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TWO PREGNANE ESTER TRIGLYCOSIDES FROM *DREGEA LANCEOLATA*

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ABSTRACT.—Two new pregnane ester glycosides designated as drelin [**3**] and ceolin [**5**] have been isolated from the dried roots of *Dregea lanceolata*. Chemical and spectroscopic evidence is consistent with the structures 11-*O*-acetylmarsdenin-3-*O*-β-D-boivinopyranosyl (1→4)-*O*-β-D-cymaropyranosyl (1→4)-*O*-β-D-cymaropyranoside and 11-*O*-acetylmarsdenin-3-*O*-β-D-cymaropyranosyl (1→4)-*O*-3-*O*-methyl-6-deoxy-β-D-allopyranosyl (1→4)-*O*-β-D-oleandropyranoside for drelin and ceolin, respectively.

Pregnane derivatives and cardenolides have been reported in several species of the Asclepiadaceae (1–5). In the course of the chemical investigation on the Asclepiadaceae, we have now focused our attention on *Dregea lanceolata*, T. Cooke (syn. *Marsdenia lanceolata*). In the present investigation of shade-dried roots of this plant, a mixture of glycosides of 2-deoxy sugars was extracted, which by cc over Si gel afforded two crystalline glycosides along with some known compounds, α-amyrin acetate, β-amyrin, and β-sitosterol. We now report the structure of two new triglycosides, drelin [**3**] and ceolin [**5**].

RESULTS AND DISCUSSION

Drelin [**3**], mp 151°, $[\alpha]_D$ +16.27°, $C_{43}H_{68}O_{16}$, responded positively to the Liebermann-Burchardt test (6), xanthydrol (7,8), and Keller-Kiliani (9) reactions, indicating it to be a steroidal glycoside with a 2-deoxy sugar residue.

The presence of a carbonyl group was shown by its reduction with $NaBH_4$ (10), and its nature as a methyl keto group was supported by the characteristic color reaction with sodium nitroprusside (11). The presence of a vicinal diol system in **3** was indicated by its positive reaction with $NaIO_4$. In the ^{1}H-nmr spectrum of **3** at 300 MHz, the presence of three anomeric protons at δ 4.82 (1H) and 4.75 (2H) along with three secondary methyl doublets (J = 6 Hz) at δ 1.35, 1.21, and 1.02, characteristic methylene signals in the region δ 2.36–2.28 (3H) and 1.86–1.80 (3H) for equatorial and axial protons, and two methoxy group singlets at δ 3.43 (6H) provided evidence that **3** is a triglycoside of 2,6-dideoxyhexoses. The ^{1}H-nmr spectrum also contained a signal of one acetyl group at δ 1.96. The triglycoside nature of **3** was further substantiated from its ^{13}C-nmr spectrum, which contained signals for three anomeric carbons at δ 99.9, 96.1, and 95.2 (5).

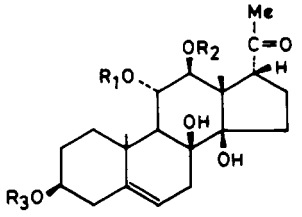
Hydrolysis of **3** with mild acid (0.025 M H_2SO_4) (12) afforded a crystalline genin **1**, mp 161–163°, $[\alpha]_D$ +21.5°, $C_{23}H_{34}O_7$, and a mixture of two sugars. The separated sugars **7** and **8** displayed characteristic color tests for 2-deoxy sugars and were identified as D-boivinose (13,14) (2,6-dideoxy-D-xylo-hexose) and D-cymarose (15) (2,6-dideoxy-3-*O*-methyl-D-ribo-hexose), respectively (pc and $[\alpha]_D$). For further characterization, **7** and **8** were oxidized with bromine H_2O to their syrupy lactones, which on treatment with phenylhydrazine yielded the known crystalline derivatives, D-boivinonic acid phenylhydrazide and D-cymaronic acid phenylhydrazide, respectively. On the basis of the above results, **3** was inferred to be a triglycoside involving D-boivinose and D-cymarose moieties only.

