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NUMMULAROGENIN, A NEW SPIROSTANE FROM *ZIZYPHUS NUMMULARIA*

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ABSTRACT.—From the C_6H_6 extract of the whole plant of *Zizyphus nummularia*, a new (25 *S*)-spirostane was isolated and characterized as nummularogenin, (25 *S*)-3α-hydroxy-5α-spirostane-2, 12-dione.

Zizyphus nummularia W. and A. (Rhamnaceae) is an herb widely distributed in India. It has a reputation for astringent action and has been used in the treatment of bilious sickness, scabies, and boils (1). Previous studies (2-4) on this plant have disclosed the presence of a flavonoid, a sapogenin, and terpenes. This paper reports the isolation from *Z. nummularia* of a new spirostane which has been shown to be (25 *S*)-3α-hydroxy-5α-spirostane-2, 12-dione, to which the trivial name nummularogenin (**1**) is given.

RESULTS AND DISCUSSION

Nummularogenin, mp 262-263°, $[\alpha]^{25}$D −78° ($CHCl_3$), was isolated by column chromatography from the C_6H_6 extract of the plant *Z. nummularia*. Molecular weight determination by mass spectral and elemental analyses established the molecular formula $C_{27}H_{40}O_5$ (M^+ 444) for **1**. This compound gave all the positive tests for a keto-steroidal sapogenin (5). The ir max (KBr) of **1** showed a broad band at 3440-60 (OH), a strong band at 1695 (six-membered ring carbonyl), and characteristic bands (6) at 828, 902, 922, and 990 cm^{-1} for a 25 (*S*))-spirostane ring system (band 922>902) (7); the pmr ($CDCl_3$, δ) spectrum exhibited absorptions of 0.72 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.98 (d, 3H, 21-Me), and 1.05 (d, 3H, 27-Me), and a signal at 4.02 was assigned to a *H*.OH (m, 1H, W½, ≏ 8.00 Hz) group. The mass spectrum showed fragments at *m/z* 444 (M^+), 429 (M^+-CH_3), 426 (M^+-H_2O), 416 (M^+-CO), 411 [M^+-(CH_3+H_2O)], 192, 189, and 139 (base peak).

Upon Wolf-Kishner reduction, **1** gave **2**, mp 189-191°, $C_{27}H_{44}O_3$ (M^+ 416). The spectral features of **2** [ir max (KBr) 3425-35 (br, OH), 985, 923, 900, 828; 923>900 cm^{-1}; pmr ($CDCl_3$, δ) 0.75 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.98 (d, 3H, 21-Me), 1.07 (d, 3H, 27-Me), and 4.00 (m, 1H, W½ 8.00 Hz, *H*.OH); ms at *m/z* 416 (M^+), 415, 398, 383, 344, 302, 287, 273, 255, 189, 139 and 137 were characteristic of saturated monohydroxy spirostanes having an OH group at C-3 with an axial conformation (8-11).

Upon Jones' oxidation, **2** yielded **3**, mp 213-215°, identical with neotigogenone (mp, mmp, and co-tlc; C=O at 1710 cm^{-1} in ir) (12). This, on $NaBH_4$-MeOH treatment, gave **4**, neotigogenin (mp, mmp, ir, and co-tlc). Thus, from this observation, **2** was shown to be 3-*epi*-neotigogenin (mp, mmp and co-tlc) (13), and accordingly, **1** contains a hydroxyl group at C-3 with an axial conformation. A 2,4-DNP derivative of **1** was formed, showing the presence of two ketonic functions in the genin.

Treatment of **1** with Jones' reagent afforded **5**, mp 125-127° (decomp), a triketo compound [2, 4 DNP; and ir max (KBr) 1705 cm^{-1}; $C_{27}H_{38}O_5$ (M^+ 442)] which showed λ max (MeOH) 268 nm (α-cyclic diketone) (14) and pmr ($CDCl_3$, δ) 0.85 (s, 3H, 18-Me), 0.92 (s, 3H, 19-Me), 0.96 (d, 3H, 21-Me), 1.04 (d, 3H, 27-Me), 3.70 (d, 24, 26-H_2), and 4.12 (ddd, 1H, 16-H) showing the presence of two ketones in the same ring and leaving the third ketone in another ring.

Upon treatment with $NaBH_4$, **1** gave agavogenin **6** (15) which, followed by mild CrO_3 oxidation, afforded manogenic acid **7**, which fixes the ketone groups at C-2 and C-12 and also the hydroxyl at C-3 in **1**. The Meerwein-Ponndorf Verley reduction (Al-isopropoxide in *iso*-PrOH solution) of **1** yielded **8**, as manogenin (mp, mmp, and co-tlc) (15), but Clemmenson reduction was ineffective.

Thus, from the above discussion, it is clear that the spirostane nummularogenin should be assigned the (25 *S*)-3α-hydroxy-5α-spirostane-2, 12-dione, structure **1**. The schematic representation of the various products are summarized in Figure 1.

