REFERENCES

1. Sanyal, P. K. and Bose, P. K. (1969) *Sci. Cult.* **35**, 332.
2. Sanyal, P. K., Basak, A., Barua, A. K. and Bose, P. K. (1975) *J. Ind. Chem. Soc.* **52**, 1213.
3. Chakraborty, D. P., Chowdhury, B. K. and Das, B. C. (1967) *Tetrahedron Letters*, 3471.
4. Ramstad, E., Lim, W. C. and Koo, W. (1968) *Tetrahedron Letters*, 811.
5. Vijayalakshmi, M., Ratnam, C. V. and Subba Rao, N. V. (1972) *Ind. J. Chem.* **10**, 564.
6. Chowdhury, B. K. and Chakraborty, D. P. (1971) *Phytochemistry* **10**, 481.
7. Price, J. R. (1956) *Fortschr. Chem. Org. Naturst* **13**, 302.
8. Chowdhury, B. K. and Chakraborty, D. P. (1971) *J. Ind. Chem. Soc.* **48**, 80.
9. Dreyer, D. L. (1968) *J. Org. Chem.* **33**, 3574.
10. Joshi, B. S. and Kamat, V. N. (1969) *Ind. J. Chem.* **7**, 635.
11. Joshi, B. S. and Kamat, V. N. (1970) *Phytochemistry* **9**, 889.
12. Chakraborty, D. P., Dutta, J. and Ghosh, A. (1965) *Sci. Cult.* **31**, 529.
13. Kureel, S. P., Kapil, R. S. and Popli, S. P. (1969) *Tetrahedron Letters*, 3857.
14. Narashimhan, N. S., Paadkar, M. V. and Chitguppi, V. P. (1968) *Tetrahedron Letters*, 5501.

Phytochemistry, 1978, Vol. 17, pp. 1817–1818. © Pergamon Press Ltd. Printed in England

0031-9422/78/1001-1817 $02.00/0

(−)-(*TRANS*-4′-RHAMNOSYLOXY-3′-METHOXYCINNAMYL)LUPININE, A NEW LUPIN ALKALOID IN *LUPINUS LUTEUS**

ISAMU MURAKOSHI†, KAZUO TORIIZUKA†, JOJU HAGINIWA†, SHIGERU OHMIYA‡ and HIROTAKA OTOMASU‡

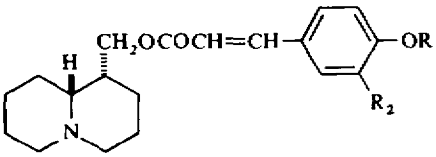
† Faculty of Pharmaceutical Sciences, University of Chiba, Yayoi-cho 1-33, Chiba, Japan 280; ‡ Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo, Japan 142

(*Received* 10 *March* 1978)

Key Word Index—*Lupinus luteus*; Leguminosae; alkaloid; glycoside; ( )-(*trans*-4′-rhamnosyloxy-3′-methoxycinnamyl)lupinine.

INTRODUCTION

We have recently isolated two ester alkaloids, (−)-(*trans*-4′-hydroxycinnamyl)lupinine (**1**) and its 4′-rhamnoside (**2**), from the young seedlings of *Lupinus luteus* [1, 2]. An enzymatic system for the formation of **1** in *Lupinus* seedlings has also been described [3]. We have now established the presence of (−)-(*trans*-4′-rhamnosyloxy-3′-methoxycinnamyl)lupinine (**4**) in the fresh seedlings of *Lupinus luteus*.



Scheme 1. Lupinine derivatives found in the fresh seedlings of *Lupinus luteus*.

(**1**) $R_1 = H, R_2 = H$
(**2**) $R_1 = Rha, R_2 = H$
(**3**) $R_1 = H, R_2 = OMe$
(**4**) $R_1 = Rha, R_2 = OMe$

RESULTS

From the EtOH extracts of the fresh 9-day-old seedlings of *Lupinus luteus*, grown in the dark at 28°, **4** was isolated as a colourless amorphous solid, $[\alpha]_D^{22}$ −78°, by repeated chromatography of the basic fraction. It gave a single grayish green with *p*-anisaldehyde–H_2SO_4 reagent for reducing sugars on TLC.

The MS spectrum of **4** showed an M^+ ion at *m/e* 491 (1%) and fragment ions corresponding to the loss of the rhamnosyl-moiety below the ion at *m/e* 345 closely resembled those of (−)-(*trans*-4′-hydroxy-3′-methoxycinnamyl)lupinine (**3**), which coexists with **4** in the same seedlings.