Genin **1**, on methanolysis by the Zemplĕn method (16,17), afforded a crystalline product **2**, which was identical in properties with 17-α-marsdenin (18), (3β, 8β, 11α, 12β, 14β-pentahydroxypregn-5-en-20-one) (mp, tlc, and $[\alpha]_D$). By the comparison of

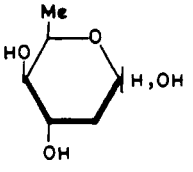
mp, tlc and [α]D with an authentic sample, **1** was found to be identical to dregenin (11-*O*-acetylmarsdenin) (19).

Direct chemical evidence for the sequence of sugar units in **3** came from a study of its very mild acid hydrolysis. The first sugar detectable after 4 days was identified as boivinose [**7**] (pc and tlc), and continued hydrolysis led to the formation of cymarose [**8**] and finally dregenin [**1**], which indicated that the boivinose sugar is the terminal sugar and cymarose was glycosidically linked to dregenin. Additional compounds presumed to be diglycosides and monoglycosides were detected but not identified during the hydrolysis.

The mass spectrum of **3** was also consistent with its triglycoside structure. Al-



1 R_1=Ac, R_2=R_3=H
2 R_1=R_2=R_3=H
3 R_1=Ac, R_2=H, R_3=Ra
4 R_1=R_2=Ac, R_3=Ra
5 R_1=Ac, R_2=H, R_3=Rb
6 R_1=R_2=H, R_3=Rb



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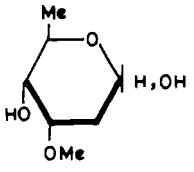

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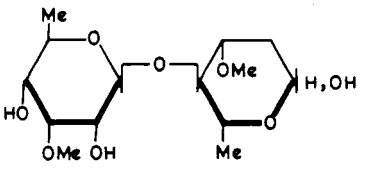

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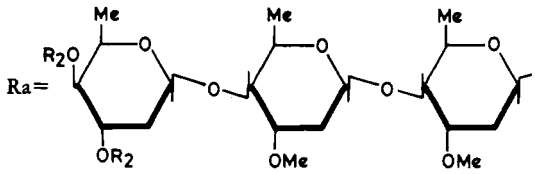

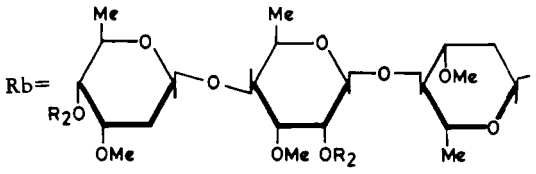

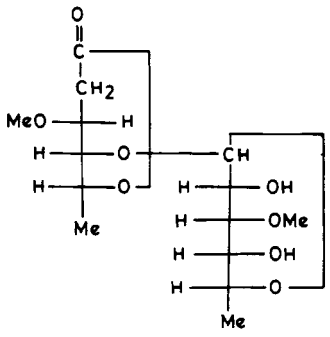

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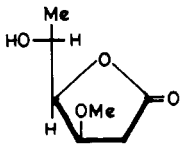

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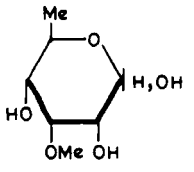

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though the mass spectrum did not exhibit the $[M]^+$ expected at *m/z* 840, there was a prominent peak at *m/z* 387 that was attributed to $[M - MeOH - aglycone]^+$; this ion presumably originated from the trisaccharide moiety of the glycoside. There was another prominent mass ion peak at *m/z* 362 attributed to [M − HOAc − trisaccharide fragment]$^+$, and peaks at *m/z* 344 $[362 - H_2O]^+$, 326 $[362 - 2H_2O]^+$, 308 $[362 - 3H_2O]^+$ and 290 $[362 - 4H_2O]^+$ were in agreement with the presence of one acetyl group and four hydroxyl groups in the aglycone moiety. The low mass region contained the expected 2,6-dideoxy-monomethoxy hexose fragment ion peaks (20) at *m/z* 145, 113, and 95.

