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(−)-(*TRANS*-4′-β-D-GLYCOPYRANOSYLOXY-3′-METHOXYCINNAMYL)-LUPININE, A NEW LUPIN ALKALOID IN *LUPINUS* SEEDLINGS

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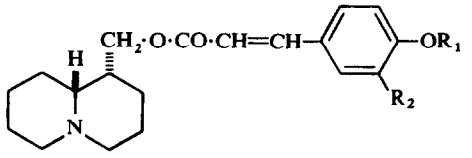
Key Word Index—*Lupinus luteus*; Leguminosae; alkaloid; glycoside; hydroxycinnamic acid; lupinine; (−)-(*trans*-4′-β-D-glucopyranosyloxy-3′-methoxycinnamyl)lupinine.

We have recently reported the presence of new lupinine-ester alkaloids, (−)-(*trans*-4′-hydroxycinnamyl)lupinine (**1**) and its derivatives (**2**–**5**) in the fresh seedlings of *Lupinus luteus* [1–4]. It has also been ascertained in our laboratory that **1** was formed from (−)-lupinine and *trans*-4-hydroxycinnamic acid by two enzymatic systems in *Lupinus* seedlings in the presence of ATP and CoA [5].

A new glucosidic lupin alkaloid, (−)-*trans*-4′-β-D-glucopyranosyloxy-3′-methoxycinnamyl)lupinine (**6**), has now been isolated from the fresh seedlings of *L. luteus*. **3** is known as a metabolite in the same young plant [6], but has never been obtained before as its glucoside.

The structure of **6** was determined by spectrometric (MS and NMR) data and by direct comparison with a synthetic sample, prepared as described in the Experimental. NMR spectra showed that both the natural and synthetic samples of **6** were present as a mixture of *trans* and *cis*-isomers in the ratio of *ca* 3:2, respectively. The conversion of *trans*-cinnamates into the *cis*-isomers is unavoidable [1–4].

No detectable amount of **6** was found in the immature and mature seeds of *L. luteus*. However, its concentration increased rapidly during the first 3–10 days growth of seedlings, analogously to those of **1**–**5**. In the later stages of the plant's growth, the concentration of **6** diminished and it again became a minor component.



1 R_1 = H; R_2 = H
2 R_1 = Rha; R_2 = H
3 R_1 = H; R_2 = OMe
4 R_1 = Rha; R_2 = OMe
5 R_1 = Glu; R_2 = H
6 R_1 = Glu; R_2 = OMe

EXPERIMENTAL

General methods. NMR spectra were determined at 100 MHz: chemical shifts are reported in δ units relative to TMS as an internal standard in $CDCl_3$ containing 5% CD_3OD. MS were obtained at 70 eV. The following solvents were used for Si gel TLC and PC: 1, CH_2Cl_2–MeOH–28% NH_4OH (60:39:1); 2, CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); 3, 7% MeOH/CH_2Cl_2–28% NH_4OH (100:1); 4, CH_2Cl_2–MeOH (3:2); 5, EtOAc–Py–H_2O (2:1:2). HPLC was carried out with solvent 6, 15% MeOH/Et_2O–H_2O–25% NH_4OH (500:9:1, v/v), using a LiChrosorb SI-100 (Merck, Particle size 10 μm, 0.3 × 50 cm) column employing a monitoring flow system (220 and 310 nm) coupled to recorder at a flow rate of 1 ml/min.

Isolation of **6**. As described in a previous paper [4], the **6**-rich fractions, obtained from the *n*-hexane-insoluble portion of the crude total alkaloid (2.1 g) in the fresh harvested 10-day-old seedlings (1.4 kg), were further purified by HPLC using solvent 6; **5** and **6** were eluted from the column at approximately 27 and 42 min, respectively, whilst **1**, **3**, **2**, **4** and (−)-lupinine appeared at positions of 5, 7, 10, 12 and 18 min, respectively, of the retention time.

The colour reactions and R_f values, on TLC in solvents 1–3, of **6** exhibited all the same as those of **5** [4]; both **5** and **6** are indistinguishable except HPLC. **6**: Colourless amorphous solid, $[\alpha]_D^{22}$ − 48.9°, $[\alpha]_{436}^{22}$ − 97.9° (*c* = 0.06, EtOH); UV λ_{max}^{EtOH} nm (log ε): 231 (4.01, shoulder), 295 (4.08), 317 (4.08); MS: *m/e* 345 (25%, M^+ − glucosyl moiety), 168 (13), 152 (100), 138 (16), 111 (14); NMR: δ 3.92 and 3.94 (3H, 2 *s*, aromatic OCH_3), 5.88 and 6.89 (2/5H each, 2 *d*, *J* = 13 Hz, CO—CH=CH (*cis*)), 6.36 and 7.59 (3/5H each, 2 *d*, *J* = 16 Hz, CO—CH=CH (*trans*)), 7.0–7.7 (3H, *m*, aromatic H), 4.91 (1H, *bd*, *J* = 6 Hz, anomeric H). From the NMR data, it was proved that **6** was a mixture of *trans* and *cis*-isomers at the ratio of *ca* 3:2, respectively.

Hydrolysis of **6** *into* **3** *and* D-*glucose*. **6** was easily hydrolysed into **3** and D-glucose with both the 3.5% HCl at 60° for 3.5 hr and the β-glucosidase (from almonds, Miles) system as described in a previous paper [4] for **5**. **3** was identified by direct comparison with **3** in nature or a synthetic sample, and the glucose by β-D-glucose oxidase system [4, 7].

