ECDYSTEROIDS FROM *VITEX GLABRATA*

KITTIPONG WERAWATTANAMETIN,

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

VERAPONG PODIMUANG,

Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

and APICHART SUKSAMRARN*

Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

Vitex glabrata R. Br. (Verbenaceae) is a tree locally known as "Kai Nao" (1). The bark and root have reportedly been used as astringents. The bark has also been claimed to be an anthelmintic and a remedy for gastro-intestinal disorders (2,3). We report here the isolation and identification of two ecdysteroids: 20-hydroxyecdysone (4) and 11α,20-dihydroxyecdysone (turkesterone) (5) from the bark of *V. glabrata*. Continuous liquid-liquid extraction provides a convenient and effective isolation of 20-hydroxyecdysone in high yield. The occurrences of 20-hydroxyecdysone and related ecdysteroids in *Vitex megapotamica* have been reported (6-8). To our knowledge, this is the first report of 11α,20-dihydroxyecdysone in Verbenaceae (9).

GENERAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Spectra were recorded with the following instruments: uv, Shimadzu UV-240; ir, Jasco A-302A; ^{1}H nmr and ^{13}C nmr, Bruker WP80; ms, Hewlett-Packard 5986.

PLANT MATERIAL.—The plant material was collected in Takhli, Nakon Sawan province, and a voucher specimen (BKF No. 82370) has been deposited at the Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperatives.

EXTRACTION AND ISOLATION.—Dried, powdered barks of *V. glabrata* (4 kg) were extracted successively with hexane and 95% EtOH in a Soxhlet apparatus. The EtOH extract was concentrated to a volume of ca. 300 ml and EtOH (400 ml) and H_2O (2 liters) were added. The filtered brownish solution was transferred to a continuous liquid-liquid extraction apparatus and extracted with $CHCl_3$, Et_2O, and finally with EtOAc from which 20-hydroxyecdysone (63 g) separated out. Tlc revealed traces of 11α,20-dihydroxyecdysone and some other materials. Recrystallization from MeOH/EtOAc gave pure 20-hydroxyecdysone, mp 240-242°. Spectroscopic (uv, ir, ^{1}H nmr, ms) comparisons with the reported data (4) confirmed the identity of this ecdysteroid.

The aqueous ethanolic solution after EtOAc extraction was extracted twice with *n*-BuOH (2.4 liters) using separatory funnels. The concentrated extract was chromatographed on a silica gel column using CH_2Cl_2/MeOH with increasing MeOH content. Short column chromatography of the CH_2Cl_2-MeOH (75:25) fractions gave 11α,20-dihydroxyecdysone (445 mg) as an amorphous solid, and its spectroscopic (uv, ir, ^{1}H nmr, ms) data are consistent with those of the reported values (5). ^{13}C-nmr (C_5D_5N) spectral assignments of this ecdysteroid are as follows: δ 18.7 (C-18), 21.4 (C-16 and C-21), 24.6 (C-19), 27.2 (C-23), 29.8 (C-26 and C-27), 31.7 (C-15), 32.6 (C-4), 39.3 (C-10), 39.6 (C-1), 42.4 (C-9), 42.5 (C-24), 43.9 (C-12), 48.0 (C-13), 49.8 (C-17), 52.2 (C-5), 68.0, 68.2 and 68.7 (C-2, C-3 and C-11), 69.5 (C-25), 76.7 (C-20), 77.4 (C-22), 84.1 (C-14), 122.1 (C-7), 164.2 (C-8), and 204.0 (C-6). Acetylation gave the 2,3,11α,22-tetraacetate and 2,3,11α,22,25-pentaacetate, the spectroscopic (uv, ir, ^{1}H nmr) data of which are also consistent with reported data (5).

Full details of the isolation and identification are available on request to the author (A.S.).

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COUMARINOLIGNOIDS, CLEOMISCOSIN A AND CLEOMISCOSIN B, FROM *AESCULUS TURBINATA*

HITOSHI TANAKA, ICHIRO KATO, KAZUHIKO ICHINO, and KAZUO ITO*

Faculty of Pharmacy, Meijo University, Yagoto, Tempaku, Nagoya 468, Japan

Aesculus turbinata B. (Hippocastanaceae) is a tall deciduous tree widely distributed in the moutains of Japan. Seeds of *A. turbinata* have been used as food. Previous chemical studies have shown that this plant contains β-sitosterol, four simple coumarins (fraxetin, fraxin, esculetin, and esculin) (1), a flavonoid (2), and triterpenoids (3). We now describe the isolation of the coumarinolignoids, cleomiscosin A and cleomiscosin B, from the bark of *A. turbinata*. Cleomiscosin A was identical with an authentic sample (4,5) by direct comparison (mmp, ms, ^{1}H-nmr, ^{13}C-nmr, and ir spectra), and cleomiscosin B was identified by direct comparison with an authentic specimen (6), which we synthesized. Cleomiscosin A has cytotoxic activity (7) and has been previously isolated from *Cleome viscosa* (Capparidaceae) (4,5), *Simaba multiflora, Soulamea soulameoides* (Simaroubaceae), and *Matayba arborecens* (Sapindaceae) (7). Cleomiscosin B is recently shown to have antihepatotoxic activity (8) and has been isolated from *C. viscosa* (5). This is the first reported isolation of the coumarinolignoids from a member of the Hippocastanaceae.

EXPERIMENTAL

PLANT MATERIAL.—*A. turbinata* was collected near Takayama, Gifu Prefecture, Japan, in July 1984. A voucher specimen is deposited at our department.

EXTRACTION AND ISOLATION.—Air-dried bark (7.56 kg) of *A. turbinata* was extracted with MeOH. The extract was evaporated, and a small amount of the concentrate was extracted with EtOAc to give fraxin (50 mg). The major portion of the concentrate was partitioned between H_2O/MeOH and hexane, and between H_2O/MeOH and EtOAc. The EtOAc soluble fraction was chromatographed on a silica gel column using C_6H_6-EtOAc (5:1) to afford scopoletin (2.4 g), esculetin (30 mg), fraxetin (1.0 g), and isoscopoletin (50 mg), mp 185-186° [lit. (9): mp 185°, lit. (10): mp 185-187°, lit. (11): mp 187-190°]. These coumarins (fraxin, fraxetin, esculetin, and scopoletin) were identified by direct comparison (mmp, ir, ms, and ^{1}H nmr) with authentic specimens. A mixture of coumarinolognoids was further purified by chromatography over silica gel using $CHCl_3$-MeOH-EtOAc (9:1:1) to provide cleomiscosin A (150 mg), mp 250-252° [lit. (4,5): mp 247-249°, lit. (7): mp 250-252°, lit. (12): mp 257°] and cleomiscosin B (15 mg), mp 273-275° [lit. (5): mp 274°, lit. (6): mp 275-276°, lit. (12): mp 275-278°].

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

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