The ^{1}H-nmr ($CDCl_3$) spectrum of **3** at 300 MHz not only confirmed that it was a triglycoside of 11-*O*-acetylmarsdenin but also helped in ascertaining the configuration of the glycosidic linkages. The two double doublets at δ 4.82 (1H, $J = 9.5$ and 2.5 Hz) and 4.75 (2H, $J = 9.5$ and 2.5 Hz) could be assigned to the three anomeric protons of the sugars. The large coupling constants (9.5 Hz) of the three anomeric protons, typical of their axial configuration, suggested D-boivinose and D-cymarose moieties in $^4C_1$ (D) conformation, also suggesting that these sugar units were linked through β-D (1↦4) glycosidic linkages (21).

To ascertain the position of the glycosidic linkage with the aglycone moiety, compound **3** was acetylated with Ac_2O in pyridine, yielding a crystalline tetraacetate **4**, mp 117°, $[\alpha]_D$ +13°, characterized from its 80 MHz ^{1}H-nmr spectrum that included signals for four acetyl groups at δ 2.08, 2.05, 1.98, and 1.95. Besides this, the C-12 methine proton doublet in **3** centered at δ 4.40 was found shifted downfield to δ 5.45 in **4** confirming that the C-12 hydroxyl group in **3** is free and that the trisaccharide unit in **3** is glycosidically linked to the C-3 hydroxyl group of the genin.

In the light of this evidence, the structure of drelin [**3**] was established as 11-*O*-acetylmarsdenin-3-*O*-β-D-boivinopyranosyl (1↦4)-*O*-β-D-cymaropyranosyl (1↦4)-*O*-β-D-cymaropyranoside.

Ceolin [**5**], mp 146–148°, $[\alpha]_D$ +4.38°, $C_{44}H_{70}O_{16}$, responded positively to the Liebermann-Burchardt test (5), xanthydrol (6,7), and Keller-Kiliani (8) reactions, indicating it to be a steroidal glycoside. In addition, the presence of three anomeric protons at δ 4.80 (1H) and 4.67 (2H) along with three secondary methyl doublets ($J = 6$ Hz) at δ 1.24, 1.12, and 1.01 as well as only two characteristic methylene signals of two protons each in the region δ 2.35–2.31 and 1.87–1.84 for equatorial and axial protons, respectively, in the ^{1}H-nmr spectrum of **5** at 400 MHz suggested it to be a triglycoside in which its two sugar units were 2,6-dideoxyhexoses but the third sugar unit was a normal 6-deoxyhexose. The ability of **5** to undergo methanolysis by the Zemplĕn method (16,17) indicated the presence of an ester function in the molecule.

To identify the sugar and the genin of **5**, it was hydrolyzed with mild acid (0.025 M H_2SO_4) (11) followed by cc, which afforded a crystalline genin **1**, mp 162–164°, $[\alpha]_D$ +21.0°, $C_{23}H_{34}O_7$, identified as dregenin (mp, mmp, $[\alpha]_D$, and co-tlc) and two syrupy sugars **8** and **9** which were identified as D-cymarose (15) (2,6-dideoxy-3-*O*-methyl-D-*ribo*-hexose) and pachybiose (18), respectively. For further characterization, **8** was oxidized with bromine H_2O to its lactone, which on treatment with phenylhydrazine yielded the known crystalline D-cymaronic acid phenylhydrazide.

To characterize the nature of second sugar **9**, it was oxidized with bromine H_2O to its lactone **10**, which on acid hydrolysis under forcing conditions using the Kiliani method (22) afforded two components, a syrupy lactone **11** and a free sugar **12**. The free sugar was identified as 3-*O*-methyl-6-deoxy-D-allose (21) by comparing its specific rotation and mobility on pc with an authentic sample. The lactone **11** on treatment with phenylhydrazine yielded the known crystalline D-oleandronic acid phenylhydrazide (23), identical with an authentic sample (mp, mmp, $[\alpha]_D$, and tlc). Sugar **9**

was thus characterized as the disaccharide pachybiose [4-*O*-(3-*O*-methyl-6-deoxy-β-D-allopyranosyl)-D-oleandropyranose].

Direct chemical evidence for the sequence of sugar units in **5** came from a study of its very mild acid hydrolysis. The first detectable sugar was identified as D-cymarose [**8**] (pc and tlc), leading to the conclusion that cymarose was the terminal sugar unit. Continued hydrolysis led to the formation of pachybiose [**9**] and finally dregenin [**1**], which indicated that pachybiose was glycosidically linked to the aglycone either at its C-3 or C-12 secondary hydroxyl group. Another compound detected during the hydrolysis was presumed to be the diglycoside.

