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BERGENIN: ISOCOUMARIN FROM THE STEMS OF *MALLOTUS REPANDUS*

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Key Word Index—*Mallotus repandus*; Euphorbiaceae; isocoumarin; bergenin.

Plant. Mallotus repandus Muell–Arg. (Tsuruakamegashiwa in Japanese)—Euphorbiaceae. Voucher specimen NO. 1399-1 deposited at the Herbarium of this University. *Source.* Unrin Prefecture, Taiwan. *Uses.* This plant is used as anti-inflammatory drug in Taiwan, but we found also antigastric-ulcer activity. *Previous work.* None.

Present work. The stems (1 kg) were extracted with hot EtOH. The extract was evaporated in vacuo and shaken with Me_2CO. The acetone insoluble fraction was extracted with *n*-BuOH and water. The acetone fraction was chromatographed on silica gel. The fraction obtained from elution with $CHCl_3$–MeOH (10:1) gave a crystalline compound, which was recrystallized to give 380 mg of colorless needles, mp 130° (from Me_2CO–hexane), $C_{14}H_{16}O_9$. (Molecular formula was measured by high resolution mass spectrometer and analytical value was in good agreement with the theoretical value.) It appeared from TLC, IR, NMR and MS data to be bergenin and was shown to be identical with an authentic sample by mmp and IR spectra. The dried stems yielded 44 g of Me_2CO extract which contained almost pure bergenin (4·4%); the *n*-BuOH fraction also gave a further 0·08% by chromatography.

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TERPENES OF THE BARK OIL OF *PINUS RADIATA*

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Key Word Index—*Pinus radiata*; Pinaceae; essential oil of bark-phloem; terpenes.

Plant. Monterey Pine (*Pinus radiata* D. Don). *Source.* Pittwater Forest, Tasmania. *Uses.* Widely used in Australia as a source of timber and paper-pulp. *Previous work.* On turpentine [1–3] and on neutral extractives from the bark [4]. *Plant part examined and present work.* A section of bark-phloem (30 × 30 cm) was removed from each of 28 trees and the essential oil was isolated by steam distillation in an average yield of 0·3%. Chromatography of each sample of oil over alumina (Woelm, neutral, Grade III) yielded the hydrocarbons on elution with light petroleum (bp 56–60°), and an oxygenated terpene fraction on elution with Et_2O–MeOH (20:1). Each hydrocarbon sample was distilled at 20 Torr to give a monoterpene hydrocarbon fraction and a residue of sesquiterpene hydrocarbons. The average composition of each sample of oil was 93% of monoterpene hydrocarbons, 1% of sesquiterpene hydrocarbons and 6% of oxygenated terpenes. The small sesquiterpene fractions were not analysed separately but were bulked together. The fractions were analysed as described below and the results are set out in Table 1.

Identification of components. (a) *Monoterpene hydrocarbons.* This fraction of each sample was analysed by GLC using column A at 70°, and the percentage of each component was determined. All samples were then bulked together and the individual components were isolated and purified by micropreparative GLC [5]. Components were identified by comparison of GLC data on columns A, B, & D and spectral data with those of authentic samples. Pure samples of the major components isolated by GLC showed the following rotations in EtOH: α-pinene $[\alpha]_D^{22}$ + 15·6°; β-pinene $[\alpha]_D^{22}$ − 16·9; and limonene $[\alpha]_D^{22}$ − 76°.

(b) *Oxygenated terpenes.* This fraction of each sample was analysed on column E programmed at 2°/min from 70° (isothermal for 5 min) to 200° (isothermal for 20 min), and the percentage of each component was determined. The individual components were isolated as in (a). Components were identified by comparison of GLC data from columns B and D and comparison of spectral data with those of authentic samples or data published in the literature [6–8].

(c) *Sesquiterpene hydrocarbons.* This fraction was analysed on column F at 135° and the percentage of each component was determined. The total was then separated into fractions on column B and then each fraction was separated further into subfractions on column C. The IR spectrum was determined if analytical GLC showed the subfraction to contain a single component. The MS of the components of each subfraction was determined by GC–MS and the modified Kovats Indices [9] were determined with column D at 155°, column E at 165° and column F at 170°. Identification was by comparison of GLC and spectral data with those of authentic samples or data in the literature [7–12].

