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CAPSUGENIN-25,30-*O*-β-DIGLUCOPYRANOSIDE: A NEW GLYCOSIDE FROM THE LEAVES OF *CORCHORUS CAPSULARIS*

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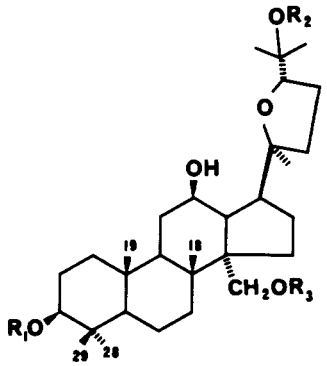
ABSTRACT.—The leaves of *Corchorus capsularis* have yielded a novel dammarane triterpene glycoside which has been characterized, on the basis of spectral data, as the 25,30-*O*-β-diglucopyranoside of 20,24-epoxy-3β,12β,25,30-tetrahydroxydammarane (capsugenin).

Previously (1,2) we have reported the isolation and identification of the bitter-tasting glycosides capsin [**1**] and isocapsin [**2**] from the leaves of jute, *Corchorus capsularis* L. (Tiliaceae). We have now isolated a more polar saponin component that has been characterized as **3**. Vacuum liquid chromatography of the glycoside mixture (1,2) gave the new compound in a yield of 7.5% of the crude mixture. Hydrolysis yielded glucose and capsugenin [**4**]. The fabms spectrum showed a quasimolecular ion peak at *m/z* 839 $[M + Na]^+$ indicating a molecular formula $C_{42}H_{72}O_{15}$, which solves for **4** plus two glucose units. An examination of the nature of the glycosidic linkages was undertaken using standard chemical techniques. Thus permethylation (3) followed by hydrolysis, reduction and acetylation and then gc-ms analysis (4) gave a single alditol product identified as 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. This revealed that the glucose units were independently attached to the genin **4** but did not indicate the points of attachment.

The ^{1}H-nmr spectrum run in pyridine-d_5 was complex with much signal bunching but did reveal two anomeric protons at δ 4.90 ($J = 7.6$ Hz) and δ 5.03 ($J = 7.5$ Hz). Acetylation of the glycoside at room temperature gave the nonaacetate **5** for which ^{1}H-nmr assignments were made from a 2D-COSY spectrum. Important features of this spectrum were the deshielding of the oxymethine proton H-3 (δ 4.50), indicating acetylation, and the nonacetylation of the H-bonded 12-OH (H-12 at δ 3.46, 12-OH at δ 5.84), which showed that neither C-3 nor C-12 was involved in glycosylation. By contrast the H-30



1 R_1=glucose, R_2=R_3=H
2 R_1=R_2=H, R_3=glucose
3 R_1=H, R_2=R_3=glucose
4 R_1=R_2=R_3=H
5 R_1=Ac, R_2=R_3=glucosetetraacetate

protons were not deshielded on acetylation, thus placing one sugar moiety at C-30. In the ^{13}C-nmr spectrum of **5** the resonance for C-25 (78.2 ppm) was deshielded by 8.2 ppm compared to the corresponding resonance in **2** (2), implying glycosylation had also occurred at this position. Assignment of carbon resonances was achieved by DEPT spectra and a partial heteronuclear 2D-COSY run in the reverse mode.

Confirmation that the glucose units were situated at C-25 and C-30 was obtained by a heteronuclear multiple band correlation (HMBC) experiment (5) on **5**. This experiment clearly shows (Figure 1) the ^3J interactions between H-1′ (δ 4.63) and C-25 (78.2 ppm) and between H-1″ (δ 4.40) and C-30 (71.6 ppm).

EXPERIMENTAL

Fabms were obtained using a VG ZAB-E mass spectrometer in the positive ion mode and using 3-nitrobenzylalcohol as matrix. The HMBC nmr experiment was performed on a modified Bruker AC300 spectrometer at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C with a relaxation delay of 1 sec and a t_1 increment of 90 μsec.

PLANT MATERIAL.—Mature leaves of *C. capsularis* were collected from Savar, Dhaka, under

