-204°]; ms (*m/z*) 416 [M]$^+$. The neutralized (Ag_2CO_3) and concentrated aqueous hydrolysate showed the presence of D-galactose, D-glucose, and D-xylose (pc, solvent C, Rf values 0.20, 0.23, and 0.37, respectively).

PARTIAL HYDROLYSIS OF **1**.—Glycoside **1** (200 mg) in 1N HCl-BuOH (1:1, 25 ml) was heated at 70° for 2.5 h. The BuOH layer was washed with H_2O and evaporated to dryness in vacuo. The residue after cc (solvent A) yielded tigogenin (5 mg), and prosapogenins PS_1 (20 mg), PS_2 (16 mg), PS_3 (20 mg), and PS_4 (70 mg).

ACIDIC HYDROLYSIS OF THE PROSAPOGENINS.—The prosapogenins PS_1, PS_2, PS_3, and PS_4 (3 mg each) were hydrolysed as above. The neutralized and concentrated hydrolysate from PS_1 and PS_2 gave D-glucose; PS_3 contained D-glucose and D-galactose, while PS_4 gave D-glucose, D-galactose, and D-xylose (pc).

PERMETHYLATES OF **1**, PS_1, PS_2, and PS_3, and PS_4.—Glycoside **1** (100 mg), PS_1, PS_2, PS_3, and PS_4 (7 mg each) were separately permethylated (8). The permethylate of **1** was purified by cc (solvent E), and those of PS_1, PS_2, PS_3, and PS_4 were purified by preparative tlc (solvent F, visualizing agent, H_2O). Hydrolysis of the permethylates of **1** (10 mg) and PS_1, PS_2, PS_3, and PS_4 (3 mg each) was performed by refluxing with N HCl-MeOH (1:1, 6 ml). The neutralized and concentrated hydrolysate from **1** showed (pc, solvent D) 2,3,4-tri-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-galactose, and 3,6-di-*O*-methyl-D-glucose. The spots corresponding to the last two sugars gave intense pink color with Wallenfel's reagent. Permethylated hydrolysates of: PS_1 gave 2,3,4,6-tetra-*O*-methyl-D-glucose; PS_2 gave 2,4,6,-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose; PS_3 gave 2,3,4,6,-tetra-*O*-methyl-D-galactose, 3,4,6-tri-*O*-methyl-D-glucose, and 2,4,6,-tri-*O*-methyl-D-glucose, while PS_4 afforded 2,3,4-tri-*O*-methyl-D-xylose, 3,4,6-tri-*O*-methyl-D-galactose, 3,4,6-tri-*O*-methyl-D-glucose, and 2,4,6-tri-*O*-methyl-D-glucose. The identities of these sugars were also confirmed by direct comparison with some available authentic samples, their color reactions with Wallenfel's reagent, and the results of $NaIO_4$ oxidation studies of the sugars as well as the glycosides.

ACKNOWLEDGMENTS

The authors are thankful to Professors T. Kawasaki and K. Miyahara Setsunan, Japan, for the fabms and ^{13}C nmr spectra and the Council of Scientific and Industrial Research, New Delhi, India, for financial assistance. We also thank the staff of the Systematic Botany Branch, Forest Research Institute, for the identification of plant material.

LITERATURE CITED

1. K.R. Kirtikar and B.D. Basu, in: "Indian Medicinal Plants," M/S Periodical Experts, Delhi, 1975, p. 2466.
2. B.S. Aswal, D.S. Bhakuni, A.K. Goel, K. Kar, B.N. Mehrotra, and K.C. Mukherjee, *Indian J. Exp. Biol.*, **22**, 312 (1984).
3. I.P. Varshney, D.C. Jain, and H.C. Srivastava, *Phytochemistry*, **21**, 239 (1982).
4. S.C. Sharma and O.P. Sati, *Phytochemistry*, **21**, 1820 (1982).
5. O.P. Sati, G. Pant, K. Miyahara, and T. Kawasaki, *J. Nat. Prod.*, **48**, 395 (1985).
6. I.P. Varshney, D.C. Jain, and H.C. Srivastava, *J. Nat. Prod.*, **44**, 662 (1981).
7. S.B. Mishra and V.K. Mohan Rao, *J. Sci. Ind. Res.*, **19**, 173 (1960).
8. S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
9. K. Wallenfel, *Naturwissenschaften*, **37**, 491 (1950).
10. S.C. Sharma, R. Chand, O.P. Sati, and A.K. Sharma, *Phytochemistry*, **22**, 1241 (1983).
11. O.P. Sati and G. Pant, *Phytochemistry*, **24**, 123 (1985).
12. H. Eggert and C. Djerassi, *Tetrahedron Lett.*, **42**, 3635 (1975).
13. S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).
14. A. Bax, R. Freeman, and S.P. Kempsell, *J. Am. Chem. Soc.*, **102**, 4849 (1980).

Received 2 June 1986