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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).

$CHCl_3$ and H_2O (1:1 by volume); the interphase was removed by filtration and evaluated for cytotoxic activity. The dried $CHCl_3$ extract (216 g from twigs, 778 g from leaves) was subjected to open column or preparative high pressure silica gel column chromatography and eluted with $CHCl_3$ and mixtures of $CHCl_3$-MeOH of increasing polarity. A total of 60 fractions (1 liter each) were collected, and pooled according to their similar constituents in tlc. Friedelan-3 β-yl acetate, friedelin, friedelan-3 β-ol, apigenin-7,4′-dimethyl ether, luteolin-7,3′,4′-trimethyl ether, (+)-syringaresinol, 2,6-dimethoxy benzoquinone, velutin, pilloin, genkwanin, cucurbitacin I, and sitoindoside I were obtained and identified on the basis of their mp, ir, uv, ms, and nmr spectral properties, and details are available from the authors.

CELL CULTURE ASSAYS.—Cytotoxic activity was assessed using cultured KB cells essentially by the method of Geran *et al.* (3). The method for evaluating activity with cultured P-388 (PS) cells was previously described by Arisawa *et al.* (16) and is based on the protocol established by the NCI. Every extract and fraction obtained in this study was evaluated in the KB assay, and every pure compound was tested in both the KB and PS bioassays.

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