# DIOSGENIN AND YAMOGENIN FROM *DIOSCOREA MULTIFLORA*

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Continued search for spiroketal glycosides that would yield aglycones such as diosgenin and hecogenin, which can be used as chief starting materials for the preparation of steroidal hormones, prompted us to analyze systematically the *Dioscorea* species found in abundance in the subtropical southern part of Brazil.

The roots of *Dioscorea multiflora* Mart Ex Griseb., which grows abundantly in the regions of Porto Alegre in the southernmost state of Rio Grande do Sul, enjoys folklore medicinal values but so far has never been studied chemically. Therefore, we became interested and found (tlc) the presence of two $C_{25}$ epimeric spiroketals—diosgenin and yamogenin—in the acid-hydrolyzed glycosidic fraction of the above plant source. Although diosgenin was isolated in yields up to 0.53% (winter, 0.53% and summer, 0.47%), the amount of yamogenin was small, so no effort was made to isolate the compound in quantity for full identification. However, complete physical, chemical, and spectral identifications of diosgenin were made, and it was converted to $\Delta^4$-tigogenone according to the method of Marker and collaborators (1) in yields up to 65%.

## EXPERIMENTAL

PLANT MATERIAL.—The roots of *D. multiflora* were collected in the metropolitan area of Porto Alegre, Brazil, in January (winter) and July (summer), 1979. The plant was authenticated by Professor Bruno E. Irgang, Department of Botany, UFRGS, and a herbarium specimen was kept in the Department of Botany.

EXTRACTION AND PURIFICATION OF THE GENINS.—The dry, powdered material (500 g) was extracted with 95% EtOH (4 liters) for 24 h in a Soxhlet. The brown extract was then concentrated in a rotatory evaporator at 50° to 250 ml until there was copious foaming. To this brick-colored concentrated extract was added $H_2SO_4$ until the solution became 10% by volume; it was hydrolyzed for 2 h and then cooled when coffee-colored solid material settled at the bottom of the flask. The solid thus separated was filtered, washed free from acid, dried, and powdered. The powdery solid (30 g) was extracted with petroleum ether, bp 30-70° (1.5 liters), in a Soxhlet for 20 h. The light yellow extract was then concentrated *in vacuo* at 30° to 30 ml, treated with active carbon (1 g), and chromatographed over neutral alumina (85 g) eluting with hexane and $C_6H_6$-$CHCl_3$ mixture (3:2). Evaporation of the $C_6H_6$-$CHCl_3$ furnished an almost colorless solid that, in tlc (silica gel, petroleum ether-$CHCl_3$, 3:1), showed an intense spot at Rf 0.54, representing diosgenin with a ghost spot at 0.55 for yamogenin. Upon three crystallizations from a petroleum ether and $Et_2O$ mixture, the solid yielded pure diosgenin in colorless needles, mp 204-207°, $M^+$ 414, $C_{27}H_{42}O_3$; pmr and cmr values and mass spectra were identical with reported values (2-6). Both the acetate, mp 198°, $M^+$ 456, and benzoate, mp 237-240°, $M^+$ 518, prepared in the usual way were found to be identical in all respects with the authentic samples provided by Dr. B. Das, CNRS, France.

The pure diosgenin thus isolated was converted into $\Delta^4$-tigogenone which crystallized from a $CHCl_3$-MeOH mixure in colorless needles, mp 186-188°, $C_{27}H_{40}O_3$, $M^+$ 412, Rf 0.67 (silica gel, petroleum ether-$CHCl_3$, 3:1), in a yield of 65%. The $\Delta^4$-tigogenone thus prepared showed the expected mass and pmr and cmr spectra.

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## NEW CONSTITUENTS OF *PRUNUS AFRICANA* BARK EXTRACT

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The $CHCl_3$ extracts obtained from the bark of *Prunus africana* (Hook.f.) Kalkm (Rosaceae) synonym *Pygeum africanum* (Hook) have been patented under various trade names and used for their activity on benign prostatic hypertrophy (1). The present research work was undertaken in order to isolate systematically all constituents from the extract, which will be then screened individually for biological activity.

In addition to substances already isolated by others [*n*-docosanol (0.39% with respect to the crude extract), *n*-tetracosanol (0.48%), fatty acids (62.3%), sitosterol (10.7%), sitostenone (2.0%), and oleanolic acid (0.66%)] (2,3), we have found the following new constituents: friedelin (1.39%), ursolic acid (2.89%), maslinic acid (trace), 2 α-hydroxyursolic acid (0.50%), epimaslinic acid (0.82%), and an acid (0.87%) that appears to be a diastereomer of 2 α-hydroxyursolic acid.

The low content of *n*-docosanol and the presence of large amounts of sterols and triterpenes in the extract stimulate new pharmacological research to establish definitely the substances responsible for the activity of the crude extract, which has been ascribed previously to sitosterol and its glucoside (2) and to *n*-docosanol (4).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Glc analyses were carried out with a C. Erba Fractovap 2300 gas chromatograph; the mass spectra were obtained by gc/ms analyses using a VG Micromass 7070 EQ mass spectrometer; the ir spectra were recorded with a Perkin-Elmer 197 spectrophotometer.

MATERIAL.—A sample of *P. africana* bark extract ("Pyg/12," a commercial $CHCl_3$ extract from Linnea Phytochemical S.A. Riazzino, Locarno, Switzerland), kindly furnished by Laboratori Baldacci S.p.A. of Pisa, Italy, was used.

The isolation of the various substances was made by standard methods before and after saponification of the crude extract. Identifications were made by comparison of gc retention times, ir and ms spectra with those of authentic samples. The acid constituents were identified as their acetates-methyl esters. Epimaslinic acid and the diastereomer of 2α-hydroxyursolic acid were identified by comparison of gc retention times (5), and by gc-ms of their acetates-methyl esters: these mass spectra were practically superimposable with those of the same derivatives of maslinic and 2α-hydroxyursolic acid, respectively.

Full details of the isolation and identification are available on request to the senior author.

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