NEOCHLOROGENIN, NEOSOLASPIGENIN, AND SOLASPIGENIN FROM *SOLANUM TORVUM* LEAVES

UMAR MAHMOOD, RAGHUNATH S. THAKUR, and GERALD BLUNDEN[1]

Central Institute of Medicinal and Aromatic Plants, P.O. Faridinagar, Lucknow-226010, India

Solanum torvum Sw. has been investigated previously for steroidal sapogenins, and neochlorogenin (1, 2), chlorogenin (2, 3), paniculogenin (1), sisalagenone (4), and torvogenin (4) have been reported. However, only neochlorogenin (1, 2), chlorogenin (2), and paniculogenin (1) have been recorded for the leaves. In this present note, we report the isolation of neosolaspigenin and solaspigenin, as well as neochlorogenin, from the leaves of *S. torvum* from India.

MATERIALS AND METHODS

PLANT MATERIALS.—*Solanum torvum* leaves were collected in September 1979 from Dehradum, Uttar Pradesh, India, and identified by Mr. N. C. Shah. A specimen is deposited in the CIMAP Herbarium, collection no. 249.

ISOLATION OF SPIROSTANES.—The air-dried leaves (5.5 kg) were powdered and extracted at room temperature by stirring for 16 h with *n*-hexane (5 x 7 liters), followed by methanol (5 x 7 liters). The methanol extract was concentrated to 500 ml; water (1 liter) was added, and the mixture extracted with chloroform (5 x 1 liters). The chloroform extracts were concentrated to dryness (122 g), and part of the extractive (60 g) was fractionated on silica gel (92 x 7 cm) using successively *n*-hexane (5 liters), *n*-hexane-benzene (1:1) (5 liters), benzene (5 liters), benzene-chloroform (3:1) (5 liters), benzene-chloroform (1:1) (5 liters), benzene-chloroform (1:3) (5 liters), chloroform (5 liters), chloroform-methanol (99:1) (7 liters), chloroform-methanol (99:2) (7 liters), chloroform-methanol (99:4) (8 liters), chloroform-methanol (95:5) (50 liters), chloroform-methanol (90:10) (6.5 liters) and chloroform-methanol (85:15) (12.5 liters). The eluate was collected in 500-ml portions. Spirostanes were detected only in the chloroform-methanol (95:5) eluate, and the serial number of these fractions is used in the text. Neochlorogenin was found in tubes 39-64 (fraction 1). The contents were mixed and evaporated to dryness; the residue was triturated with boiling benzene to remove colored materials, and the mixture was then filtered. The insoluble residue was dissolved in hot methanol, and, on cooling, crystals of neochlorogenin were obtained, which were contaminated with traces of unidentified compounds. Purification was effected by preparative tlc on air-dried silica gel G layers, 500 μm wet thickness, using chloroform-ethanol (95:5) as the development solvent. The sapogenin bands were located as opaque zones by spraying them with distilled water (5). After drying, the bands were removed and the sapogenin extracted with chloroform. The extract was evaporated to dryness, the residue was dissolved in hot methanol, and, on cooling, neochlorogenin was obtained.

Tubes 65-78 contained a mixture of neochlorogenin and solaspigenin, 78-89 held a mixture of solaspigenin and neosolaspigenin (fraction 2), and 90-110 contained neosolaspigenin with a trace of solaspigenin (fraction 3). Fractions 2 and 3 were evaporated to dryness separately, their residues were triturated with boiling benzene, as above, and the spirostanes were crystallized from chloroform.

A Bruker 270 MHz instrument determined nmr spectra in $CDCl_3$ or C_5D_5N. The ms were recorded on a JEOL JMS-D 300 at an ionizing potential of 70 eV.

RESULTS AND DISCUSSION

A methanol extract of air-dried *S. torvum* leaves yielded, after silica gel column chromatography, three spirostane fractions. Fraction 1, after purification by preparative tlc, yielded neochlorogenin (mp, ir, ^{1}H-nmr), which has been recorded previously for this species (1,2). The ms fragmentation patterns of fractions 2 and 3 showed that both probably contained 3,6,23-trihydroxyspirostanes, as their fragments were very similar to those recorded for hispigenin, paniculogenin (6), solaspigenin, and neosolaspigenin (7). The M^+ was observed at *m/z* 448 and other important peaks at *m/z* 363, 345, 327, 289, 271, and 253. The peak at *m/z* 363 is characteristic of 23-hydroxyspirostanes (8).

