

## A NEW SAPOGENIN FROM *BOUSSINGAULTIA GRACILIS*

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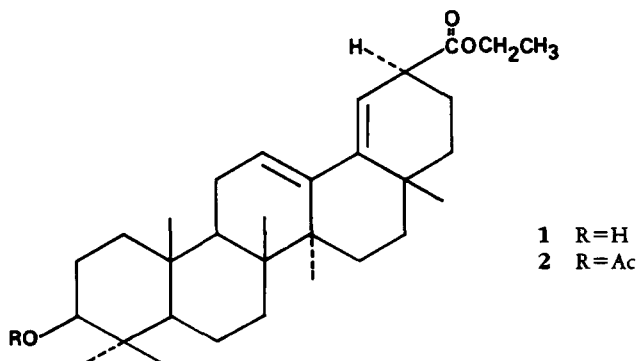
*Boussingaultia gracilis* Miers var. *pseudobaselloides* Bailey (Basellaceae) is a folk medicine used as an analgesic and for the symptomatic treatment of diabetes mellitus in Taiwan. The hypoglycemic activity of this herb was demonstrated in alloxan-induced diabetic mice (1-3). The *n*-hexane and *n*-BuOH fractions, as well as the aglycone fraction resulting from acid hydrolysis of the *n*-BuOH fraction, all show significant hypoglycemic effects in mice (4).

The EtOH extract of the ground plant was defatted with *n*-hexane, then partitioned with H<sub>2</sub>O-saturated *n*-BuOH to afford a crude saponin fraction which upon acid hydrolysis in EtOH/H<sub>2</sub>O yielded a mixture of at least seven aglycones.

Chemical investigation of this plant was first conducted on the aglycone fraction. After cc separation on Si gel eluting with increasing percentages of Me<sub>2</sub>CO in *n*-hexane, six pure sapogenins were successfully crystallized. Among the six, five were identified as known compounds which were larreagenin A (5), 3 $\beta$ -hydroxy-30-noroleana-12,19-dien-28-oic acid (6) and its ethyl ester (7-9), ursolic acid, and 28-ethyl hydrogen 3 $\beta$ -hydroxyolean-12-ene-28,29-dioate (10). This paper reports the isolation and

characterization of a new triterpenoid sapogenin which was elucidated as ethyl 3 $\beta$ -hydroxy-30-noroleana-12,18-dien-29-oate [1].

Elution with *n*-hexane-Me<sub>2</sub>CO (10:1) yielded compound **1** which showed a molecular ion at *m/z* 468 in ms. The uv spectrum showed  $\lambda$  max at 249 nm, indicating the presence of a heteroannular conjugated diene system in the molecule. The ir spectrum revealed the presence of carbonyl (1700 cm<sup>-1</sup>) and hydroxyl (3300 cm<sup>-1</sup>) functions, the latter being confirmed by the formation of a monoacetate **2**. <sup>1</sup>H nmr exhibited a quartet at  $\delta$  4.10 (2H) typical for a methylene of an ethoxy group. Two olefinic protons were observed at  $\delta$  5.08 (1H, t) and  $\delta$  6.30 (1H, br s); the latter could be assigned to H-19 and accounted for by the anisotropic effect of a neighboring carbonyl function. <sup>13</sup>C nmr showed a carbonyl function of ester type at 176.4 ppm and four olefinic carbons at 120.83, 127.09, 134.75, and 137.74 ppm. From the spectroscopic data mentioned above, compound **1** was determined to be ethyl 3 $\beta$ -hydroxy-30-noroleana-12,18-dien-29-oate. Retro-Diels-Alder fragmentation at C ring could satisfactorily explain the significant peaks at *m/z* 260, 247, 207, 187, and



173 in ms. This compound could be an artifact derived from 3 $\beta$ -hydroxy-30-noroleana-12,18-dien-29-oic acid during hydrolysis in EtOH. Compound **1** was then hydrolyzed with NaOH in dioxane to show a new sapogenin spot that was found correspondent to a spot of sapogenin obtained from acid hydrolysis of the *n*-BuOH fraction in dioxane. Therefore, the genuine sapogenin in this case should be 3 $\beta$ -hydroxy-30-noroleana-12,18-dien-29-oic acid, which has not been previously recorded in the literature.

### EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—All melting points are uncorrected and were obtained on a Laboratory micro Met-Temp apparatus. Ir spectra were obtained on a Shimadzu IR-435 spectrophotometer. Uv spectra were recorded on a Shimadzu UV-210 double beam spectrophotometer. Electron impact mass spectra were obtained on a JEOL JMS-D100 mass spectrometer. Nmr spectra were obtained on either a JEOL JNM-PMX60 or a JEOL FX-100 spectrometer.

**EXTRACTION AND FRACTIONATION.**—The leaves and stems were coarsely chipped. The powdered plant material (2.5 kg) was extracted with 95% EtOH at room temperature. Solvent was removed in vacuo at 40°; the residue was suspended in H<sub>2</sub>O and then defatted with *n*-hexane. The H<sub>2</sub>O layer was extracted with H<sub>2</sub>O-saturated *n*-BuOH. The *n*-BuOH extract was hydrolyzed with alcoholic HCl for 6 h, then chromatographed over 200 g of Si gel using a gradient elution of increasing Me<sub>2</sub>CO concentration in *n*-hexane. Six compounds were observed based on tlc (developed by *n*-hexane-Me<sub>2</sub>CO, 20:1).

**ETHYL 3 $\beta$ -HYDROXY-30-NOROLEANA-12,18-DIEN-29-OATE [1].**—Crystallized from MeOH as colorless needles, mp 107–108°; uv (EtOH)  $\lambda$  max (log  $\epsilon$ ) 249 (4.0) nm; ir (KBr)  $\nu$  max (cm<sup>-1</sup>) 3300, 1700, 1430; <sup>1</sup>H nmr (100 MHz, CDCl<sub>3</sub>)  $\delta$  0.76 (3H, s, Me), 0.80 (3H, s, Me), 0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.98 (3H, s, Me), 1.20 (3H, t, 31-Me), 1.27 (3H, s, Me), 3.24 (1H, t,

3-H), 3.48 (1H, s, 3-OH), 4.10 (2H, q, 30-H), 5.08 (1H, t, 12-H), 6.30 (1H, br s, 19-H); <sup>13</sup>C nmr (100 MHz, CDCl<sub>3</sub>) 79.01 (C-3), 120.83 (C-12), 127.09 (C-19), 134.75 (C-18), 137.74 (C-13), 176.43 (C-29); eims *m/z* (rel. int. %) [M]<sup>+</sup> 468 (22), 260 (39), 247 (99), 207 (27), 187 (43), 173 (100).

**ACETYLATION OF 1.**—Compound **1** (20 mg) was treated with Ac<sub>2</sub>O and pyridine at room temperature overnight. The residue thus obtained was purified by Si gel thick layer chromatography (CHCl<sub>3</sub>) to give compound **2** as a colorless crystalline solid, <sup>1</sup>H nmr (60 MHz, CDCl<sub>3</sub>)  $\delta$  2.0 (3H, s, Ac); eims *m/z* [M]<sup>+</sup> 510.

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