the atmosphere changed to N_2. After 72 hr at room temp. separated crystals (790 mg) were collected and recrystallized from 10% HOAc; mp 250–282° (decomp.). Found: C, 56.79; H, 5.28; N, 6.50. calc. for $C_{10}H_{11}NO_4$: C, 57.43; H, 5.30; N, 6.70%. **2** was synthesized by the method of ref. [2]: mp 240–269° (decomp.). Found: C, 58.58; H, 5.69; N, 6.57. Calc. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27%.

Feeding of radioactive **3**. *Ca* 1 g fr. wt of 31 day-old callus was incubated in 1.7 ml of culture medium containing DL-[β-^{14}C]DOPA (10 μCi, 54 mCi/mmol, NEN, Boston) for 24 hr at 27° in a flask with a centre well in which a small tube containing 0.5 ml scintillamine-OH was placed to trap evolved CO_2. After incubation, the sample was washed × 3 with dist. H_2O and extracted × 4 with hot 80% EtOH. The EtOH extracts were conc and subjected to cellulose TLC developed with PhOH–H_2O (25:8). Authentic samples of **1**, **2** and **4** were co-chromatographed and the spots were detected with ninhydrin. Cellulose powder of each area corresponding to **1**, **2** and **4** on the chromatograms was collected and extracted × 4 with 80% EtOH. The radioactivity of a portion of extracts was counted in Tritosol scintillator [17] or ACS-II (Amersham). Radiolabelled **1**, **2** and **4** were co-crystallized with authentic specimens (61, 60 and 22.3 mg, respectively) from 10% HOAc for **1** and **2**, and from *n*-PrOH–H_2O (3:2) for **4** [5]. The recrystallization was repeated until the crystals showed constant sp. act.

REFERENCES

1. Bell, E. A., Nulu, J. R. and Cone, C. (1971) *Phytochemistry* **10**, 2191.
2. Daxenbichler, M. E., Kleiman, R., Weisleder, D., VanEtten, C. H. and Carlson, K. D. (1972) *Tetrahedron Letters* 1801.
3. Komamine, A. (1962) *Bot. Mag. (Tokyo)* **75**, 228.
4. Obata-Sasamoto, H., Nishi, N. and Komamine, A. (1981) *Plant Cell Physiol.* **22**, 827.
5. Saito, K., Komamine, A. and Senoh, S. (1975) *Z. Naturforsch.* **30c**, 659.
6. Saito, K., Komamine, A. and Senoh, S. (1976) *Z. Naturforsch.* **31c**, 15.
7. Ellis, B. E. (1976) *Phytochemistry* **15**, 489.
8. Müller, P. and Schütte, H.-R. (1968) *Z. Naturforsch.* **23b**, 491.
9. O'Donovan, D. G. and Kenneally, M. F. (1967) *J. Chem. Soc. C.* 1109.
10. Stolle, K. and Gröger, D. (1968) *Arch. Pharm.* **301**, 561.
11. Battersby, A. R., Binks, R. and Huxtable, R. (1967) *Tetrahedron Letters* 563.
12. Leete, E. and Braunstein, J. D. (1969) *Tetrahedron Letters* 451.
13. Kapadia, G. J., Rao, G. S., Leete, E., Fayez, M. B. E., Vaishnav, Y. N. and Fales, H. M. (1970) *J. Am. Chem. Soc.* **92**, 6943.
14. Coutts, I. G. C., Hamblin, M. R. and Tinley, E. J. (1979) *J. Chem. Soc. Perkin Trans.* 1, 2744.
15. Saito, K. and Komamine, A. (1976) *Eur. J. Biochem.* **68**, 237.
16. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15**, 473.
17. Fricke, U. (1975) *Analyt. Biochem.* **63**, 555.

Phytochemistry, Vol. 21, No. 2, pp. 476–478, 1982.
Printed in Great Britain.