Synthesis of **6** *from* **3**. **6** was synthesized in 30% yield from **3** [3, 8], and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylbromide as described in a previous paper [4] for the synthesis of **5**. The synthetic product was found to be completely identical with those of the natural product in its MS, NMR and chromatographic behaviour.

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REFERENCES

1. Murakoshi, I., Sugimoto, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1975) *Phytochemistry* **14**, 2714.
2. Murakoshi, I., Kakegawa, F., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1977) *Phytochemistry* **16**, 2046.
3. Murakoshi, I., Toriizuka, K., Haginiwa, J., Ohmiya, S. and

Otomasu, H. (1978) *Phytochemistry* **17**, 1817.
4. Murakoshi, I., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1979). *Chem. Pharm. Bull. (Tokyo)* **27**, 151.
5. Murakoshi, I., Ogawa, M., Toriizuka, K. Haginiwa, J., Ohmiya, S. and Otomasu, H. (1977) *Chem. Pharm. Bull. (Tokyo)* **25**, 527.
6. Podkowinska, H. and Wiewiorowski, M. (1965) *Bull. Acad. Polon. Sci. Ser. Sci. Biol.* **13**, 623.
7. Kabasakalian, P., Kalliney, S. and Westcott, A. (1974) *Clin. Chem.* **20**, 606.
8. Podkowinska, H. and Wiewiorowski, M. (1967) *Bulll Acad. Polon Sci. Ser. Sci. Chim.* **15**, 467.

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RUDRAKINE, A NEW ALKALOID FROM *ELAEOCARPUS GANITRUS**

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Key Word Index—*Elaeocarpus ganitrus*; Elaeocarpaceae; alkaloid; rudrakine.

INTRODUCTION

In continuation of our search for chemical constituents from different *Elaeocarpus* species [1–3], we have isolated a minor alkaloid, rudrakine, from the leaves of *Elaeocarpus ganitrus* Roxb. (Sanskrit: Rudraksh). *E. granitrus*, which grows in the Himalayan region, is valued in India for its attractive fruit stones and also for its medicinal properties [4, 5]. The present communication describes the structural elucidation of rudrakine.

RESULTS AND DISCUSSION

Rudrakine, $C_{16}H_{23}NO_3$, M^+ 277, mp 159–60°, was characterized as an indolizidine alkaloid like other C_{16}-alkaloids of *Elaeocarpus* species [6] from its MS which showed the base peak at *m/e* 122 (ion **a**) and an important peak at *m/e* 97 (ion **b**). The similarity of the UV and IR spectra of rudrakine (λ_{max} 272 nm, ε 7300; ν_{max} 1660 cm^{-1}) with those reported for pseudoepi-isoelaeocarpiline (**1**) (λ_{max} 275 nm, ε 7600; ν_{max} 1665 cm^{-1}) suggested the presence of a dihydro-γ-pyrone chromophore in the molecule. Recognition of the third oxygen function as OH from the IR band at 3450 cm^{-1}, and the absence of any olefinic or aromatic proton and presence of a secondary C-Me group (3H, $d\,\delta$ 1.2; $J = 7$ Hz) from the PMR spectrum of the alkaloid, led to a tetracyclic structure for rudrakine similar to that of **1** but differing from the latter by the presence of a OH group and the absence of 14,15 double bond. The position of the OH group in the tetracyclic skeleton was considered to be at C-14 on biogenetic grounds and MS evidence. The genesis of the ion species **a** and **b** on electron impact and their abundance as discerned from the spectrum of rudrakine clearly indicates that the OH is not attached to any of the carbon atoms of the indolizidine part of the molecule. Again, the presence of the secondary C-Me group excludes the possibility of its linkage to C-16. Of the remaining 3 sites in ring A, C-14 is the most appropriate site for this group, as C_{16}-alkaloids of *Elaeocarpus* species are known to be derived from 6 acetate and one ornithine units [6]. The placement of the OH group at C-14 also rationalizes the appearance of a small but significant MS peak at *m/e* 207 (ion **c**). The structure of rudrakine was thus established as **2**.

A number of C_{16}-dienone alkaloids including (±)-elaeocarpine (**3**) and (±)-isoelaeocarpine (**4**) as minor constituents have been isolated from the leaves of *E. sphaericus* (Gaertn.) K. Schum. of New Guinea [7], a plant reported to be synonymous with *E. ganitrus* Roxb. of India [8]. An ecological variation is manifested in the Indian species which reveals the presence of aromatic alkaloids **3** and **4** as major constituents [1, 9], absence of dienone alkaloids and the presence of a new alkaloid rudrakine. Isolation of rudrakine is indeed of biogenetic significance because it appears to be an intermediate which by loss of H_2O can give rise to both conjugated and non-conjugated dienone alkaloids like elaeocarpiline (**5**) and pseudoepi-isoelaeocarpiline (**1**).

EXPERIMENTAL

Mps were determined in open capillary and are uncorr. UV spectra were measured in MeOH; IR in Nujol and PMR at 60 MHz.

Isolation of alkaloids. Dried and powdered leaves (10 kg) of *E. ganitrus* Roxb. were defatted with petrol (60–80°) and then extracted with EtOH (95%) by cold percolation. The extract was concd under red. pres. to a dark brown syrup, stirred with citric acid (5%) and filtered. The filtrate was made alkaline with NH_4OH (pH 9) and exhaustively extracted with $CHCl_3$.

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