Table 1. Composition of the bark oil of *Pinus radiata*

Compound	Mean %	Range %	Identification
Monoterpene hydrocarbons			
α-Pinene	27·0	61·8–10·5	GC, MS, IR
Camphene	0·2	0·5–0·1	GC
β-Pinene	54·6	70·9–28·8	GC, MS, IR
3-Carene	0·3	1·9–0·0	GC, MS, IR
Sabinene	1·7	11·1–0·2	GC, MS, IR
α-Phellandrene*	trace	trace–0·0	GC
Myrcene	1·1	1·8–0·5	GC, MS, IR, UV
Limonene	4·4	10·0–0·5	GC, MS, IR
β-Phellandrene	2·6	12·3–0·7	GC, MS, IR
γ-Terpinene	0·1	2·1–0·0	GC, MS
cis-β-Ocimene*	0·2	1·2–0·0	GC, MS, UV
Terpinolene	0·9	12·2–0·0	GC, MS, IR
p-Cymene	trace	trace–0·0	GC, MS
α,*p*-Dimethylstyrene	trace	trace–0 0	GC, MS, UV
Oxygenated terpenes			
Fenchone	0·02	0·04–0·00	MS
Citronellal	0·97	1·86–0·07	GC, MS, IR
{Camphor* / Pinocamphone*}	0·02	0·04–0·00	GC, MS
Isopinocamphone	0·03	0·10–0·00	GC, MS
Linalool	0·58	1·49–0·05	GC, MS
trans-Dihydro-α-terpineol*	0·12	0·49–0·08	GC
Isopulegol	0·19	0·36–0·06	GC, MS
α-Fenchol	0·05	0·10–0·02	GC, MS
Bornyl acetate	0·04	0·08–0·01	GC, MS
{Thymyl methyl ether / Terpinen-4-ol}	0·24	2·08–0·04	GC, MS, IR, UV / GC, MS
Myrtenal	0·02	0·07–0·01	GC, MS, UV
Pulegone	0·01	0·02–0·00	GC, MS
trans-Pinocarveol	0·18	0·48–0·02	GC, MS
{α-Terpineol / Borneol}	1·01	1·87–0·27	GC, MS / GC, MS
Piperitone	0·34	1·10–0·02	GC, MS
Citronellol	0·85	1·85–0·16	GC, MS
Myrtenol	0·03	0·07–0·01	GC, MS
Anethole*	0·03	0·07–0·01	MS
p-Cymen-8-ol	0·04	0·19–0·01	GC, MS
Geraniol	0·01	0·04–0·00	GC, MS
δ-Cadinol*	0·09	0·23–0·01	MS
α-Cadinol	0·09	0·42–0·00	GC, MS
Thymol	0·02	0·06–0·01	GC, MS
13-Epimanool	0·24	2·45–0·00	MS, IR
Sesquiterpene hydrocarbons			
α-Copaene	0·02	—	GC, MS
β-Elemene	0·03	—	GC, MS, IR
Caryophyllene	0·05	—	GC, MS, IR
trans-α-Bergamotene	0·13	—	GC, MS, IR
Aromadendrene	0·04	—	GC, MS
γ-Muurolene*	0·04	—	GC, MS
β-Selinene	trace	—	GC, MS
α-Muurolene	0·25		GC, MS
γ-Cadinene	0·09	—	GC, MS
Calamenene	trace	—	MS, UV
δ-Cadinene	0·25	—	GC, MS, IR

* Tentative.

GLC systems. Combined GC–MS was carried out with a Pye 104 gas chromatograph fitted with column F and linked via a Llewellyn separator to an EAI Quad 300 mass spectrometer. Analytical and micropreparative GLC was carried out on a Philips PV4000 instrument fitted with the following columns: A. 3m × 2·3mm i.d.; 20% β,β'-Oxydipropionitrile: B. 3m × 2·3mm i.d.; 10% Carbowax 20M: C. 3·5m × 2·3mm i.d.; 10% SF-96 (95%) + Igepal 880 (5%): D. 2m × 2·3mm i.d.; 10% Apiezon L. Analytical GLC was also carried out with the Pye 104 instrument fitted with the following capillary columns: E. 72m × 0·76mm; Carbowax 20M: F. 72m × 0·76mm; SF-96 (95%) + Igepal 880 (5%). All columns were of stainless steel, and the support used in packed columns was acid-washed, 60–80 mesh, DMCS treated Chromosorb W. The percentage compositions were calculated from peak areas found using a Series 200 Disc integrator.

Biological significance. There is evidence [13] that the wood-wasp *Sirex noctilio* F. is attracted to volatiles released from the bark of *P. radiata* and most of the compounds listed in Table 1 have been tested [14] as possible attractants.

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