0031-9422/82/020476-03$03.00/0

LIGNANS OF *HORSFIELDIA IRYAGHEDHI*

L. M. V. TILLEKERATNE,* D. T. JAYAMANNE, K. D. V. WEERASURIA and A. A. LESLIE GUNATILAKA†

Department of Chemistry, University of Colombo, P.O. Box 1490, Colombo-3, Sri Lanka; † Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

(*Received* 26 *May* 1981)

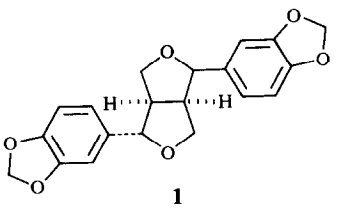
Key Word Index—*Horsfieldia iryaghedhi*; Myristicaceae; (+)-asarinin; (−)-dihydrocubebin; lignans; dodecanoylphloroglucinol.

Abstract—A detailed chemical investigation of extracts of the bark, leaf and timber of *Horsfieldia iryaghedhi* collected in Sri Lanka, led to the isolation of (+)-asarinin, dodecanoylphloroglucinol and (−)-dihydrocubebin.

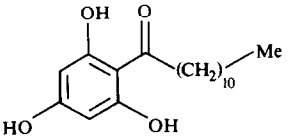
INTRODUCTION

Horsfieldia iryaghedhi Warb. (= *Myristica horsfieldia*, *M. iryaghedhi*; *Sinhala*, Iryaghedhi), a plant indigenous to Sri Lanka is a member of the nutmeg family (Myristicaceae) [1]. The chemical constituents of nutmeg (*Myristica fragrans*) have been studied extensively due to their pharmacological properties [2]. Previous chemical investigation of *H. iryaghedhi* seeds has resulted in the isolation of (+)-asarinin **1** and dodecanoylphloroglucinol **2** [3]. However, no detailed

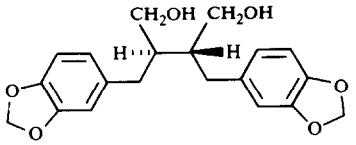
*To whom correspondence should be addressed.



1



2



3

studies of the chemical constituents of other parts of this plant have thus far been reported and the present paper deals with a detailed investigation of the bark, leaf and timber.

RESULTS AND DISCUSSION

The petrol soluble fraction of the MeOH extract of the bark gave mainly a single compound which deposited colourless needles on crystallization from Et_2O–petrol. From the spectral data it was shown to be (+)-asarinin **1**.

CC of the Et_2O-soluble fraction of the MeOH extract gave three solid compounds. The first compound eluted, on further purification by prep. TLC and recrystallization from $CHCl_3$–petrol gave colourless needles, which were identified as dodecanoylphloroglucinol **2** from its spectral characteristics. The second compound eluted was found to be a further quantity of (+)-asarinin. The third compound eluted, when further purified by CC and recrystallized from petrol–Et_2O, gave colourless needles, mp 101–102°. This compound was identified as (−)-dihydrocubebin **3** from its spectral characteristics. Although the isolation of (−)-dihydrocubebin from a natural source has been reported earlier [4–6], this is the first reported isolation of this lignan from a member of the Myristicaceae. Dihydrocubebin has been reported [6] to show antimicrobial activity against *Mycobacterium smegmatis*.

EXPERIMENTAL

General. Mps are uncorr. NMR spectra were recorded in $CDCl_3$ at 60 MHz with TMS as int. standard. Si gel (Merck) plates (0.25 mm) were used for TLC and for prep. TLC; these were 1 mm thick. Petrol refers to the fraction bp 60–80°.

Extraction, fractionation and isolation. Bark (2.7 kg) of *H. iryaghedhi* collected at Kanneliya, Sri Lanka in August 1978 was air dried, powdered and extracted with hot MeOH for 110 hr. Removal of solvent gave a dark reddish brown gum (520 g). A portion of this residue (260 g) was successively extracted with petrol, Et_2O and EtOAc. The petrol extract on concn gave a white amorphous solid (320 mg), which on recrystallization from Et_2O–petrol gave (+)-asarinin **1** as colourless needles, mp 123–124°, lit. 122.5–123° [3]; IR ν_{max} (KBr) cm^{-1}: 2860, 1600, 1485, 1440, 1370, 1260; NMR, δ 2.80–4.20 (6 H, *m*, –CH –CH_2–O–), 4.40 (1H, *d*, J = 7.5 Hz, Ar–CH–O–), 4.80 (1 H, *d*, J = 5 Hz, Ar–CH–O–), 5.90 (4H, *s*, –O– CH_2–O–), 6.75–6.80 (6H, *m*, aromatic H); MS: *m/z* (rel. int.) 354 (88), 203 (22), 178 (19), 161 (38), 150 (33), 149 (100), 136 (11), 135 (54), 131 (26). The viscous oil left after the removal of a white amorphous solid from the petrol extract was chromatographed on a column of Si gel (50 g) with mixtures of petrol and Et_2O as eluents to obtain a further quantity (70 mg) of (+)-asarinin.

