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LIMONOIDS, ALKALOIDS, AND A COUMARIN FROM THE ROOT AND STEM BARKS OF *TETRADIUM GLABRIFOLIUM*

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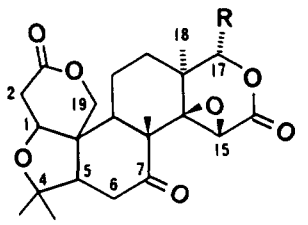
Tetradium glabrifolium (Champ. ex Benth.) Hartley (formerly *Euodia meliaefolia* Benth., *Euodia glauca* Miq.) (Rutaceae) is a shrub to medium-sized tree occurring throughout eastern Asia from Japan, China, and northeastern India to Sumatra and the Philippines (1). In previous studies this species has been reported to contain "berberine-like" alkaloids (2), limonoids (3-5), and an anthraquinone (6). We have recently examined samples of root and stem bark and from these have isolated a range of secondary metabolites typical of the Rutaceae. The identification of several of these compounds and a discussion of their chemotaxonomic significance have been reported elsewhere (7). In this paper we record the presence of further limonoids (one novel) and alkaloids and give additional spectroscopic data on two of the previously reported alkaloids.

Si gel column chromatography of the petroleum extract of the stem bark yielded β-sitosterol, the coumarin, (−)-mexoticin, and the benzophenanthridine alkaloid, chelerythrine (7). Similar treatment of an EtOAc extract gave six further compounds, three alkaloids, and three limonoids.

Of the limonoids, one was identified as the previously reported limonin [**1**] (3,7). The major limonoid differed from **1** only in oxidation of the furan nucleus and was identified as isolimonexic acid [**2**] (8) on the basis of the following: (a) the mass spectrum revealed common fragments at *m/z* 347 and 332 for the limonin-type nucleus, the highest fragment (M^+-15) in **2** was 32 amu (2×O) higher than in **1**, and the base peak in **1** (*m/z* 95 for the furan ring) was absent from **2**; (b) additional absorption bands in the ir spectrum of **2** for OH (3250 cm^{-1}) and C=O (between 1770-1730 cm^{-1}); and (c) two 1H doublets at δ 7.55 and 6.20 in the ^{1}H-nmr spectrum indicative of the dihydrofuranone system found in **2**.

The third limonoid also possessed a dihydrofuranone nucleus, but, in this case, the two protons resonated at δ 6.28 and 6.08, indicative of the alternative dihydrofuranone system to **2** (see **3**). Further differences relating to the limonoid nucleus were: (a) the absence of the ABX system seen for H-5 and H-6 in **1** and **2** and its replacement by a singlet at δ 2.87 (H-5) and a doublet at δ 5.73 for H-7 showing coupling to a 7-hydroxyl proton, (b) the H-19 protons were almost equivalent, (c) one methyl resonance (8-Me) was strongly shielded, (d) in the ir spectrum absorption bands for OH (3240 cm^{-1}) and C=O (1770-1740 cm^{-1}) were similar to **2**, (e) the mass spectrum showed M^+-CH_3 as base peak with small fragments for $M^+-CH_3-H_2O$, $M^+-C_5H_3O_4-CO$ (*m/z* 363), which is identical to the base peak in rutaevin [**4**] (9), and *m/z* 345 (*m/z* $363-H_2O$), but no *m/z* 95. Comparison of the ^{1}H-nmr spectrum with that obtained for authentic rutaevin [**4**] showed only the differences expected for the oxidation of the furan nucleus leading to the identification of the isolated compound as rutaevinexic acid [**3**], which appears to be novel.

The identification of the three al-



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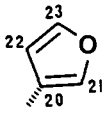

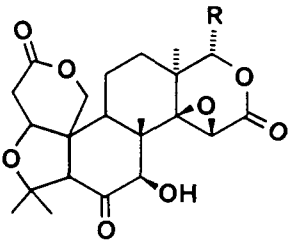

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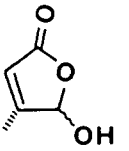

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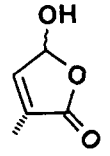

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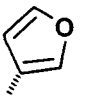

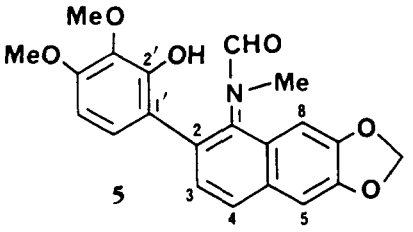

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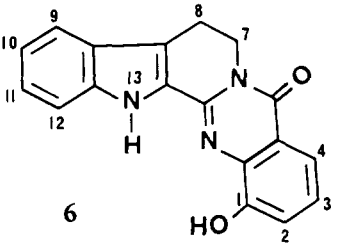

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kaloids as decarine, arnottianamide [**5**], and 1-hydroxyrutaecarpine [**6**] has been reported previously (7). In the course of this study, detailed high-field ^{1}H-nmr studies were performed on both **5** and **6**. These appear to offer useful additional data over those published previously and are, therefore, recorded in the Experimental section.

