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NEW PREGNANE GLYCOSIDES FROM *CARALLUMA TUBERCULATA*

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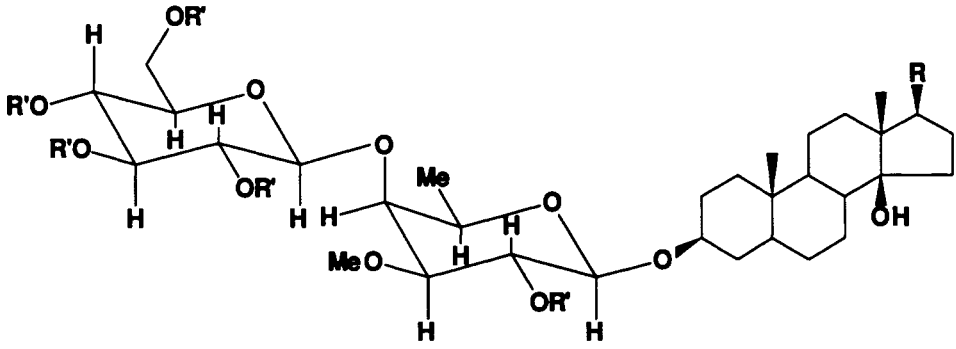
ABSTRACT.—Two new pregnane glycosides, named caratubersides A and B, were isolated from the whole plant of *Caralluma tuberculata*. Their structures were determined as 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-desoxy)-galactopyranosyl]-14-hydroxy-14β-pregnane-20-one [**1**] and 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-desoxygalactopyranosyl)]-14,20-dihydroxy-14β-pregnane [**2**] mainly on the basis of spectroscopic studies. Their stereochemistry has been determined by nOe difference, COSY 45, and 2D *J*-resolved NOESY ^{1}H-nmr and DEPT ^{13}C-nmr experiments.

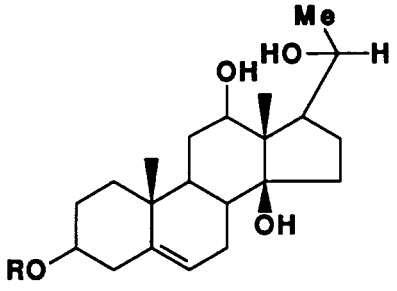
Caralluma tuberculata N.E. Brown (Asclepiadaceae) (syn. *Boucerosia aucheriana* Decn.) is a succulent perennial herb occurring wild and also cultivated throughout Pakistan (1–3). This plant is eaten raw or cooked as a vegetable and is also reputed to be a cure for diabetes and rheumatism (4). In 1967, Nikaido *et al.* (5) reported the isolation and structure elucidation of two genins, boucerin [**4**] and dihydroboucerin [**5**], from *B. aucheriana* Decn., a synonym of *C. tuberculata*. After hydrolysis of the glycosides, these authors also identified cymarose, sarmentose, oleandrose, and digitoxose in the hydrolysate. Apart from this work no other chemical investigation on this species is reported in the literature. This paper presents the isolation and characterization of two new pregnane glycosides.

RESULTS AND DISCUSSION

The EtOH extract of the whole plant of *C. tuberculata* yielded, after evaporation, fractionation, and repeated chromatographic separation on Si gel columns, a mixture of **1** and **2**. The mixture gave positive tests with Liebermann-Burchard reagent (for sterols), vanillin sulfate (for genins), and Molisch reagent (for sugars). Glycosides **1** and **2** were separated by preparative hplc employing a Bond-Pak C-18 reversed-phase column, with MeOH-H_2O (80:20) as the mobile phase.



1 R=-$\overset{O}{\overset{\|}{C}}CH_3$, R′=H
2 R=-CH(OH)CH$_3$, R′=H
3 R=-$\overset{O}{\overset{\|}{C}}CH_3$, R′=Ac



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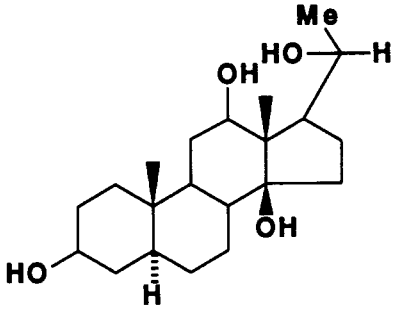

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Caratuberside A [**1**] was obtained as a white, crystalline substance, mp 170–171°, $[\alpha]^{20}D$ +60° (c = 0.66 in MeOH). On acidic hydrolysis with 1% HCl, **1** yielded a crystalline genin as well as glucose and another sugar. Glucose was identified by tlc as well as hplc. The structure of the second sugar was determined as 6-deoxy-3-*O*-methyl-β-D-galactose on the basis of extensive nmr studies including ^1H nmr (COSY-45, *J*-resolved, NOESY, nOe difference) and ^{13}C nmr (broad band and DEPT experiments) on **1** as described below.