The inertness of **5** to $NaIO_4$ reagent, in contrast to its deacylated product **6** which reacted with this reagent, indicated that the C-12 hydroxyl group is free and is present in a vicinal diol arrangement with the C-11 hydroxyl group. Thus, it was concluded that the sugar moiety was linked to dregenin at C-3 hydroxyl group.

The ^{1}H-nmr ($CDCl_3$) spectrum of **5** at 400 MHz was in full agreement with the derived structure, further indicating the configuration of the glycosidic linkages. A one-proton doublet ($J = 10$ Hz) and a two-proton double doublet ($J = 10$ and 2 Hz) could be assigned to the three anomeric protons of the three sugars, the configuration of C-1 in all the three sugars being identical. Their large coupling constants ($J = 10$ Hz) were typical of an axial proton on a hexopyranose unit in the $^4C_1$ (D) conformaiton, also suggesting that all the three sugar units were linked through a β-D (1↦4) glycosidic linkages (21).

The mass spectrum of **5**, like that of drelin, did not exhibit an $[M]^+$ ion, but the highest recorded ion at *m/z* 344 was interpreted as $[M - \text{sugars} - HOAc - H_2O]^+$, followed by the subsequent losses of three H_2O molecules, giving fragment ion peaks at *m/z* 326 $[344 - H_2O]^+$, 308 $[344 - 2H_2O]^+$ and 290 $[344 - 3H_2O]^+$, which were in agreement with the presence of an acetyl group and four hydroxyl groups in its genin moiety. The position of an acetyl group at C-11 was also confirmed by the fragment at *m/z* 222 $[M - \text{sugars} - HOAc - 140]^+$. The mass spectrum of **5** also contained the common fragments of 2,6-dideoxy-monomethoxyhexose (20) at *m/z* 145, 113, and 95 and of 6-deoxy normal hexose (24) at *m/z* 161, 129, and 111.

The foregoing chemical and spectroscopic evidence thus confirmed the structure of ceolin [**5**] as 11-*O*-acetylmarsdenin-3-*O*-β-D-cymaropyranosyl (1↦4)-*O*-3-*O*-methyl-6-deoxy-β-D-allopyranosyl (1↦4)-*O*-β-D-oleandropyranoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The general procedures were as reported earlier (25) except that ^{1}H-nmr spectra were recorded on 400 MHz (Brüker WM), 300 MHz (Brüker AM), and 80 MHz (FT-80A) spectrometers in $CDCl_3$ and the mass spectra on an AEI.MS-30 mass spectrometer. The plant *D. lanceolata* was collected in Maharastra and identified by Dr. S.L. Kapoor, National Botanical Research Institute, Lucknow India; a voucher specimen, Herbarium No. 68836, is preserved at the institute.

PLANT EXTRACTION AND ISOLATION.—Shade-dried powdered roots of *D. lanceolata* were extracted and fractionated with solvents of different polarities, as reported earlier (25). Repeated cc of the $CHCl_3$ extract (180.05 mg) and $CHCl_3$-EtOH (4:1) extract (310.95 mg) over Si gel using $CHCl_3$-MeOH (94:6) as eluent afforded drelin [**3**] (80 mg). Ceolin [**5**] (50 mg) was isolated from $CHCl_3$-EtOH (3:2) extract (270.85 mg) using $CHCl_3$-MeOH (88:12) as eluent.

DRELIN [**3**].—Drelin: mp 151° (MeOH/Et_2O), $[\alpha]^{25}D$ +16.27° ($c = 0.7375$, MeOH) (found C 61.12, H 7.93; $C_{43}H_{68}O_{16}$ requires C 61.42, H 8.09). It gave a green color in Liebermann-Burchardt test and produced pink in xanthydrol and a blue in Keller-Kiliani reactions. It also gave positive color tests with tetranitromethane and sodium nitroprusside. It reacted positively with $NaIO_4$ reagent and also underwent reduction with $NaBH_4$ and alkaline hydrolysis with methanolic KOH. ^{1}H nmr (300 MHz) δ 5.47–5.45 (1H, m, H-6), 5.35 (1H, t, $J = 8$ Hz, H-11), 4.82 (1H, dd, $J = 9.5$ and 2.5 Hz, H-1′ of S_3), 4.75 (2H, dd, $J = 9.5$ and 2.5 Hz, H-1′ of S_1 and S_2), 4.40 (1H, d, $J = 8$ Hz, H-12), 3.88 (3H, m, H-5′