Similar treatment of the smaller root bark sample yielded **1** as the major component, together with two alkaloid bands. One of the latter was characterized as the common furoquinoline γ-fagarine (7). The other proved to be a mixture of two compounds in a 2:1 ratio and, while they were not separated, ^{1}H-nmr and eims analysis enabled them to be identified as 8-methoxy-*N*-methylflindersine and dictamnine, respectively (10).

Limonoids with oxidized furan rings are relatively rare in the Rutaceae (11), and it has been suggested that they are artifacts caused by air oxidation (12). However, the specific formation of different dihydrofuranone moieties in **2** and **3** suggests that, in this case, they are probably true natural products. A further interesting feature of *T. glabrifolium* is that the benzophenanthridines appeared to be concentrated in the stem bark. This contrasts with the situation in the closely allied genus *Zanthoxylum*, where root bark is generally by far the richest source of this type of alkaloid (13).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a Kofler hot stage apparatus. Ir spectra were recorded as KBr discs on a Pye-Unicam SP3-200 instrument. Specific rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H-nmr spectra were obtained with Bruker WH-250 or WH-360 spectrometers, using TMS as internal standard. Chemical shifts were recorded in δ (ppm). Eims and accurate mass measurements were determined on an AEI MS 902 spectrometer at 70 eV and cims on a Hewlett Packard 5890/5988A spectrometer.

PLANT MATERIAL.—*T. glabrifolium* was collected in Hong Kong in 1985. A voucher specimen, D. Tsang 851, has been deposited at the Herbarium of the Chinese University of Hong Kong.

EXTRACTION AND SEPARATION.—The ground stem bark (1.7 kg) was extracted successively with petroleum ether (bp 60-80°), EtOAc, and then MeOH. The petroleum ether extract was concentrated and subjected to column chromatography over Si gel eluting with petroleum ether containing increasing amounts of EtOAc to give β-sitosterol (120 mg), then (−)-mexoticin (8 mg), and finally chelerythrine (11 mg). Similar treatment of the EtOAc extract yielded, in order of elution, **6** (3 mg), decarine (213 mg), **5** (11 mg), **1** (24 mg), **3** (25 mg), and **2** (215 mg).

Ground root bark (400 g) was treated in the same manner. From the EtOAc extract the following were obtained: a mixture of 8-methoxy-*N*-methylflindersine and dictamnine (15 mg), γ-fagarine (5 mg), and **1** (74 mg).

CHARACTERIZATION OF ISOLATED COMPOUNDS.—*Limonin* [**1**].—Needles from EtOAc, mp 224-226°; $[\alpha]_D-106°$ (*c* 0.063, MeOH; cims *m/z* 471 $(M+1)^+$; eims *m/z* (rel. int.) 455 (2), 347 (100), 95 (37); ^{1}H nmr (DMSO-d_6, 250 MHz) 7.72 (1H, d, J=1 Hz, H-21), 7.66 (1H, dd, J=3.5, 1.8 Hz, H-23), 6.50 (1H, dd, J=1.8, 1 Hz, H-22), 5.46 (1H, s, H-17), 4.92, 4.47 (2H, ABq, J=13 Hz, H-19), 4.10 (1H, s, H-15), 4.06 (1H, d, J=3.6 Hz, H-1), 3.11 (1H, t, J=15 Hz, H-6_{ax}), 2.72 (1H, d, J=16.1 Hz, H-2), 2.60 (1H, dd, J=16.1, 3.6 Hz, H-2), 2.53-2.41 (2H, m, H-9, H-6_{eq}), 2.27 (1H, dd, J=15, 3 Hz, H-5), 1.17, 1.09, 1.01, 0.97 (4×Me).

Isolimonexic acid [**2**].—Needles from EtOAc, mp 295°; $[\alpha]_D-65°$ (*c* 0.13, MeOH); eims *m/z* (rel. int.) 487 (44), 469 (6), 347 (13), 332 (2); ^{1}H nmr (DMSO-d_6, 250 MHz), 8.02 (1H, s, 23-OH), 7.55 (1H, s, H-22), 6.20 (1H, s, H-23), 5.24 (1H, s, H-17), 1.19, 1.15, 1.01, 1.01 (4×Me).