Caratuberside A [**1**] displayed no absorption in the uv region indicating the absence of any conjugated double bonds in the molecule. The ir spectrum (KBr) revealed peaks at max 3300 (OH), 2900 (CH_2), 1690 (C=O), 1100 (C-O) cm^{-1}. In the negative ion fabms the compound showed an $[M-H]^-$ peak at *m/z* 655. In the eims spectrum the highest peak at *m/z* 477 was due to the removal of the terminal glucose unit, indicating the 6-deoxy-3-*O*-methyl-D-galactose is directly attached to the aglycone. There was also a strong peak at *m/z* 317 due to the aglycone without oxygen at C-3 and a base peak at *m/z* 299 $[317-H_2O]^+$.

The aglycone was obtained as a crystalline compound by acidic hydrolysis, and its structure was confirmed by spectroscopy. Because *C. tuberculata* belongs to the Asclepiadaceae, which are known to contain pregnane glycosides, and because the pregnane genins, boucerin and dihydroboucerin, have actually been isolated from the acid hydrolysate of the glycosides of this plant, it was expected that the aglycone could possibly be a pregnane type steroid. The ^1H- and ^{13}C-nmr spectra of **1** confirmed this assumption. However, the ^{13}C-nmr spectrum showed that an oxygen function at C-12 commonly found in pregnane aglycones of the Asclepiadaceae is missing (C-12, 39.44 ppm). The chemical shift of C-13 at 49.49 ppm with only one hydroxyl-bearing vicinal carbon atom (C-14, 84.76 ppm) and the chemical shift of C-11 at 21.63 ppm also support the absence of oxygen function in the neighboring C-12. This fact is further confirmed in the mass spectral data discussed above.

The ^1H-nmr of **1** in CD_3OD reveals methyl singlets at δ 0.83 (3H, H-19), 1.08 (3H, H-18), and 2.33 (3H, H-21). The last peak indicates the presence of a $COCH_3$ group in the compound. A doublet at δ 1.28 (3H, J = 6 Hz) is assigned to the secondary methyl group in the 6-deoxy sugar, and a singlet at δ 3.49 is assigned to the OMe group of the methylated sugar. A triplet at δ 2.90 (J = 5.6 Hz) can be assigned to H-17 vicinal to the ketonic group. This chemical shift and coupling are very close to those reported for H-17 in 13β,14-dihydroxy-14β-pregn-5-en-20-one (6). It is, therefore, concluded that the configuration of **1** at C-17 is the same as in this latter compound; i.e., the side chain at C-17 is β-oriented. The spectrum further shows two anomeric proton signals at δ 4.35 (d, J = 7.7 Hz) and 4.60 (J = 7.6 Hz). The latter is assigned to the anomeric proton of glucose, whereas the former is assigned to the H-1 signal of the methylated 6-desoxy sugar. In both cases the coupling constants indicate diaxial cou-

pling, hence β-glycosidic linkages. It also proves that the hydroxylic groups at the neighboring carbon atoms in the two sugars (C-2′ and C-2″) have the same equatorial configuration. It may be noted here that the Keller-Killiani test for 2-desoxy sugars gave negative results with **1**. This means that the 6-desoxy sugar possessed an OH group at C-2′.

Decoupling experiments, as well as COSY-45 experiments, show that the H-1′ doublet at δ 4.35 is coupled with a double doublet at δ 3.59 (J = 7.7, 10 Hz) due to H-2′. This means that $J_{2',3'}$ is equal to 10 Hz, and, therefore, H-3′ must also be axially oriented. The H-3′ signal becomes clear only in the ^{1}H-nmr spectrum of caratuberside A pentaacetate [**3**] where this signal is not shifted downfield because it bears a methoxy rather than a hydroxy group. It absorbs as a double doublet at δ 3.23 (J = 10, 2.5 Hz), which indicates that the methoxy group at C-3′ is equatorial and H-3′ is axial. The smaller $J_{3',4'}$ (2.5 Hz) also shows that H-4′ is equatorial. H-4′ absorbs as a broad doublet at δ 4.17; the $J_{4',5'}$ is very small and could not be calculated. H-5′ absorbs as a broad quartet at δ 3.65 (J = 6.3 Hz) which is coupled to the doublet at δ 1.28 as shown by the decoupling experiment or irradiation of the methyl doublet at 1.28 when this quartet is converted into a broad singlet.