of S_1, S_2, and S_3), 3.79 (3H, m, H-3′ of S_1, S_2, and S_3), 3.60 (1H, m, H-3), 3.43 (6H, s, 2 × OMe of S_1 and S_2), 3.30–3.22 (3H, m, H-4′ of S_1, S_2, and S_3), 3.22 (1H, m, H-17), 2.36–2.28 (3H, m, H-2′eq of S_1, S_2, and S_3), 2.17 (3H, s, 17-COMe), 1.96 (3H, s, 11-OAc), 1.86–1.80 (3H, m, H-2′ax of S_1, S_2, and S_3), 1.35 (3H, d, J = 6 Hz, Me-6′), 1.21 (3H, d, J = 6 Hz, Me-6′), 1.10 (3H, s, Me-18), 1.06 (3H, s, Me-19), 1.02 (3H, d, J = 6 Hz, Me-6′); ^{13}C nmr (90 MHz) δ 39.1 (t, C-1), 29.0 (t, C-2), 79.5 (d, C-3), 38.2 (t, C-4), 139.2 (s, C-5), 117.8 (d, C-6), 33.5 (t, C-7), 72.0 (s, C-8), 45.1 (d, C-9), 34.5 (s, C-10), 72.9 (d, C-11), 73.5 (d, C-12), 57.5 (s, C-13), 88.5 (s, C-14), 35.8 (t, C-15), 19.1 (t, C-16), 58.3 (d, C-17), 18.5 (q, C-18), 18.2 (q, C-19), 31.2 (q, C-21), 99.9 (d, C-1′), 96.1 (d, C-1′), 95.2 (d, C-1′), 61.0 (q, 2 × OMe); ms *m/z* (rel. int.) $[M]^+$ not observed, $[M - \text{sugars} - \text{HOAc}]^+$ 362 (8.0), $[362 - H_2O]^+$ 344 (15.6), $[362 - 2H_2O]^+$ 326 (21.0), $[362 - 3H_2O]^+$ 308 (23.0), $[362 - 4H_2O]^+$ 290 (6.4), $[M - \text{sugars} - 138]^+$ 284 (28.6), 242 (21.0), 224 (28.2), $[284 - H_2O]^+$ 266 (47.4), 250 (22.0), 179 (23.4), $[179 - H_2O]^+$ 161 (87.3), $[M - \text{sugars} - 284]^+$ 138 (4.1), $[138 - H_2O]^+$ 120 (51.0), 97 (18.6); sugar fragments [trisaccharide ion − MeOH]$^+$ 387 (8.2), 289 (6.8, 257 (8.4), [monosaccharide]$^+$ 145 (4.2), $[145 - \text{MeOH}]^+$ 113 (7.6), $[113 - H_2O]^+$ 95 (8.8).

