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A NOVEL PREGNANE GLYCOSIDE FROM *PERIPLOCA CALOPHYLLA*

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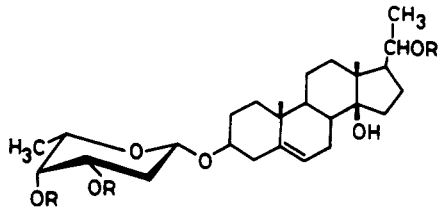
Pregnane derivatives and cardenolides have been reported to be present in several species of the Asclepiadaceae family (1–4). In earlier investigations of the twigs of *Periploca calophylla* Falc. four novel glycosides, locin (5), plocin (6), plocinin (7), and calocin (8), as well as triterpenoids (9) were reported. We now report the isolation of the new glycoside calocinin [**1**].

Calocinin [**1**], $C_{27}H_{44}O_6$, mp 250–255°, $[\alpha]_D$ +16°, isolated from the combined $CHCl_3$-EtOH (4:1 and 3:2) extracts of the twigs of *P. calophylla*, responded positively to the Liebermann-Burchardt test (10), to xanthydrol (11,12), and to the Keller-Kiliani reaction (13), indicating it to be a steroidal glycoside of a 2-deoxy sugar. In addition, characteristic methylene signals in the region δ 2.25–2.14 (1H) and 1.72–1.68 (1H) along with a secondary methyl doublet at δ 1.34 (J = 6 Hz) and an anomeric proton at δ 4.64 (dd, J = 10 and 2.5 Hz) in the ^{1}H nmr spectrum of **1** suggested it to be a monoglycoside of 2,6-dideoxy hexose.

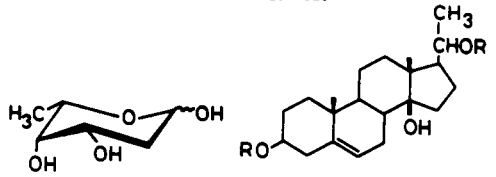
Mild acid hydrolysis of calocinin [**1**] with 0.025 M H_2SO_4 (14) afforded a crystalline genin **3**, $C_{21}H_{34}O_3$, mp 198–201°, $[\alpha]_D$ −50°, and the sugar **5** as a viscous syrup, $[\alpha]_D$ −58.5°. A measurement of the relative mobility of **5** using digitoxose as a reference (15) indicated it to be 2,6-dideoxy-L-fucose (2,6 dideoxy-L-lyxo hexose) (16), and this assignment was confirmed by oxidation and conversion of the resulting lactone to its phenylhydrazide, identical with L-fuconic acid phenylhydrazide (17).

The pregnane genin **3** (C_{21}) was characterized as calogenin (3β,14β,20ϵ-trihydroxypregn-5-ene) by co-chromatography (tlc) with an authentic sample. This finding was confirmed by preparation of its diacetate **4**, $C_{25}H_{38}O_5$, mp 163–165°, $[\alpha]_D$ −18°, identical with di-*O*-acetylcalogenin (mmp, $[\alpha]_D$, tlc).

The eims of the glycoside **1** did not exhibit a molecular ion. The highest peak at *m/z* 419 corresponded to the loss of the C-17 hydroxyethyl chain, indicating that the sugar was glycosidically linked to the C-3 hydroxyl group and



1 R=H
2 R=Ac



5 **3** R=H
4 R=Ac

not to the C-20 hydroxyl group of calogenin. The spectrum also showed peaks for the genin moiety at *m/z* 334, 289 $[334-CH(OH)CH_3]^+$, 271 $[289-H_2O]^+$, 253 $[289-2H_2O]^+$, accounting for all three oxygen functions in the genin. The base peak at *m/z* 131 for the fucosyl cation was accompanied by peaks at *m/z* 113, 95, 72, and 68 which could be interpreted as arising from the characteristic fragmentation pathway of 2,6 dideoxy hexose (18).

The ^{1}H-nmr spectrum of the glycoside **1** at 400 MHz not only confirmed the derived structure but also established the configuration of the glycosidic linkage. The large coupling constant ($J = 10$ Hz) of the anomeric proton double doublet at δ 4.64 attributed to its axial configuration suggested that the sugar was present in $^1C_4$ (L) conformation and linked to the genin through a β-glycosidic linkage. The two singlets at δ 1.24 (3H) and δ 1.02 (3H), a one-proton quartet at δ 3.75 ($J = 6$ Hz), and two three-proton doublets centered at δ 1.30 ($J = 6$ Hz) and δ 1.34 ($J = 6$ Hz) were attributed to the two tertiary *C*-methyl groups (C-18 and C-19), carbinol methine proton at C-20, and to the C-21 secondary methyl group of C-17 hydroxyethyl chain in the pregnane genin and C-6′ secondary methyl group in the 6-deoxy hexose moiety, respectively. A triplet at δ 3.15 (1H) and two multiplets (1H each) centered at δ 3.32 and δ 5.32 were assigned to protons at C-4′ and C-5′ of the sugar moiety and C-6 of the genin moiety, respectively.