The configuration of the methyl group (H-6′) was determined by nOe difference measurements of **3**. Irradiation of the H-5′ quartet at δ 3.49 resulted in 13.6% increase of the signal at δ 3.2 (H-3′) and 10.2% increase in the intensity of the doublet at δ 4.37 (H-1′). The intensity of the methyl doublet at δ 1.2 also increased by 19.3%. On the other hand, irradiation of the H-3′ double doublet at δ 3.2 led to 5.6% nOe of H-1′ at 4.37 but no increase in the intensity of methyl doublet at δ 1.2. This indicates that H-1′, H-3′, and H-5′ are axial, and 6-Me is equatorial. Thus, the methylated sugar was identified as 6-deoxy-3-*O*-methyl-D-galactose. The ^{13}C-nmr spectrum of **1** (Table 1) supports its proposed structure. It clearly shows the absence of olefinic carbon atoms, indicating that there is no carbon double bond in the compound. The assignments were aided by DEPT experiments as well as correlation with ^{13}C-nmr spectra of similar types of compounds (6).

Caratuberside B [**2**] was also obtained as a white crystalline compound, mp 182° (dec), which on acidic hydrolysis gave a pure genin as well as glucose and 6-deoxy-3-*O*-methyl-β-D-galactose. Caratuberside B does not show a carbonyl absorption in the ir spectrum. In the ^{13}C-nmr spectrum the peak assigned to the carbonyl carbon (C-20) of **1** is missing. Instead, a peak at 65.30 ppm is present indicating an additional hydroxyl-bearing carbon which, according to the DEPT spectrum, is secondary. These observations together with the $[M-H]^-$ peak at *m/z* 657 in the negative ion fab spectrum suggested that the carbonyl group in **1** has been reduced to a secondary alcohol in **2**. This is supported by the observation that the peak at 63.01 ppm (C-17) is shifted upfield to 57.08 ppm and the C-21 methyl peak from 32.37 ppm in **1** to 21.53 ppm in **2**. The correlation between **1** and **2** was confirmed by reduction of **1** to **2** with $NaBH_4$. Thus, the structure of **2** is proved to be 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-(3-*O*-methyl-6-desoxygalactopyranosyl]-14,20-dihydroxy-14β-pregnane.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on Electrothermal Gallenkamp apparatus (capillary) and are uncorrected. Optical rotations were measured with a JASCO Dip-140 digital polarimeter. Tlc was carried out on Si gel PF 254 precoated glass plates (Merck 70–230 mesh ASTM) using the following solvent systems: (A) $CHCl_3$-MeOH-H_2O (7.5:2.5:0.2), (B) *n*-BuOH-HOAc-H_2O (12:3:5). Paper chromatography for sugars was run on Whatman paper No. 1 using solvent system (C) EtOAc-MeOH-H_2O-HOAc (65:15:15:20). A saturated solution of aniline phthalate in *n*-BuOH was used as a staining agent. Hplc analysis was performed with a LDC Constrametric model III instrument using a Bonda-Pak C-18 reversed-phase column (25 cm length, 1 mm diameter) and a monitor

TABLE 1. ^{13}C-nmr Data of Caratubersides A [**1**] and B [**2**] in C_5D_5N (75.43 MHz).

Carbon	Compound	
	1	**2**
C-1	37.49	37.63
C-2	29.96	30.10
C-3	71.53	72.03
C-4	34.74	34.88
C-5	44.48	44.65
C-6	29.21	29.40
C-7	28.14	28.18
C-8	40.63	40.91
C-9	49.65	50.05
C-10	36.07	36.18
C-11	21.63	22.86
C-12	39.44	40.91
C-13	49.49	49.09
C-14	84.76	83.83
C-15	32.02	33.01
C-16	24.84	18.85
C-17	63.01	57.08
C-18	12.22	12.36
C-19	15.73	15.48
C-20	216.39	65.30
C-21	32.37	21.53
C-1′	102.41	102.51
C-2′	76.80	76.84
C-3′	85.50	85.58
C-4′	77.30	77.48
C-5′	70.48	70.55
C-6′	17.76	17.82
C-1″	105.51	105.58
C-2″	76.12	76.17
C-3″	78.34	78.41
C-4″	71.94	71.61
C-5″	78.51	78.59
C-6″	63.18	63.25
OMe	59.50	59.05