Rutaevinexic acid [**3**].—Needles from EtOAc, mp 253°; $[\alpha]_D-148°$ (*c* 0.062, MeOH); ir 3440, 3240, 2980, 1770-1740, 1710, 1460, 1390, 1280, 1170, 1130, 1100, 1060, 960 cm^{-1}; eims *m/z* (rel. int.) 503 (100), 485 (3), 363 (11), 345 (9); ^{1}H nmr (DMSO-d_6, 360 MHz), 8.22 (1H, s, 21-OH), 6.28 (1H, s, H-22), 6.08 (1H, s, H-21), 5.73 (1H, d, J=4.6 Hz, H-7), 5.19 (1H, s, H-17), 4.52 (1H, br.s, H-1), 4.31, 4.27 (2H, ABq, J=12.5 Hz, H-19), 4.20 (1H, d, J=4.6 Hz, 7-OH), 4.00 (1H, s, H-15), 3.04 (1H, d, J=14 Hz, H-2), 2.87 (1H, s, H-5), 2.59 (1H, dd, J=14, 2.1 Hz, H-2), 1.26, 1.26, 1.13, 0.57 (4×Me).

Arnottianamide [**5**].—Uv, ir, eims in agreement with published data (14); ^{1}H nmr (Me_2CO-d_6, 250 MHz), 8.05 (1H, s, CHO), 7.81, 7.28 (2H, ABq, J=8.3 Hz, H-3, H-4), 7.34 (1H, s, H-8), 7.04 (1H, s, H-5), 6.81, 6.60 (2H, ABq, J=8.6 Hz, H-6′, H-5′), 6.15 (2H, s, O-CH_2-O), 3.88, 3.79 (2×OMe), 2.95 (NMe).

1-Hydroxyrutaecarpine [**6**].—Uv, ir, eims in agreement with published data (15). ^{1}H nmr ($CDCl_3$, 360 MHz), 8.51 (1H, s, OH), 7.72 (1H, dd, J=8, 1 Hz, H-9), 7.66 (1H, dd, J=8, 1.4 Hz, H-4), 7.53 (1H, dd, J=8, 1 Hz, H-12), 7.33 (1H, dt, J=8, 1 Hz, H-10), 7.32 (1H, t, J=8 Hz, H-3), 7.21 (1H, dd, J=8, 1.4 Hz, H-2), 7.12 (1H, dt, J=8, 1 Hz, H-11), 4.57 (2H, t, J=7 Hz, H-7), 3.31 (2H, t, J=7 Hz, H-8).

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INTERNATIONAL SYMPOSIUM ON PLANT NITROGEN METABOLISM

The Phytochemical Society of North America will hold its 28th Annual Meeting at the University of Iowa, Iowa City, on 26-30 June 1988. The meeting will feature an international symposium on recent developments in primary and secondary nitrogen phytochemistry, as well as contributed oral and poster sessions in all areas of phytochemistry. Participation at this conference is not restricted to PSNA members.

The symposium will include the following plenary papers:

"An Overview of Plant Nitrogen Metabolism from an Agronomic Standpoint"
Prof. Dale G. Blevins (University of Missouri-Columbia)

"Synthesis, Transport and Utilization of Products of Symbiotic N_2 Fixation"
Prof. J.S. Pate (University of Western Australia)

"Genetics and Molecular Biology of Higher Plant Nitrate Reductases"
Dr. Andris Kleinhofs (Washington State University)

"The Use of Mutants Lacking Glutamine Synthetase and Glutamate Synthase To Study Their Role in Plant Nitrogen Assimilation"
Prof. Peter J. Lea (University of Lancaster, UK)

"Assimilation of Ammonia by Glutamate Dehydrogenase?"
Prof. David Rhodes (Purdue University)

"Sites of Action of Herbicides in Amino Acid Metabolism: Primary and Secondary Physiological Effects"
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"Metabolism of 1-Aminocyclopropane-1-carboxylic Acid in Relation to Ethylene Biosynthesis"
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"Structure and Expression of Wheat and Rice Seed Protein Genes"
Prof. T.W. Okita (Washington State University)

"The Biological Activity of Polyhydroxyalkaloids and Related Compounds from Plants"
Dr. Linda E. Fellows (Royal Botanic Gardens, Kew, UK)

"Primary and Secondary Metabolism of Polyamines in Plants"
Prof. Hector E. Flores (Louisiana State University)

"Biosynthesis of Alkaloids Using Plant Cell Cultures"
Prof. Dr. Meinhart H. Zenk (Universität München, West Germany)

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