CEOLIN [**5**].—Ceolin: mp 146–148° ($MeOH/Et_2O$), $[\alpha]^{25}D$ +4.38° (c = 0.45, MeOH) (found C 61.28, H 7.95; $C_{44}H_{70}O_{17}$ requires C 61.82, H 8.19). It gave positive Liebermann-Burchardt test and produced pink in the xanthydrol and blue in Keller-Kiliani reactions. It also gave positive color tests with tetranitromethane and sodium nitroprusside. It underwent reduction with $NaBH_4$ and alkaline hydrolysis with methanolic KOH. ^{1}H nmr (400 MHz) δ 5.48 (1H, m, H-6), 5.36 (1H, J = 8 Hz, H-11), 4.80 (1H, J = 10 Hz, H-1′ of sugar), 4.67 (2H, dd, J = 10 and 2 Hz, 2 × H-1′ of sugars), 4.50 (1H, d, J = 8 Hz, H-12), 3.87–3.82 (3H, m, H-5′ of sugar), 3.80 (1H, m, H-3), 3.62–3.60 (3H, m, H-3′ of sugar), 3.45 (3H, s, OMe), 3.43 (3H, s, OMe), 3.42 (3H, s OMe), 3.24–3.20 (3H, m, H-4′ of sugar), 2.35–2.31 (2H, m, H-2′eq), 2.18 (3H, s, 17-COMe), 1.97 (3H, s, 11-OAc), 1.87–1.84 (2H, m, H-2′ax), 1.24 (3H, d, J = 6 Hz, Me-6′), 1.12 (3H, d, J = 6 Hz, Me-6′), 1.07 (3H, s, Me-18), 1.05 (3H, d, J = 6 Hz, Me-6′), 1.02 (3H, s, Me-19); ms *m/z* (rel. int.) $[M]^+$ not observed, $[M - \text{sugars} - \text{HOAc} - H_2O]^+$ 344 (13), $[344 - H_2O]^+$ 326 (23), $[344 - 2H_2O]^+$ 308 (11), $[344 - 3H_2O]^+$ 290 (3), $[M - \text{sugars} - 138]^+$ 284 (29), $[284 - H_2O]^+$ 266 (66), $[266 - \text{Me}]^+$ 251 (12), $[266 - H_2O]^+$ 248 (50), $[M - \text{sugars} - \text{HOAc} - 140]^+$ 222 (10), $[248 - \text{Ac}]^+$ 205 (12), $[222 - H_2O]^+$ 204 (13), 179 (14), $[179 - H_2O]^+$ 161 (20), $[161 - \text{Me}]^+$ 156 (25), $[M - \text{sugars} - \text{HOAc} - 222]^+$ 140 (22), $[M - \text{sugars} - 284]^+$ 138 (22), $[138 - H_2O]^+$ 120 (40), $[120 - \text{Me}]^+$ 105 (70), $[140 - \text{Ac}]^+$ 97 (73), sugar fragments $[M - \text{genin fragment} - S_3 - \text{MeOH}]^+$ 273 (13), $[273 - \text{MeCHO}]^+$ 229 (11), $[229 - \text{MeOH}]^+$ 197 (14), [monosaccharide]$^+$ 161 (20), [monosaccharide]$^+$ 145 (63), $[161 - \text{MeOH}]^+$ 129 (26), $[145 - \text{MeOH}]^+$ 113 (41), $[129 - H_2O]^+$ 111 (45), $[113 - H_2O]^+$ 95 (76).

MILD ACID HYDROLYSIS OF DRELIN [**3**].—To a solution of **3** (10 mg) in 80% aqueous dioxane (1 ml) was added 0.05 M H_2SO_4 (1 ml), and the solution was warmed for 30 min at 50°, then concentrated under reduced pressure to remove dioxane. The usual workup as reported earlier (6) afforded genin **1** (2 mg), which crystallized form $MeOH/Me_2CO$ as colorless needles, mp 161–163°, $[\alpha]^{25}D$ +21.5° (c = 0.15, MeOH).

The aqueous hydrolysate afforded a mixture of two sugars that were isolated through cc affording **7** (1.2 mg), $[\alpha]^{25}D$ +4.0° (c = 0.175, H_2O), and **8** (3.6 mg), $[\alpha]^{25}D$ +50° (c = 0.16, H_2O). Both gave positive coloration in the xanthydrol and Keller-Kiliani reactions. The [α]D, tlc, and pc comparisons of **7** and **8** showed them to be identical to D-boivinose [lit. (13,14) [α]D +3.8° (H_2O)] and D-cymarose [lit. (15) [α]D +53.4° (H_2O)], respectively.

MILD ACID HYDROLYSIS OF CEOLIN [**5**].—To a solution of **5** (10 mg) in 80% aqueous dioxane (1 ml) was added 0.05 M H_2SO_4 (1 ml) as in the acid hydrolysis of **3**, affording genin **1**, which was crystallized from $MeOH/Me_2CO$ as colorless needles (4 mg), mp 162–164°, $[\alpha]^{25}D$ +21.0° (c = 0.15, MeOH). The aqueous hydrolysate was concentrated under reduced pressure, and a mixture of two sugars was obtained. These sugars were isolated through cc [$CHCl_3$-MeOH (98:2)] over Si gel, affording **8** (1.2 mg), $[\alpha]^{25}D$ +53.2° (c = 0.12, H_2O), and **9** (2.3 mg), $[\alpha]^{25}D$ −9.2° (c = 0.16, H_2O). Both gave positive coloration in the xanthydrol and Keller-Kiliani reactions. The optical rotation, tlc, and pc comparison of **8** and **9** showed them to be identical to D-cymarose [lit. (15) [α]D +55° (H_2O)] and pachybiose [lit. (18) [α]D −9.4° (H_2O)], respectively.

