DIOSCIN AND GRACILLIN FROM *TAMUS COMMUNIS*

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In our investigation of the chemical composition of Dioscoreaceae (1-3), we report here the isolation of two known spirostane triglycosides, dioscin and gracillin (4), from the rhizomes of *Tamus communis* L. While gracillin was never isolated from *T. communis*, dioscin was isolated, previously only by enzymatic hydrolysis of a more complex saponin (5), and identified by paper chromatography. In studies currently in progress (6), we have not found more complex saponins with diosgenin as the aglycone.

EXPERIMENTAL

PLANT MATERIALS.—The rhizomes of *T. communis* were collected at Agerola (Naples) in September. Voucher specimens of the plants are deposited in the Herbarium at Naples University, Faculty of Pharmacy.

EXTRACTION AND ISOLATION.—Lyophilized rhizomes of *T. communis* (750 g) were extracted in a Soxhlet apparatus with light petroleum (bp 40-70°), $CHCl_3$, $CHCl_3$-MeOH (9:1), and then with MeOH.

The residue from the $CHCl_3$-MeOH extract (14 g) was chromatographed on a Si-gel column ($CHCl_3$ and $CHCl_3$-MeOH in varying proportions) and rechromatographed on a Sephadex LH-20 column (MeOH) to give 90 mg of a mixture of dioscin and gracillin. The mixture of two triglycosides was separated by preparative hplc (μ-bondapack C-18 column, 30 cm×7.8 mm id, H_2O-MeOH 25:75) to afford 24.5 mg of dioscin and 10 mg of gracillin.

STRUCTURAL ELUCIDATION.—Dioscin and gracillin were identified as diosgenin-*bis*-α-L-rhamnopyranosyl-(1↦2 and 1↦4)-β-D-glucopyranoside and as diosgenin-α-L-rhamnopyranosyl-(1↦2)-β-D-glucopyranosyl-(1↦3)-β-D-glucopyranoside, respectively, by spectral (^1H and ^{13}C nmr, fabms) and chemical data (methanolysis and glc analysis of the persilylated methyl glycosides of the sugars).

The exact position of the linkages of terminal sugar units in gracillin was clarified by partial acid hydrolysis followed by permethylation and methanolysis of the resulting glycosides. The permethylated derivatives were gas-chromatographed in comparison with authentic standards.

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THE FLAVONOIDS OF *STEVIA MICROCHAETA*, *STEVIA MONARDIFOLIA*, AND *STEVIA ORIGANOIDES*

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In our continuing chemical studies of *Stevia* species (Compositae, Eupatorieae) (1,2), we report the isolable flavonoids from three North and Central American species namely, *Stevia microchaeta* Sch. Bip., *Stevia monardifolia* H.B.K., and *Stevia origanoides* H.B.K. All the flavonoids recorded have been isolated

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previously although some are new to the Compositae. The flavonoids isolated are hispidulin (3), 5,7,4′-trihydroxy 3,6,-dimethoxyflavone (4), 5,7-dihydroxy 3,6,4′-trimethoxyflavone (Santin) (4), eupatorin (3), centaureidin (3), luteolin 7-*O*-β-D-glucoside (3), luteolin 4′-*O*-β-D-glucoside (3), cirsimaritin-4′-*O*-β-D-glucoside (3), quercetin 3-*O*-β-D-glucoside (3), quercetin-3-*O*-β-D-galactoside (3), quercetin-3-*O*-α-L-arabinoside (3), and quercetin-3-*O*-α-L-rhamnosyl-D-galactoside (3).

These species show a flavonoid glycoside pattern similar to the previously reported North and Central American species *Stevia nepetifolia* H.B.K. aff. (2), though this species yielded only one aglycone, centaureidin. Of the species presently considered, *S. microchaeta*, which is generally considered to be one of the more primitive members of the genus, was notable for its lack of methoxylated flavonoid aglycones. *S. origanoides*, however, showed a much greater diversity of methoxylated aglycones and was particularly rich in the common flavonoid quercetin-3-*O*-β-D-galactoside. All the North and Central American species investigated contained a greater diversity of flavonoids than the South American species which were also investigated during this study (6).

EXPERIMENTAL

PLANT MATERIALS.—All plant material investigated was collected in Mexico: *S. microchaeta*, from central Oaxaca; *S. monardifolia*, in the region bounded by Pachuca, Orizaba, Taxco, and Toluca; and *S. origanoides*, from central Oaxaca. Voucher specimens of each plant are deposited in the Herbarium of the University of Texas at Austin.

EXTRACTION AND ISOLATION OF FLAVONOIDS.—The dried leaves of *S. microchaeta* (250 g), *S. monardifolia* (9 g), and *S. origanoides* (200 g) were extracted and the flavonoids isolated using standard procedures (1,2,5). The compounds isolated from *S. microchaeta* were as follows: luteolin-7-*O*-β-D-glucoside (23 mg), luteolin-4′-*O*-β-D-glucoside (42 mg), quercetin-3-*O*-β-D-glucoside (15 mg), and quercetin-3-*O*-β-D-galactoside (30 mg). Compounds isolated from *S. monardifolia* were hispidulin (1.2 mg), centaureidin (1.0 mg), quercetin-3-*O*-β-D-glucoside (2.2 mg), quercetin-3-*O*-α-L-arabinoside (0.9 mg), and quercetin-3-*O*-α-L-rhamnosyl-D-galactose (1 mg); those isolated from *S. origanoides* were hispidulin (8 mg) 5,7,4′ trihydroxy 3,6,dimethoxyflavone (6.3 mg), santin (14 mg), eupatorin (5 mg), centaureidin (26 mg), cirsimaritin-4-*O*-β-D-glucoside (8 mg), quercetin-3-*O*-β-D-glucoside (15 mg), and quercetin-3-*O*-β-D-galactoside (600 mg).

All flavonoids were identified by standard spectral methods (uv, ^{1}H nmr, ms) and hydrolytic data as well as by authentic sample comparison and color reaction procedures (5,7). Where authentic samples were not available, as for 5,7,4-trihydroxy 3,6, dimethoxyflavone and santin, these compounds were further clarified by 360 MHz, ^{1}H nmr and ms data and reference to the appropriate literature (4,8,9).

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