The sugar obtained by controlled hydrolysis of **4** with 3% HCl was identified as L-rhamnose by co-chromatography on PC and by Si-TLC. The aglycone was also confirmed as **3** by comparing MS, TLC and HPLC of the natural compound with synthetic material [6].

The PMR spectrum of **4** revealed the presence of a methyl-group of the rhamnosyl-moiety at δ 1.27 (3H, *d*, $J = 6$ Hz), anomeric proton at δ 5.50 (1H, *bs*) and two sharp peaks of three protons for a methoxyl group at δ 3.86 and 3.88.

From these results, the structure of **4** can be represented as (−)-(*trans*-4′-rhamnosyloxy-3′-methoxycinnamyl)lupinine (**4**), a new natural product. It was subsequently inferred from its large negative optical rotation ($[\alpha]_D^{22}$ −78.5; 3; −8.5°) that the configuration at the anomeric center of the rhamnosyl-unit in **4** involves an α-L-rhamnosidic linkage (see [2]). Furthermore, the PMR spectrum of **4** showed two pairs of AB-doublets due to *cis* and *trans* olefinic protons of the cinnamyl-moiety: from the data of chemical shifts, coupling constants and absorption intensities, it was proved that **4** was a mixture of *cis* and *trans* isomers at the ratio of *ca* 1:3, respectively. The conversion of *trans*-cinnamates, such as **1** [1], **2** [2], **3**, **4** and desmethoxyabresolines [4], into the *cis*-isomers during a treatment of the samples in daylight is unavoidable.

The concentration of **4** in the dry seeds of *Lupinus luteus* is extremely low, but its concentration increased rapidly along with those of **1**, **2** and **3** during the first 4–9 day's growth of seedlings at 28°.

---

* This work was presented partly at the 96th Annual Meeting of the Pharmaceutical Society of Japan, 6 April 1976 (Meeting Abstracts, II, p. 210).

EXPERIMENTAL

General methods. TLC was performed in the following solvent systems: 1. CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); 2. CH_2Cl_2–MeOH–28% NH_4OH (60:39:1); 3. Py–AcOEt–AcOH–H_2O (36:36:7:21); 4. *n*-BuOH–AcOH–H_2O (3:1:1); 5. Me_2CO–H_2O–$CHCl_3$–MeOH (15:1:2:2); 6. $CHCl_3$–MeOH (3:2). HPLC was carried out with solvent 7, 15% MeOH·Et_2O–H_2O–28% NH_4OH (500:10:1, v/v), as eluent using a LiChrosorb SI 100 (Merck, particle size 10 μm, 0.3 × 50 cm) column employing a monitoring flow system (310 nm) coupled to recorder at a flow rate of 1 ml/min. PMR spectra were measured at 100 MHz in $CDCl_3$ containing 5% CD_3OD using TMS as an internal standard. MS were taken with a direct inlet system at 70 eV and the optical rotation in MeOH.

Isolation of **4**. The fresh seedlings (1.2 kg) of *Lupinus luteus*, grown in the dark for 9 days at 28°, were macerated in EtOH and the basic fraction (2.05 g) was treated essentially as previously described for the isolation of **2** [2]: **4** is always present as a mixture in **2**-rich fractions.

The fractions (0.18 g) containing **2** and **4**, which appeared in the early eluates with solvent 2, were further purified by a Si gel CC (Merck, type 60, 230–400 mesh, 2 × 31 cm) using 20% MeOH·Et_2O–H_2O–28% NH_4OH (500:25:0.5), 20 ml fractions being collected. **4** (16 mg) was obtained from fractions 28–35 as a colourless amorphous solid, whilst **2** (51 mg) appeared in fractions 15–22, which gave one spot by analytical TLC on Si gel in solvents 1 and 2, and one peak by HPLC with solvent 7. The R_f values on Si gel TLC for lupinine, **1**, **2**, **3** and **4** obtained in solvent 1 were 0.18, 0.42, 0.12, 0.48 and 0.12, respectively, and in solvent 2 were 0.26, 0.64, 0.58, 0.65 and 0.58, respectively. However, **2** and **4** were indistinguishable on the TLC with solvents 1 and 2, but **4** (13 min) was separated clearly from **2** (10 min) by HPLC with solvent 7.

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., Ogawa, M., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1977) *Chem. Pharm. Bull.* **25**, 527.
4. Rother, A. and Schwarting, A. E. (1978) *Phytochemistry* **17**, 305.