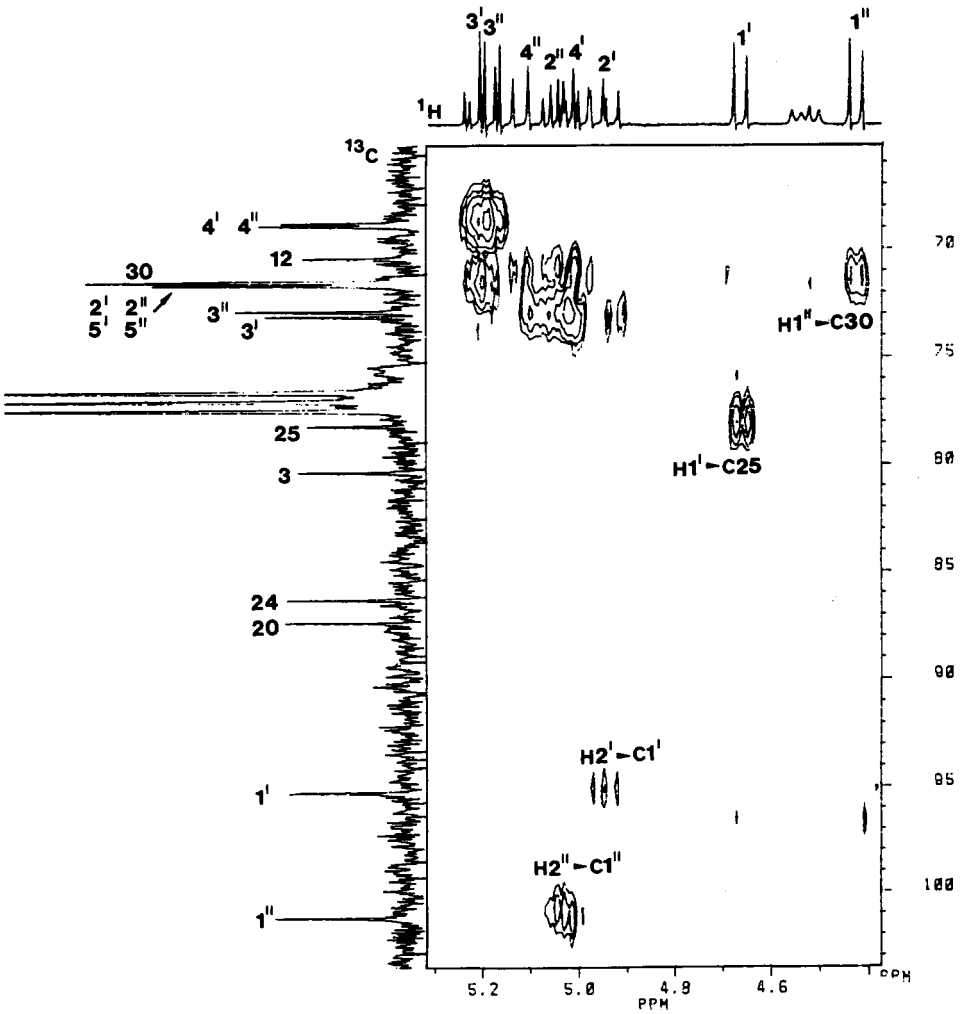


FIGURE 1. Partial heteronuclear multiple bond correlation (HMBC) 2D spectrum for compound **5** showing ^3J interactions between the anomeric protons of the two glucose units and C-25 and C-30 of the aglycone.

TABLE 1. ^{13}C- and ^{1}H-nmr Assignments for Compound **5**.

Carbon	^{13}C	^{1}H	Carbon	^{13}C	^{1}H
C-1	38.9 t		C-26	23.4 q[b]	1.09 s
C-2	23.8 t		C-27	28.4 q[b]	1.26 s
C-3	80.3 d	4.50 dd (11.0, 5.6)	C-28	15.1 q	0.84 s[c]
C-4	39.7 s		C-29	22.5 q	0.90 s[c]
C-5	56.5 d		C-30	71.6 t	3.34/4.21 ABq (9.9)
C-6	20.5 t		C-1′	95.2 d	4.63 d (7.9)
C-7	35.3 t		C-2′	71.4 d	4.92 dd (9.5, 7.9)
C-8	41.7 s		C-3′	80.3 d	5.18 t (9.5)
C-9	50.4 d		C-4′	68.9 d	4.98 dd (10.0, 9.5)
C-10	37.1 s		C-5′	71.5 d	3.63 ddd (10.0, 5.7, 2.5)
C-11	32.1 t		C-6′	62.4 t	4.16 dd (12.0, 5.7)
C-12	70.4 d	3.46 dt (4.6, 10.2)			4.06 dd (12.0, 2.5)
C-13	48.9 d		C-1″	101.2 d	4.40 d (7.9)
C-14	52.0 s		C-2″	71.5 d	5.00 dd (9.5, 7.9)
C-15	31.7 t		C-3″	72.9 d	5.17 t (9.5)
C-16	24.8 t		C-4″	68.9 d	5.08 t (9.5)
C-17	48.8 d		C-5″	71.6 d	3.66 ddd (9.5, 4.5, 2.8)
C-18	15.7 q[a]	0.97 s	C-6″	62.0 t	4.20 dd (12.3, 4.5)
C-19	17.6 q[a]	0.92 s	Ac	170.6, 170.5	2.05, 2.03 (×2)
C-20	87.3 s			170.3 (×3)	2.00 (×3), 1.99
C-21	22.3 q	1.22 s		169.5, 169.4	1.97, 1.96
C-22	31.3 t			169.2, 169.1	
C-23	28.4 t			21.3, 20.7	
C-24	86.3 d	3.78 dd (10.2, 5.3)		20.7 (×2),	
C-25	78.2 s			20.6 (×4),	
				20.0	

[a–c]Assignments with the same superscript are interchangeable.

the auspices of the Bangladesh Jute Research Institute. A voucher specimen is deposited in the herbarium of the Department of Botany, Dhaka University.