$NaBH_4$ REDUCTION OF **3**.—Drelin [**3**] (3 mg) was dissolved in MeOH (1.2 ml). $NaBH_4$ (3 mg) was added, and the mixture was kept for 2 h at room temperature. After usual workup as reported earlier (26), the residue showed complete consumption of **3** and a spot of lower mobility [tlc, $CHCl_3$-MeOH (85:15)].

HYDROLYSIS OF **1** BY THE ZEMPLÉN METHOD.—To a solution of **1** (3 mg) in absolute MeOH (1 ml) was added NaOMe (0.15 ml), and the mixture was kept at room temperature. When the reaction was complete (tlc), it was neutralized with IR 120 H resin and filtered. MeOH was removed under reduced pres-

sure, yielding a viscous product **2** which crystallized from MeOH/Me_2CO, mp 263–265°, $[\alpha]^{25}D$ −9.5° (c = 0.306, MeOH). Its physical constants were in good agreement with those of 17α-marsdenin (3β, 8β, 11α, 12β, 14β-pentahydroxypregn-5-en-20-one) [lit. (18) mp 263–266°, $[\alpha]D$ −9.0° ± 2° (c = 0.88, MeOH)]. The crystalline genin **1** was found to be identical with dregenin, mp 162°, $[\alpha]^{25}D$ +20° (c = 0.1, MeOH).

D-BOIVINONIC ACID PHENYLHYDRAZIDE.—A solution of **7** (1.2 mg) in H_2O (0.3 ml) was mixed with Br_2 (4 μl) and shaken in a stoppered flask in the dark for 24 h at room temperature. The excess of Br_2 was then removed under reduced pressure. The acidic mixture was made neutral with freshly precipitated Ag_2CO_3, and the suspension was filtered. In the filtrate, H_2S was passed to remove Ag^+ ions, and the suspension was again filtered. The filtrate was evaporated to dryness under reduced pressure, yielding a syrupy lactone that gave a violet coloration with the $NH_2OH/FeCl_3$ reagent. A solution of this lactone (1 mg) in absolute EtOH (0.025 ml) was mixed with freshly distilled phenylhydrazine (0.02 ml), and the mixture was heated for 30 min at 100°. The viscous mass was cooled and repeatedly triturated with absolute Et_2O (to remove excess of phenylhydrazine), yielding the D-boivinonic acid phenylhydrazide which crystallized from MeOH/Et_2O as colorless needles (0.5 mg), mp 133–135° [lit. (27) mp 134–136°].

D-CYMARONIC ACID PHENYLHYDRAZIDE.—A solution of **8** (3 mg) in H_2O (0.6 ml) was mixed with Br_2 (11 μl) and shaken in a stoppered flask in dark for 24 h at room temperature. Workup of the reaction was carried out as discussed in the oxidation of **7** and finally gave a syrupy lactone (2.2 mg) that gave a violet coloration with NH_2OH-$FeCl_3$ reagent. A solution of this lactone (2 mg) in absolute EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) as in D-boivinonic acid phenylhydrazide, affording D-cymaronic acid phenylhydrazide, crystallized from MeOH/Et_2O as colorless needles (1.0 mg), mp 152–154° [lit. (15) mp 155°].

OXIDATION OF PACHYBIOSE [**9**] WITH BROMINE H_2O.—A solution of **9** (2.0 mg) in H_2O (0.5 ml) was oxidized with Br_2 (8 μl) as in the oxidation of **7**, affording a syrupy lactone **10**, which gave a violet coloration with $NH_2OH/FeCl_3$ reagent.