The Et_2O extract on removal of solvent gave a dark reddish brown gum (23.5 g) which was chromatographed on a column of Si gel (350 g) with mixtures of petrol and Et_2O as eluents. The first fraction eluted was a dark red gum, which when treated with Et_2O–petrol, gave a white amorphous solid (414 mg). It was further purified by prep. TLC to obtain dodecanoylphloroglucinol **2** (36 mg) as colourless needles from petrol–$CHCl_3$, mp 126–127°, lit. 125–126° [3]; IR ν_{max} (KBr) cm^{-1}: 3380, 3240, 2910, 2840, 1640, 1620, 1600, 1470, 1210, 1180; NMR, δ 0.9 (3H, *br s*, $-CH_3$), 1.33 (18H, *br s*, $-CH_2-$), 3.1 (2H, *t*, J = 7 Hz, $-\overset{O}{\overset{\|}{C}}-CH_2-$), 6.03 (2H, *br s*, aromatic H), 8.65 (1H, *br s*, Ar–OH, exchangeable D_2O), 11.5 (2H, *br s*, Ar–OH, exchangeable D_2O); MS: *m/z* (rel. int.) 308 (60), 290 (58), 206 (11), 205 (22), 192 (11), 191 (11), 181 (10), 169 (30), 168 (56), 164 (11), 163 (10), 155 (14), 154 (60), 153 (100), 152 (10). The second and third fractions from the column gave a further crop of (+)-asarinin (33 mg).

The fourth fraction on concn gave a dark reddish brown oil (4.7 g), which was rechromatographed on a column of Si gel (75 g) using mixtures of petrol and $CHCl_3$ and mixtures of $CHCl_3$ and EtOAc as eluents. The latter system gave a reddish brown oil which on standing gave reddish brown crystals (210 mg). Recrystallization from petrol–Et_2O gave (−)-dihydrocubebin **3** as colourless needles, mp 101–102°, lit. 100–102° [4]; $[\alpha]_D^{28}$ −36.8° ($CHCl_3$; c = 2.2); IR ν_{max} (KBr) cm^{-1}: 3250, 2880, 1605, 1480, 1435, 1355, 1240, 1185, 1095, 1030, 920, 870, 808, 770; NMR δ 1.85 (2H, *m*, –CH–), 2.66 (4H, *d*, J = 7 Hz, Ar–CH_2–), 3.6 (6H, *m*, $-CH_2OH$), 5.9 (4H, *s*, –O–CH_2–O–), 6.62 (6H, *s*, aromatic H); MS: *m/z* (rel. int.) 358 (14), 340 (11), 204 (8), 192 (5), 187 (8), 161 (5), 149 (19), 136 (39), 135 (100), 105 (18), 83 (6), 77 (14), 71 (7), 57 (10), 55 (5), 51 (6), 43 (7), 41 (6), 32 (7), 28 (12).

The fifth fraction gave a light brown solid (0.59 g), further purification of which by prep. TLC gave a further quantity (65 mg) of (−)-dihydrocubebin.

The EtOAc extract on purification by column and prep. TLC failed to give any pure compounds.

Detailed investigation of the leaves and timber of *H. iryaghedhi* according to the method of ref. [3] also resulted in the isolation of compounds, **1–3**.

Acknowledgements—The authors express their gratitude to Prof. S. Balasubramaniam, Department of Botany, University of Peradeniya, Sri Lanka, for identification of plant material. Financial assistance from the National Science Council of Sri Lanka and from the University of Colombo, Sri Lanka, is gratefully acknowledged.