Nmdr experiments were helpful in the confirmation of these assignments. Irradiation of the double doublet at δ 4.64 corresponding to the C-1 anomeric proton caused the collapse of the multiplicity of the methylene signal in the region δ 2.25–2.14 and 1.72–1.68. Similarly, irradiation of the two doublets at δ 1.30 and δ 1.34 corresponding to the C-21 and C-6′ methyl group caused collapse of the C-20 methine proton quartet into a singlet at δ 3.75 and the C-5′ methine proton multiplet into a doublet at δ 3.32, respectively. Conversely, irradiation of the C-20 carbinol methine proton quartet at δ 3.75 resulted in the collapse of doublet into a singlet at δ 1.30. These results indicated that the sugar was a 2,6-dideoxy hexose, and the pregnane genin bears a C-17 hydroxyethyl chain.

Acetylation of **1** with Ac_2O in pyridine yielded an amorphous triacetate **2**, $[\alpha]_D + 12°$, also characterized from its 400-MHz ^{1}H nmr spectrum which consisted of signals for three acetyl groups as 3H singlets at δ 2.0, 2.02, and 2.05 in addition to two tertiary methyl group singlets at δ 0.9 and 1.0 ppm. Besides this, the C-20 methine proton quartet in **1** centered at δ 3.75 shifted downfield to δ 4.71 in **2**, confirming that the C-20 hydroxyl group is free and that the sugar in **1** is glycosidically linked to the C-3 hydroxyl group of the genin.

In the light of the foregoing evidence the structure of calocinin [**1**] is established as 3-*O*-β-L,2,6 dideoxy fucopyranoside.

EXPERIMENTAL

The general procedures were the same as reported earlier (8), except that the optical rotations were measured on a Perkin Elmer 241 automatic polarimeter, the 400-MHz ^{1}H nmr spectra on a Bruker instrument, and the mass spectra on an AEI MS-30 mass spectrometer. The plant *P. calophylla* was collected on Dec. 12, 1985 from Kempti Falls, Dehradun, Uttar Pradesh, India and identified by Dr. S.L. Kapoor, National Botanical Research Institute, Lucknow. A voucher specimen, Herbarium No. 116828, is preserved at the Institute.

PLANT EXTRACTION.—The extraction of the shade-dried, powdered twigs of *P. calophylla* was carried out as described earlier (8) and shaken with petroleum ether, Et_2O, $CHCl_3$, $CHCl_3$-EtOH (4:1), and $CHCl_3$-EtOH (3:2) to fractionate its glycosides of different polarities. Repeated cc of $CHCl_3$-EtOH (4:1 and 3:2) extracts over Si gel using $CHCl_3$/MeOH as eluent afforded calocinin [**1**] (55 mg).

CALOCININ [**1**].—Mp 250–255° (MeOH/Et_2O), $[\alpha]^{25}_D + 16°$ ($c = 0.13$, MeOH); found C 69.79, H 9.41; $C_{27}H_{44}O_6$ required C 69.83, H

9.48%. It gave a pink color in xanthydrol and blue color in the Keller-Kiliani reaction and also underwent $NaIO_4$ oxidation. Ir ν max (KBr) cm^{-1} 3490–3400, 2940, 1440, 1390, 1130, 1070, 820, 600; ^1H nmr (pyridine-d_5) δ 5.32 (1H, m, H-6), 4.64 (1H, dd, J = 10 and 2.5 Hz, H-1'), 3.75 (1H, q, H-20), 3.65 (1H, m, H-3'), 3.32 (1H, m, H-5'), 3.15 (1H, t, H-4'), 2.25–2.14 (1H, m, H-2'eq), 1.72–1.68 (1H, m, H-2'ax), 1.64–1.50 (methylene of aglycone), 1.34 (3H, d, J = 6 Hz, 6'-Me), 1.30 (3H, d, J = 6 Hz, 21-Me), 1.24 (3H, s, 18-Me), 1.02 (3H, s, 19-Me); ms *m/z* (rel. int.) $[M]^+$ (not observed) $[M-CHOHMe]^+$ 419 (11), $[M-sugar]^+$ 334 (15), $[M-CHOHMe-sugar]^+$ 289 (35), $[289-H_2O]^+$ 271 (30), $[271-H_2O]^+$ 253 (25); genin fragments $[249-2H_2O]^+$ 213 (9), $[137-H_2O]^+$ 119 (8); sugar fragments 148 (2), $[148-OH]^+$ 131 (100), $[131-H_2O]^+$ 113 (55), $[113-H_2O]^+$ 95 (15), $[148-C_3H_6O-H_2O]^+$ 72 (20), $[148-H_2O-MeCHO-H_2O]^+$ 68 (25).