KILIANI HYDROLYSIS OF **10**.—Compound **10** (2.3 mg) was dissolved in Kiliani mixture [0.12 ml, HOAc-H_2O-HCl (7:11:2)], heated at 100° for 1 h, cooled, and evaporated to dryness over KOH in a vacuum desiccator. Usual workup (23) afforded a lactone **11** and a free sugar **12**. The free sugar was crystallized from Me_2CO (1.0 mg), mp 118–120°, $[\alpha]^{25}D$ +3.5° (c = 0.1, H_2O) and was found to be identical to 3-*O*-methyl-6-deoxy-D-allose [lit. (21) mp 119–121°, $[\alpha]D$ +3.8°].

D-OLEANDRONIC ACID PHENYLHYDRAZIDE.—A solution of **11** (0.8 mg) in absolute EtOH (0.04 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) as in the preparation of D-boivinonic acid phenylhydrazide, affording D-oleandronic acid phenylhydrazide, crystallized from MeOH/Et_2O as colorless needles, mp 133°, $[\alpha]^{25}D$ −18.2° (c = 0.685, MeOH) [lit. (23) mp 134–135°, $[\alpha]D$ −20.6° (MeOH)].

VERY MILD ACID HYDROLYSIS OF **3**.—To a solution of **3** (15 mg) in 80% aqueous dioxane (2.5 ml) was added 0.005 M H_2SO_4 (2.5 ml), and the solution was kept at room temperature. After 4 days, it showed three spots on tlc identical in mobility with boivinose [**7**], (R_f 1.0, taken as reference), unreacted starting compound **3** (R_f 3.3), and a new spot (R_f 10.0), presumably diglycoside. After 7 days two more new spots appeared, one identical in mobility with cymarose [**8**] (R_f 6.0) and the other presumed to be monoglycoside (R_f 12.3). The hydrolysis was complete in 9 days (tlc). Workup of the hydrolysate followed by cc over Si gel afforded an amorphous product that was found to be identical to dregenin [**1**] ([α]D, tlc) and two pure viscous syrups **7** (1.5 mg) and **8** (2.0 mg), identified as D-boivinose and D-cymarose, respectively, by comparison with authentic samples ([α]D, tlc).

VERY MILD ACID HYDROLYSIS OF **5**.—To a solution of **5** (12 mg) in 80% aqueous dioxane (1.8 ml) was added 0.005 M H_2SO_4 (1.8 ml), and the solution was kept at room temperature. After 4 days, the reaction mixture showed three spots on tlc, identical in mobility with D-cymarose [**8**] (R_f 1.00, taken as reference), unhydrolyzed starting material **5** (R_f 0.12), and the diglycoside (R_f 0.33). This hydrolysis was complete in 6 days, showing two additional new spots, one comparable with pachybiose [**9**] (R_f 0.51) and the other of dregenin [**1**] (R_f 1.94). The reaction mixture was then worked up followed by cc, affording dregenin [**1**] (2.5 mg) ([α]D, tlc) and two chromatographically pure reducing sugars as viscous syrups, **8** (2 mg) and **9** (2.4 mg), identified as D-cymarose and pachybiose, respectively, by comparison with authentic samples ([α]D, tlc).

ACETYLATION OF COMPOUND **3**.—Compound **3** (8 mg) on acetylation with pyridine (0.5 ml) and Ac_2O (0.4 ml) at 100° for 4 h afforded a crystalline tetraacetate **4** (7.5 mg), mp 117°, $[\alpha]^{25}D$ +13° (c = 0.53, $CHCl_3$). ^1H nmr (80 MHz) δ 5.45 (1H, d, J = 8 Hz, H-12), 5.45–5.35 (1H, m, H-6), 5.35

(1H, t, $J=8$ Hz, H-11), 5.05–4.95 (1H, dd, $J=9.5$ and 2.5 Hz, H-1′ of S_3), 4.70–4.60 (2H, dd, $J=9.5$ and 2.5 Hz, H-1′ of S_1 and S_2), 3.45 (3H, s, OMe of cym), 3.40 (3H, s, OMe of cym), 2.15 (3H, s, 17-COMe), 2.08 (3H, s, OAc), 2.05 (3H, s, OAc), 1.98 (3H, s, OAc), 1.95 (3H, s, OAc), 1.15 (3H, s, Me-18) and 1.05 (3H, s, Me-19).

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