REFERENCES

1. Trimen, H. (1974) *A Hand-book of the Flora of Ceylon*, Part III, p. 435. M/S, Periodical Experts, Delhi.
2. Claus, E. P. and Tyler, V. E. Jr. *Pharmacognosy*, 5th edn, p. 223. Lea & Febiger, Philadelphia.
3. Kitagawa, I., Nakanishi, T., Ito, Y., Sultanbawa, M.U.S. and Yosioka, I. (1972) *Chem. Pharm. Bull.* **20**, 2278.
4. Dwuma-Badu, D., Ayim, J. S. K., Dabra, T. T., ElSohly, H. N., Knapp, J. E., Slatkin, D. J. and Schiff, P. L. Jr. (1975) *Lloydia* **38**, 343.
5. Dwuma-Badu, D., Ayim, J. S. K., Dabra, T. T., ElSohly, H. N., Knapp, J. E., Slatkin, D. J. and Schiff, P. L. Jr. (1976) *Lloydia* **39**, 60.
6. Anjaneyulu, A. S. R., Madhusudhana Rao, A., Krameswara Rao, V., Ramachandra Row, L., Pelter, A. and Ward, R. S. (1975) *Tetrahedron Letters* 2961.

Phytochemistry, Vol. 21, No. 2, pp. 478–480, 1982.
Printed in Great Britain.

0031-9422/82/020478-03$03.00/0

COELONIN, A 9,10-DIHYDROPHENANTHRENE FROM THE ORCHIDS *COELOGYNE OCHRACEA* AND *COELOGYNE ELATA*

PRIYALAL MAJUMDER, SWAPNA LAHA and NABANITA DATTA

Department of Chemistry, University College of Science, Calcutta 700009, India

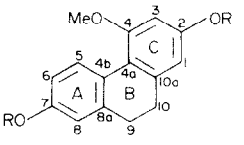
(*Received* 12 *June* 1981)

Key Word Index—*Coelogyne*; Orchidaceae; orchid; coelonin; 9,10-dihydrophenanthrene.

Abstract—2,7-Dihydroxy-4-methoxy-9,10-dihydrophenanthrene was isolated and identified from the whole plant of *Coelogyne ochracea* and *C. elata*.

The isolation of physiologically active alkaloids like dendrobine and a number of its structural analogues from the orchids of the genus *Dendrobium* [1] prompted us to investigate chemically a series of high-altitude Himalayan orchids. In this communication we report the structure elucidation of a new phenolic compound, coelonin, isolated from two such orchids, *Coelogyne ochracea* and *C. elata*.

Coelonin, $C_{15}H_{14}O_3$ ($M^{\cdot+}$ 242) was isolated as an amorphous solid in extremely poor yield from the $CHCl_3$ and MeOH extracts of *C. ochracea* and *C. elata*. The UV spectrum of coelonin shows resemblance to those of 9,10-dihydrophenanthrenes [2]. It responds to colour reactions characteristic of a phenolic compound. This is supported by its IR spectrum showing absorption for hydroxyl group (ν_{max} 3620 cm^{-1}) and usual bands for aromatic nucleus. The ^1H NMR spectrum of coelonin shows a four-proton singlet at δ 2.60 which is typical [2, 3] of the four equivalent protons of the 9- and 10-methylene groups of 9,10-dihydrophenanthrenes. The spectrum also displays signals for an aromatic methoxyl (δ 3.75), two exchangeable protons at δ 4.83 and five aromatic protons at δ 8.02 (1H, *d*, $J = 8$ Hz), 6.67 (1H, *dd*, $J_1 = 8$ Hz and $J_2 = 3$ Hz), 6.51 (1H, *d*, $J = 3$ Hz), 6.33 (1H, *d*, $J = 3$ Hz) and 6.30 (1H, *d*, $J = 3$ Hz). The downfield aromatic proton is reminiscent [4, 5] of the H-4 or H-5 of a 9,10-dihydrophenanthrene and its appearance as a clear doublet having a coupling constant of 8 Hz implies that it must have an *ortho* aromatic proton with the *meta* position being substituted. The chemical shifts and the splitting patterns of the remaining four aromatic protons when considered along with the above observation and the presence of two phenolic hydroxyl groups and an aromatic methoxyl nicely fit in with structure **1a** for coelonin. In the alternative 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene formulation the two phenolic OH protons would be expected to resonate at different fields, as is observed in structurally similar compounds [6].



1a R=H
1b R=Ac
1c R=Me