EXPERIMENTAL

ISOLATION AND PURIFICATION.—Defatted, air dried, and powdered whole plant oof *Z. num-*

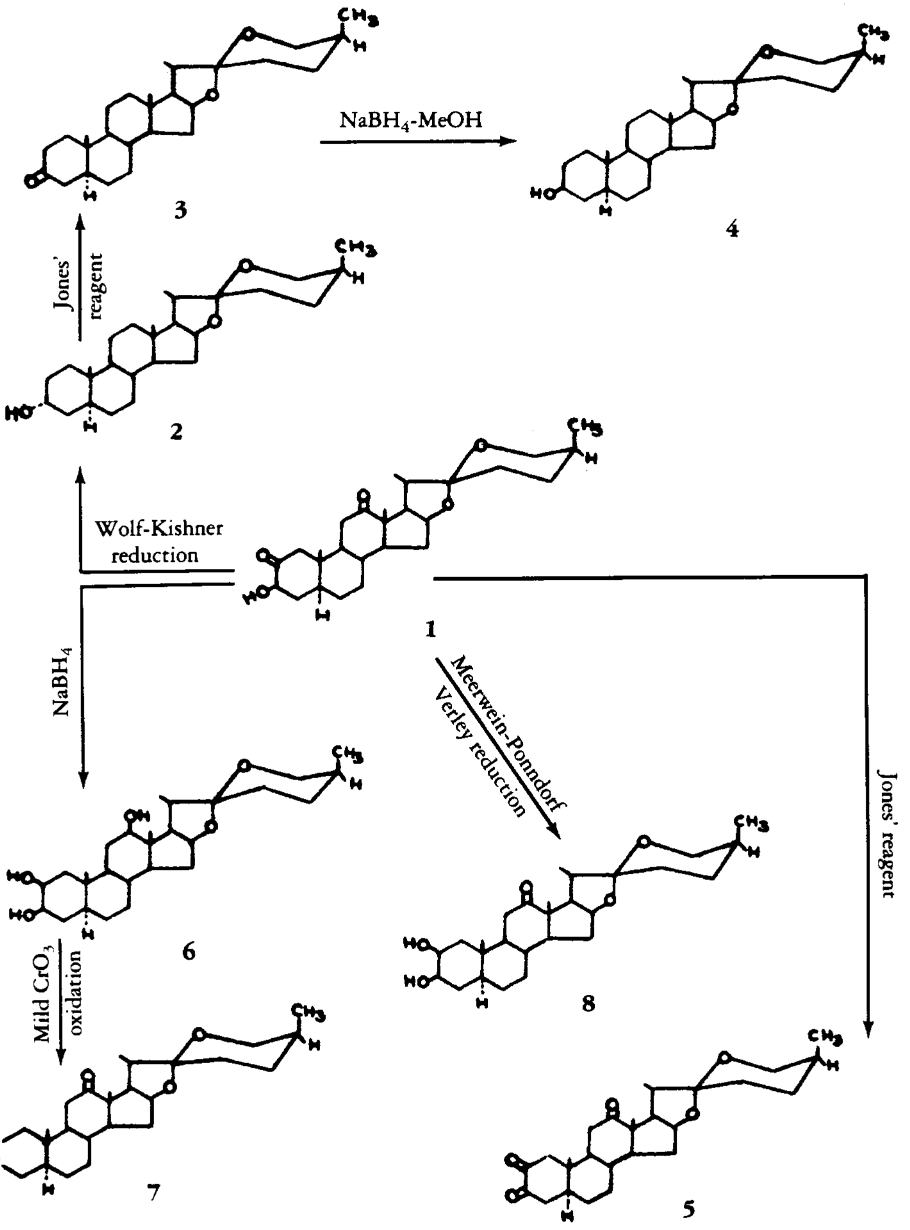

FIGURE 1. Schematic representation of the various products of nummularogenin.

mularia (4 kg) was exhaustively extracted with C_6H_6 under reflux for 160 h. The solvent was distilled to leave a white, amorphous compound that was purified by passing through a column of Al_2O_3 ($CHCl_3$-C_6H_6, 8:2) and crystallized as white needles, mp 262-263° (Found: C, 73.00; H, 9.10; $C_{27}H_{40}O_5$ required: C, 72.97; H, 9.00%).

3-EPI-NEOTIGOGENIN FROM NUMMULAROGENIN.—A solution of 1 g Na in 20 ml EtOH was added to 200 mg of the spirostane and 3 ml of 85% hydrazine hydrate. The mixture was heated in a sealed tube at 18° for 11 h. After cooling, it was poured into H_2O and the precipitate extracted with Et_2O. The ethereal solution was washed with H_2O and dilute HCl, respectively. The Et_2O was removed, the product crystallized from aqueous MeOH (mp 189-191°) and identified as 3-*epi*-neotigogenin. (Found: C, 77.90; H, 10.52; $C_{27}H_{44}O_3$ reqired: C,' 77.88; H, 10.58%).

MANOGENIC ACID FROM AGAVOGENIN.—100 mg of agavogenin (obtained from nummularogenin) in 10 ml HOAc was added to a solution of 100 mg chromic anhydride in 2 ml of 90% HOAc. After standing at room temperature for 3 h, H_2O was added, and the product was extracted with Et_2O and washed with H_2O. It was extracted with KOH, and the alkaline layer was acidified with dilute HCl and again extracted with Et_2O. The solvent was removed, and the residue was crystallized from MeOH-Me_2CO, mp 266-269° (decomp). The dimethylester (CH_2N_2) was obtained as needles (MeOH), mp 161-62°.

ATTEMPTED MILD CLEMMENSEN REACTION WITH NUMMULAROGENIN.—Nummularogenin (100 mg) in 20 ml of EtOH was added to 2 g of two-mesh Zn. The mixture was heated under reflux, and 1 ml. HCl was added every h for 5 h. H_2O was added and extracted with Et_2O. The Et_2O was concentrated and cooled to give needles, mp 262-263° (undepressed on admixture with nummularogenin).

Meerwein-Ponndorf Verley reduction of 100 mg of nummularogenin yielded manogenin (mp, mmp, and co-tlc).

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