All the flavonoids were identified by standard spectral data (uv, ir, ms, ^{1}H nmr) as well as by authentic sample comparison (mp, tlc) (7-12). Full details of the isolation and identification of the compounds are available on request to the senior author.

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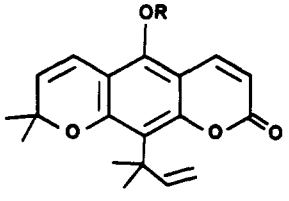
BIOLOGICALLY ACTIVE COUMARINS FROM *ENKLEIA SIAMENSIS*

ZEYUAN LUO, LADDAWAN BOONYARATANAKORNKIT,[1] CHUN-TAO CHE, CLEMENS A.J. ERDELMEIER, HARRY H.S. FONG, and N.R. FARNSWORTH

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

Enkleia siamensis Kurz (Thymeliaceae) is a climbing shrub commonly distributed throughout the northeastern region of Thailand. An ethanolic extract of the dried roots was found to be active in the P-388 in vitro cytotoxicity assay (ED_{50} 0.1 μg/ml). Bioassay-directed fractionation of the total alcoholic extract resulted in the isolation of three coumarins, clausarin, nordentatin, and daphnoretin.

Clausarin and nordentatin were obtained from the petroleum ether fraction of the ethanolic extract. Clausarin was identified by comparing its spectra (^{1}H nmr, uv, ir, ms) with published data (1,2). Nordentatin exhibited spectral properties (^{1}H nmr, ir, uv, ms) indicating a demethylponcitrin structure. When methylated with CH_2N_2, it gave poncitrin (co-tlc). Since dentatin has been proved to be identical with poncitrin (**1**), which is a linear structure (3-8), nordentatin must also have a linear structure (**2**) rather than



1 R=CH_3
2 R=H

[1]Present Address: Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

an angular one as orginally proposed (9). The third coumarin compound, daphnoretin, was isolated from the $CHCl_3$-soluble fraction of the ethanolic extract. It was identified by its spectral properties (^1H nmr, ^{13}C nmr, uv, ir, ms) (10) and by a direct comparison with an authentic sample (co-tlc and ms).

Both clausarin and nordentatin were significantly toxic to P-388 cells in vitro (ED_{50} 1.13 μg/ml and 0.26 μg/ml, respectively). Daphnoretin was, however, inactive in this assay system, although it has been shown to be antineoplastic against Ehrlich ascitic carcinoma (11,12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined using a Kofler hot-stage apparatus. Ir spectra were recorded with a Nicolet MX-1 FT-IR spectrophotometer and uv spectra on a Beckman DU-7 spectrophotometer. Nmr data were collected on a Nicolet NMC-360 or a Nicolet NMC-200 instrument. Mass spectra were obtained with a Varian MAT 112S double-focusing spectrometer operating at 70 eV.

PLANT MATERIAL.—The roots of *E. siamensis* were collected in the Ubol Province of Thailand in March 1985. A voucher specimen of the plant was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Chukalongkorn University, Bangkok, Thailand.

EXTRACTION AND FRACTIONATION.—The dried, powdered roots (4.5 kg) were extracted with 95% EtOH to give 450 g of extractive (P-388 ED_{50} 0.1 μg/ml) after the removal of the solvent in vacuo. The syrupy residue was then suspended in $MeOH/H_2O$ and partitioned with petroleum ether. The aqueous MeOH layers were further extracted with $CHCl_3$. Chromatography of the petroleum ether fraction (22 g, ED_{50} 4.6 μg/ml) over a column of silica gel 60 gave several cytotoxic fractions. Fraction F-12 was then separated over another silica gel 60 column to afford clausarin (23 mg). Fraction F-13 yielded crystals of nordentatin (34 mg). Daphnoretin (480 mg) was obtained by repeated chromatography of the $CHCl_3$ fraction.

Details of the isolation procedure and the spectral data of all isolates are available upon request.

BIOLOGICAL EVALUATION.—The P-388 (9PS) cytotoxicity assay was performed according to the NIH protocol (13). The isolates were solubilized as their PVP-coprecipitates (14) for bioassay. A control using PVP alone was shown to have no effect on cell growth.

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