TRI-*O*-ACETYLCALOCININ [**2**].—Compound **1** (12 mg) on acetylation with Ac_2O (0.15 ml) in pyridine (1.1 ml) at room temperature and usual workup yielded **2** as an amorphous residue (12 mg), $[\alpha]^{25}D$ +12° (c = 0.08, $CHCl_3$) which failed to crystallize. ^1H nmr δ 5.38 (1H, m, H-6), 4.71 (1H, q, H-20), 4.64 (1H, dd, J = 10 and 2.5 Hz, H-1'), 4.21 (1H, t, H-4'), 2.05 (3H, s, OAc), 2.03 (3H, s, OAc), 2.02 (3H, s, OAc), 1.32 (3H, d, J = 6 Hz, 6'-Me), 1.22 (3H, d, J = 6 Hz, 21-Me), 1.02 (3H, s, 18-Me), 0.9 (3H, s, 19-Me).

MILD ACID HYDROLYSIS OF CALOCININ [**1**].—To a solution of **1** (25 mg) in 80% aqueous dioxane (1.7 ml) was added 0.025 M H_2SO_4 (1.7 ml), and the solution was warmed for 30 min at 50°. Dioxane was removed under reduced pressure, and the aqueous portion was repeatedly extracted with $CHCl_3$-MeOH (99:1). The organic layer was washed in sequence with H_2O, 2 N Na_2CO_3, again with H_2O, dried over Na_2SO_4, and evaporated to afford genin **3** as colorless needles (11 mg), mp 198–201° (MeOH/Et_2O), $[\alpha]^{25}D$ −50° (c = 0.15, MeOH); found C 75.38, H 10.10; $C_{21}H_{34}O_3$ required C 75.45, H 10.18%. It was identified as calogenin [lit. (8) mp 202–205°] by mmp and tlc comparison with the authentic sample. ^1H nmr δ 5.35 (1H, m, H-6), 3.84 (1H, q, J = 6 Hz, H-20), 3.51 (1H, m, H-3), 1.2 (3H, d, J = 6 Hz, 21-Me), 1.1 (3H, s, 18-Me), 0.75 (3H, s, 19-Me).

The aqueous hydrolyzate was neutralized with freshly prepared $BaCO_3$, filtered, and concentrated under reduced pressure to afford the syrupy sugar **5** (7 mg): $[\alpha]^{25}D$ −58.5° (c = 1.0, ω), which gave a pink coloration in xanthydrol and blue coloration in the Keller-Kiliani reaction and reacted with $NaIO_4$. Sugar **5** was identified as 2-deoxy-L-fucose ($[\alpha]D$ −60.2°) by comparing its relative mobility with that of digitoxose.

DI-*O*-ACETYL CALOGENIN [**4**].—Crystalline **3** (4 mg) acetylated in the usual manner afforded acetate **4** (4 mg): mp 163–165°; $[\alpha]^{25}D$ −18° (c = 0.3, MeOH); found C 71.68, H 9.01; $C_{25}H_{38}O_5$ required C 71.77, H 9.09%.

OXIDATION OF 2-DEOXY-L-FUCOSE [**5**] WITH Br_2 WATER.—A solution of **5** (6 mg) in H_2O (0.4 ml) was mixed with Br_2 (6 μl), and the mixture was shaken in a stoppered flask in the dark for 24 h at room temperature. Excess Br_2 was then removed under reduced pressure, and the acidic mixture was made neutral with freshly precipitated Ag_2CO_3. H_2S was passed through the filtrate to remove Ag^+ ions. This filtrate was evaporated to dryness under reduced pressure yielding L-fuconic acid lactone (4.5 mg), $[\alpha]^{25}D$ +28.5° (c = 1.0, Me_2CO), showing a violet coloration with NH_2OH-$FeCl_3$ spray reagent (19).

L-FUCONIC ACID PHENYLHYDRAZIDE.—A solution of L-fuconic acid lactone (4.5 mg) in absolute EtOH (0.06 ml) was heated with phenylhydrazine (0.06 ml) at 100° for 30 min. The cooled viscous mass was triturated with absolute Et_2O (to remove excess phenylhydrazine), yielding L-fuconic acid phenylhydrazide (3 mg): mp 165–168° (MeOH/Me_2CO), $[\alpha]^{25}D$ −7.0° (c = 1.0, ω); found N 10.96; $C_{12}H_{18}N_2O_4$ required N 11.02%.

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