III machine with refractive index detector. Uv was recorded in MeOH on a Shimadzu uv-240 spectrophotometer. Ir spectra were recorded in KBr on Jasco A-302 infrared spectrophotometer. Electron impact mass spectra were determined on a Finnigan MAT 312 Varian MAT 112 double focusing mass spectrometer connected to a PDP 11/34 (DEC) computer system. Fast atomic bombardment mass spectral analysis was performed on the Varian MAT 312 fitted with a standard source and a high-field magnet. ^{1}H-nmr spectra were obtained from a Bruker AM-300 (300 MHz) spectrometer using both CD_3OD and C_5D_5N as solvents and TMS as an internal standard (s, singlet; d, doublet; q, quartet; m, multiplet). ^{1}H chemical shifts are reported from TMS and coupling constants are in Hz. ^{13}C-nmr spectra were measured in C_5D_5N and CD_3OD at 75.43 MHz with TMS as an internal standard using the Bruker AM-300 spectrometer. High-field ^{1}H-nmr spectroscopy was performed on a Bruker WM 400 instrument equipped with an Aspect 2000 computer. Two-dimensional COSY-45 experiments were performed at 400 MHz.

PLANT MATERIAL.—The whole plant of *C. tuberculata* (25 kg) was purchased from the vegetable market of Karachi, and identification was kindly carried out by Prof. Dr. S.I. Ali, Department of Botany, University of Karachi. A voucher specimen is deposited on a herbarium sheet in our department.

EXTRACTION AND SEPARATION.—The fresh material was chopped into small pieces and extracted with EtOH after percolation at room temperature for 15 days. The EtOH extract was evaporated under reduced pressure, which furnished a dark green thick semisolid residue (122 g). The extract so obtained was

partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract (73 g) was redissolved in a small quantity of MeOH, then poured into cold Et_2O to precipitate the crude glycoside mixture. This was repeated several times, and the yellow precipitate was collected by filtration. The crude mixture (43 g) gave a positive test with Liebermann-Burchard, vanillin sulfate, and ceric sulfate reagents, whereas Keller-Kiliani and xanthydrol tests for 2-deoxy sugars were negative. The crude glycoside (36 g) was subjected to cc and eluted with $CHCl_3$-MeOH (9:1) which gave a mixture of two components. These two components were detected on tlc (PF 254) with ceric sulfate reagent after development with $CHCl_3$-MeOH-H_2O (7.5:2.5:0.5). The mixture of these two components was separated after repeated hplc on a reversed-phase Bonda-Pak C-18 semi-preparative column by using MeOH-H_2O (80:20) as a solvent with flow rate 5 mm/min, yielding caratuberside A (50 mg) and caratuberside B (23 mg).

CARATUBERSIDE A.—Caratuberside A [**1**] was obtained as a white, crystalline substance which was recrystallized from aqueous MeOH, mp 170–171°, $[\alpha]^{20}D$ +60° (*c* = 0.66, MeOH). Ir (KBr) ν max 3300 (OH), 2900 (CH_2), 1690 (C=O), 1100 (C-O) cm^{-1}; ^1H nmr (CD_3OD) δ 0.83 (3H, s, 19-Me), 1.08 (3H, s, 18-Me), 1.28 (3H, d, *J* = 6.0 Hz, 6-Me of methyldesoxygalactose), 2.23 (3H, s, 21-Me), 2.90 (1H, t, *J* = 5.6 Hz, H-17), 3.49 (3H, s, O-Me of methyldesoxygalactose), 3.3 (1H, m, H-3), 4.44 (1H, s, 14-OH), 4.35, 4.60 (two anomeric protons, *J* = 7.7 Hz and 7.6 Hz); ^{13}C-nmr see Table 1; fabms (negative ion mode) *m/z* $[M-H]^-$ 655 (100%); eims *m/z* (rel. int.) 477 (2), 345 (20), 317 (53), 299 (100), 281 (36), 219 (27), 161 (38).