PURIFICATION OF **3**.—A portion (0.95 g) of the previously isolated bitter glycoside mixture (1,2) was subjected to vacuum liquid chromatography over Si gel. Elution of the column with $CHCl_3$ followed by $CHCl_3$ with increasing amounts of EtOAc and finally with MeOH gave, from the MeOH fraction, **3** (68 mg).

CAPSUGENIN-25,30-*O*-β-DIGLUCOPYRANOSIDE[**3**].—Recrystallized from aqueous MeOH as plates: mp 190–191°; $[\alpha]_D$ −13° (MeOH, $c = 1$), R_f 0.32 (Si gel, solvent *n*-BuOH saturated with H_2O); fabms *m/z* $[M + Na]^+$ 839; ir (KBr disc) ν max 3600–3100, 2930–2840, 1450, 1375, 1250, 1000, 1075, 1038 cm^{-1}; ^{1}H nmr (250 MHz, pyridine-d_5) δ 6.54 (s, 1H, 12-OH), 5.03 (d, $J = 7.5$ Hz, 1H, anomeric-H), 4.90 (d, $J = 7.6$ Hz, 1H, anomeric-H), 4.56, 4.45 (ABq, $J = 9.6$ Hz, 2H, 30-CH_2), 4.25 (dd, $J = 11.7$, 6.6 Hz, 1H, H-24), 3.80 (m, 1H, H-12), 3.59 (m, 1H, H-3), 1.55, 1.47, 1.43, 1.19, 0.93, 0.83, 0.82 (7 × s, 7 × 3H, 7 × Me).

HYDROLYSIS OF **3**.—Compound **3** (10 mg) was refluxed with 0.5 M TFA for 16 h. The reaction mixture was cooled and diluted with H_2O to give a precipitate which was recrystallized from MeOH as needles, mp 230–231° [identical with **4** (1) by mmp, tlc, ir]. The aqueous supernatant yielded glucose.

PREPARATION OF 1,5-DIACETYL-2,3,4,6-TETRAMETHYLGLUCITOL.—Compound **3** (15 mg) was dissolved in dry DMSO (2 ml) and transferred to a vial sealed with a rubber septum. The vial was flushed with N_2, and 2 M dimsyl sodium in DMSO (12 ml) was added dropwise by syringe. The mixture was agitated in an ultrasonic bath for 30 min and left to stand. The mixture was cooled to 0° and MeI (3 ml) added dropwise, the mixture being stirred at room temperature for 1 h. Excess MeI was removed and the reaction mixture diluted with H_2O (15 ml) and extracted with $CHCl_3$ (4 × 15 ml).

The $CHCl_3$-soluble material was concentrated and treated with 90% v/v aqueous HCOOH (2 ml) at 100° for 1 h. The reaction mixture was concentrated and reacted with 2 M TFA (4 ml) at 100° for 16 h. The cooled reaction mixture was extracted with $CHCl_3$ and the aqueous phase subjected to co-distillation with EtOH until neutral, whereupon $NaBH_4$ (12 mg) was added. After 2 h

the solution was acidified (pH 3.5) and passed through a Dowex 50 (H^+) resin column, concentrated to dryness, and co-distilled with MeOH. The residue was acetylated [dry pyridine-Ac_2O (1:1), 2 ml, 30 min at 100°) and subjected to gc-ms (column, SGE BP-1, 12 m × 0.25 mm i.d.; temperature program from 100° to 300° at 10°/min. The only product eluting (Rt 9.89 min) was identified (eims) as 1,5-diacetyl-2,3,4,6-tetramethylglucitol.

CAPSUGENIN-25,30-*O*-DIGLUCOPYRANOSIDE NONAACETATE [**5**].—Compound **3** (25 mg) was acetylated using normal procedures (dry pyridine/Ac_2O, 6 h, room temperature) to yield **5** (28 mg) as needles: mp 260–261°; ir (KBr disc) ν max 3360, 2950, 2930, 1750, 1630, 1450, 1438, 1230, 1165, 1035, 975, 900, 695 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) and ^{13}C nmr (75.5 MHz, $CDCl_3$) see Table 1.

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