CONSTITUENTS OF THE BARK OF *EUPTELEA POLYANDRA*

TAKAO KONOSHIMA, TAKATSUGU MATSUDA, MIDORI TAKASAKI, JOHJI YAMAHARA, MUTSUO KOZUKA,* TOKUNOSUKE SAWADA,

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

and TETURO SHINGU

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Arise, Ikawadani-cho, Nishi-ku, Kobe 673, Japan

As part of an investigation of crude drugs and plants for antiinflammatory activity (1, 2), we have investigated the bark of *Euptelea polyandra* Sieb. et Zucc. (Eupteleaceae). Fractionation of the MeOH extract of the bark, guided by the fertile-egg test (3), yielded an Et_2O-soluble fraction which showed the activity. However, it showed no activity at 1500 mg/kg in the CCl_4-induced liver damage test using dd-mice (4). Purification of the Et_2O-soluble fraction gave nine compounds. These compounds were inactive in the fertile-egg test, indicating that the activity of the fraction might be due to other constituents or other factors. Two additional compounds were isolated from the other fractions as described in the Experimental section. Eupteleoside A and B were reported from the leaves of this plant (5), but neither of them was obtained during the present study on the bark.

EXPERIMENTAL

PLANT MATERIAL.—*E. polyandra* was collected in Kyoto, Japan, in June 1984. Voucher specimens have been preserved in the herbarium of Kyoto Pharmaceutical University.

EXTRACTION AND ISOLATION.—Fresh bark (5 kg) was shredded and extracted with MeOH at room temperature. The extract was concentrated to give crude crystals (ca. 50 g). The crystals were recrystallized from MeOH-$CHCl_3$, repeatedly, to yield 3-*O*-acetyloleanolic aldehyde (2 g) (6). The mother liquors were combined, and the solvent was evaporated off. The residue was chromatographed on silica gel with hexane and hexane-$CHCl_3$, and 3-*O*-acetyloleanolic aldehyde (7 g), 3-*O*-acetylerythrodiol (620 mg) (7), and 3-*O*-acetyloleanolic acid (1.05 g) (8) were isolated. The MeOH extract, separated from the crude crystals, was concentrated, and the concentrate was suspended in H_2O. The suspension was extracted successively with Et_2O, EtOAc, and *n*-BuOH. The Et_2O-soluble fraction (32 g) was chromatographed on silica gel with hexane, hexane-$CHCl_3$, $CHCl_3$, and $CHCl_3$-MeOH. The compounds obtained after several chromatographic steps were 3-*O*-acetyllupeol (200 mg) (9), 3-*O*-acetyloleanolic aldehyde (5 g), olean-12-ene-1,3-dione (200 mg) (10), 3-*O*-acetyloleanolic acid (2 g), β-sitosterol (500 mg) (11), betulinic acid (1.2 g) (11), oleanolic acid (300 mg) (11), 2α-hydroxyoleanolic acid (maslinic acid) (600 mg) (12), and 3-*O*-β-D-glucosyl-β-sitosterol (370 mg) (11). In addition to these compounds, (+)-catechin (1 g) (11) was isolated from a portion (ca. 40 g) of the EtOAc extract (400 g) by silica gel chromatography. Identification of the above compounds was achieved by comparison with authentic samples and/or by comparison with derivatives and by comparison with the reported data. Details of the experimental work may be obtained upon request to the senior author.

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STUDIES IN THE THYMELAEACEAE III. CONSTITUENTS OF *GYRINOPS WALLA*[1]

YEH SCHUN and GEOFFREY A. CORDELL*

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

The genus *Gyrinops*, in the family Thymelaeaceae, is composed of eight species of trees or shrubs, occurring mostly in the Malaysian Islands (2). No medicinal properties have been reported for members of the genus, and there have hitherto been no phytochemical studies. As part of our program to investigate plants for their anticancer constituents, the twigs and leaves of *Gyrinops walla* Gaertn. were exhaustively extracted with petroleum ether and MeOH, and the MeOH extract was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract displayed activity against Eagle's carcinoma of the nasopharynx in cell culture (KB) (3) showing ED_{50} 5.6 and 0.75 μg/ml for twigs and leaves, respectively. Bioactivity directed fractionation of the $CHCl_3$ extract through column chromatography on silica gel and preparative tlc afforded two previously known active principles, 2,6-dimethoxybenzoquinone (NSC-56336) (4, 5) showing activity in KB and PS with ED_{50} 3.7 and 0.0026 μg/ml, respectively, while for cucurbitacin I (NSC-521777) (6), the activities were 0.03 and 0.0014 μg/ml, respectively.

A number of inactive constituents were also isolated and characterized on the basis of their identical spectral properties with previously published data and/or authentic samples. These compounds were friedelan-3β-yl acetate (7, 8), friedelin, friedelan-3β-ol, apigenin-7,4′-dimethyl ether (9), luteolin-7,3′,4′-trimethyl ether (10), (+)-syringaresinol (11), velutin (12), pilloin (13), genkwanin, sitoindoside I (14), and mangiferin (15). The cucurbitacin I content of the $CHCl_3$ extract was 0.0018% for the twigs, and 0.015% for the leaves, and this may explain the different KB activity originally observed for the twig and leaf plant samples. This is the first isolation of a cucurbitacin from the Thymelaceaceae, and is also the first isolation of sitoindoside I and mangiferin from this plant family.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined by means of a Kofler hotplate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrometer. The ir spectra were determined on a Nicolet MX-1 interferometer. ^{1}H-nmr spectra were recorded on a Nicolet NMC 360 instrument using TMS as an internal standard. Mass spectra were obtained with a Varian MAT 112S double focussing spectrometer operating at 70 eV. Column chromatography used silica gel purchased from E. Merck, Darmstadt, W. Germany, and preparative tlc plates were obtained from Analtech, Newark, DE.

PLANT MATERIAL.—The twigs and leaves of *G. walla* (Thymelacaceae) used in this study were obtained from Sri Lanka in August 1981, and a herbarium specimen is deposited in the National Herbarium, Washington, DC. Both plant parts were air dried and extracted separately.

EXTRACTION, FRACTIONATION AND PURIFICATION.—Air-dried, ground twigs (24.5 kg) and leaves (22.5 kg) of *G. walla* were extracted exhaustively with petroleum ether and MeOH, the MeOH extract gave a yellow precipitate on standing at room temperature, and on repeated crystallization from MeOH, mangiferin was obtained. The MeOH extract was evaporated in vacuo and partitioned between

[1]For the previous paper see Borris and Cordell (1).