ACID HYDROLYSIS OF CARATUBERSIDE A.—Caratuberside A (10 mg) was refluxed with 1% HCl (10 ml) in a boiling H_2O bath for 5 h. Thereafter, 10 ml H_2O was added and concentrated to 5 ml, and the reaction mixture was kept in a boiling H_2O bath for 30 min and extracted with $CHCl_3$. The organic extract so obtained was washed with H_2O and neutralized with 2 N Na_2CO_3, and the aqueous phase was again extracted with $CHCl_3$. On evaporation of $CHCl_3$, the precipitate was filtered off, and the filtrate was evaporated to dryness, then crystallized from aqueous MeOH to give the genin of caratuberside A (1.5 mg). The spectra of the isolated compound were compared with the spectra of caratuberside glycoside A and the spectral data of 13,14-dihydroxy-14-β-pregnane-5-ene-20-one (6). Colorless, sharp crystals ($CHCl_3$), mp 205–210°; ir ν max ($CDCl_3$) 3400–3500 (OH), 1690 (C=O) cm^{-1}; eims *m/z* 318 $[M]^+$, 301 $[M-H_2O]^+$, 296, 281, 271, 253, 228, 43 (base peak); ^1H nmr (300 MHz, $CDCl_3$) δ 1.00 (6H, s, 18-Me, 19-Me), 2.25 (3H, s, 21-Me), 2.95 (1H, t, 5.6 Hz, H-17), 3.47 (1H, m, H-3), 4.40 (1H, s, 14-OH); ^{13}C nmr (75.43 MHz, C_5D_5N) δ 37.49 (C-1), 29.96 (C-2), 17.49 (C-3), 34.71 (C-4), 44.49 (C-5), 29.20 (C-6), 28.13 (C-7), 40.59 (C-8), 49.69 (C-9), 36.04 (C-10), 21.62 (C-11), 39.44 (C-12), 49.46 (C-13), 83.79 (C-14), 32.00 (C-15), 25.80 (C-16), 62.89 (C-17), 12.22 (C-18), 15.72 (C-19), 218.39 (C-20), 32.29 (C-21).

The combined aqueous phase of the reaction mixture was concentrated under reduced pressure, tested for carbohydrates by tlc using EtOAc-MeOH-H_2O-HOAc (65:15:15:20) as a solvent system, and sprayed with freshly prepared aniline phthalate reagent. R_f values were calculated as 0.55 (glucose) and 0.73 (methyldesoxygalactose) (also confirmed by hplc).

ACETYLATION OF CARATUBERSIDE A.—Acetylation of caratuberside A (5 mg) was carried out in C_5H_5N (1 ml) and Ac_2O (2 ml), and the reaction mixture was left at room temperature overnight, then poured into ice H_2O and extracted with EtOAc. The EtOAc extract was concentrated in vacuum followed by Si gel cc (1% MeOH in $CHCl_3$) of the residue to give caratuberside A pentaacetate (2.32 mg). It forms colorless needles with $CHCl_3$; mp 190°; ir ($CDCl_3$) ν max 1725 (OAc), 1100 (C-O) cm^{-1}; eims *m/z* 523 $[M-OCOMe]^+$, 481, 439, 377, 177, 145, 74 (base peak); ^1H nmr (400 MHz, $CDCl_3$), δ 1.00 (6H, s, 18-Me and 19-Me), 1.99, 2.01, 2.057, 2.051, 2.10 (pentaacetate), 2.96 (1H, t, *J* = 5.6 Hz, H-17), 4.44 (1H, s, 14-OH).

REDUCTION OF CARATUBERSIDE A.—Compound **1** (5 mg) in MeOH (10 ml) was treated with an aqueous solution of $NaBH_4$ (15 mg), and the reaction mixture was stirred for 3 h. Dilute H_2SO_4 was then introduced into the reaction mixture. After neutralization and preparative layer chromatography [Si gel PF 254, $CHCl_3$-MeOH-H_2O (7.5:2.5:0.2)] of the reduction product, a pure compound (13.5 mg) was obtained which was found to be identical (^1H-nmr, ^{13}C-nmr, ir, ms, tlc) with caratuberside B.

CARATUBERSIDE B.—Colorless, shiny crystalline compound: mp 182–185° (dec) from aqueous MeOH; ir ν max (KBr) 3300–3400 (OH), 1100 (C-O) cm^{-1}; fabms negative ion at *m/z* 657 $[M-H]^-$; eims *m/z* (rel. int.) 345 (4), 317 (7.5), 301 (5.8), 299 (20), 283 (16), 257 (6), 55 (100); ^1H nmr (300 MHz, C_5D_5N) δ 0.70 (3H, s, 19-Me), 0.90 (3H, d, *J* = 9.0 Hz, 18-Me), 1.60 (3H, d, *J* = 6.3 Hz, 6-Me of methyldesoxygalactose), 2.20 (3H, s, 21-Me), 3.70 (3H, s, OMe of galactose), 4.34 and 4.60 (dd, *J* = 9.6 and 9.8 Hz, anomeric protons); ^{13}C-nmr see Table 1.

ACID HYDROLYSIS OF CARATUBERSIDE B.—Caratuberside B (10 mg) was hydrolyzed as described

for caratuberside A. The identities of the genins and sugars were confirmed by chromatography and spectroscopy.

ACKNOWLEDGMENTS

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