The ^{1}H-nmr spectrum of fraction 3, after acetylation, in $CDCl_3$ closely

[1]Visiting British Council Fellow; permanent address: School of Pharmacy, Portsmouth Polytechnic, King Henry I Street, Portsmouth, Hants PO1 2DZ, U.K.

matched that of neosolaspigenin triacetate (7). Resonances were observed at δ0.75(3H, s; C-18 Me), δ0.89(3H, s; C-19 Me), δ1.07(3H, d, $J \simeq 6.5$ Hz; C-21 Me), δ1.17(3H, d, $J \simeq 6.5$ Hz; C-27 Me), δ1.98(3H, s; OCOCH_3), δ1.99(3H, s; OCOCH_3), δ2.01(3H, s; OCOCH_3), δ3.35(1H, d, $J \simeq 11$ Hz; C-26βH), δ3.97(1H, dd, $J \simeq 11$ Hz and 2.5 Hz; C-26αH), δ4.40(1H, q, $J \simeq 7.5$ Hz; C-16H), δ4.58(2H, m; C-6βH and C-3αH), and δ4.69(1H, t, W½≃7 Hz; C-23αH). The signals for the C-3α, C-6β, and C-16 protons of neosolaspigenin triacetate were not resolved in the original 90 MHz nmr spectrum recorded (7). The presence of a small amount of the 25R-epimer, solaspigenin triacetate, was detected by a small "triplet" at δ3.44(C-26αH; "25R-") and a small "double doublet" at δ3.53(C-26βH; "25R-") (9).

In the ^{1}H-nmr spectrum of fraction 3 in C_5D_5N signals were observed at δ0.82(3H, s; C-18 or C-19 Me), δ0.84(3H, s; C-18 or C-19 Me), δ1.46(3H, d, $J \simeq 6.5$ Hz; C-21 Me), δ1.51(3H, d, $J \simeq 6.5$ Hz; C-27 Me), δ3.47(1H, d, $J \simeq 11$ Hz; C-26 βH), δ3.56(1H, td, $J_{6\beta 7\alpha} = J_{6\beta 5\alpha} \simeq 10$ Hz and $J_{6\beta 7\beta} \simeq 5$ Hz; C-6 βH), δ3.81(1H, m, W½≃22 Hz; C-3 αH), δ3.94(1H, t, W½≃7 Hz; C-23 αH), δ4.10(1H, dd, $J \simeq 11$ Hz and 2.5 Hz; C-26 α H) and δ4.55(1H, q, $J \simeq 7.5$ Hz; C-16 H). From the shape (10) and chemical shifts (11) of the *CH*OH signals and from the methyl shifts (12) the 3β- and 6α-hydroxy groups were assigned. The "triplet" nature of the signals at δ3.94 confirmed the axial orientation of the hydroxy group at C-23. The occurrence of a small amount of the 25R-epimer was observed again by the presence of a small "double doublet" at δ0.71(C-27 Me). From all the data available, fraction 3 was concluded to be neosolaspigenin containing a small proportion of solaspigenin.

The ^{1}H-nmr spectra of fraction 2 in both C_5D_5N and $CDCl_3$ showed that it, like fraction 3, was a mixture of solaspigenin and neosolaspigenin, although the proportion of solaspigenin was higher, as shown by the relative intensity of the "doublet" at δ0.71(C-27 Me) in the spectrum in C_5D_5N. Separation of the two epimers was not possible due to shortage of material.

To our knowledge solaspigenin and neosolaspigenin have been reported only once previously (6). In this study, the spirostanes were extracted from the air-dried leaves, and it is possible that the compounds had been produced by enzymatic hydrolysis of saponins during the time that the plant material was in transit, after collection and before being air-dried.

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