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

ISAMU MURAKOSHI†, FUSAKO KAKEGAWA†, 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

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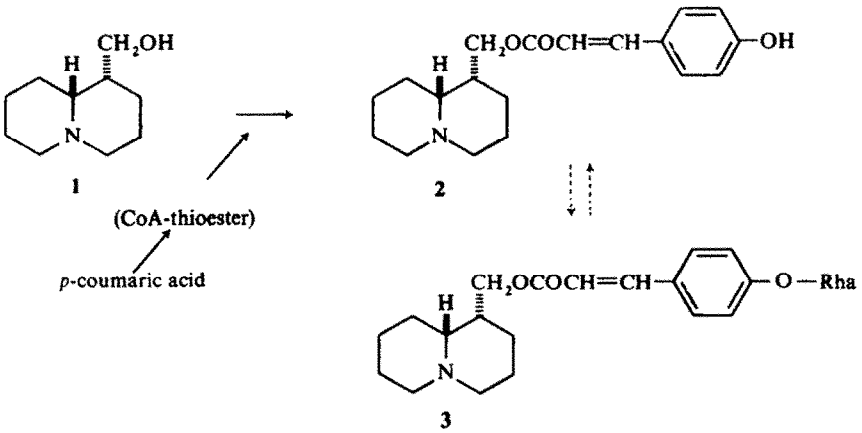
We have previously reported a new lupin alkaloid, (−)-(*trans*-4′-hydroxycinnamyl) lupinine (**2**), in *Lupinus* seedlings [1]. **2** accumulates only in relatively young seedlings: no detectable amount of **2** was found in the mature and immature seeds, and in the later stages of the plant's growth, However, its concentration increased rapidly during the first 4–8 day's growth and fell gradually to a very low level during further growth. The enzymatic conversion of (−)-lupinine (**1**) to **2** has also been demonstrated by a cell free system from *Lupinus* seedlings in the presence of adenosine triphosphate (ATP) and coenzyme A (CoA) as cofactors, as shown in Scheme 1 [2].



Scheme 1. Biosyntheses of 2 and 3 by enzymes in *Lupinus* seedlings. (------ :possible biosynthetic pathway).

This paper describes evidence for the presence of **2** as a rhamnoside, (−)-(*trans*-4′-rhamnosyloxycinnamyl) lupinine (**3**), in varying concentration at different times in the seedling's growth of *Lupinus luteus*.

RESULTS AND DISCUSSION

From the EtOH extracts of the fresh 7-day-old seedlings of *Lupinus luteus* a colourless amorphous solid (**3**) was isolated by repeated chromatography of the basic fraction. It gave a single reddish bright yellow immediately with Dragendorff's reagent on TLC.

In the MS spectrum of **3** an M^+ ion was exhibited at *m/e* 461 and fragment ions corresponding to the loss of rhamnosyl moiety below the ion at *m/e* 315 were very similar to those of **2**. Controlled hydrolysis of **3** with 3% HCl gave rhamnose and **2**. The NMR spectrum of **3** revealed the presence of a methyl group of rhamnosyl unit at δ 1.26 (3H, *d*, J = 6Hz) and anomeric proton at δ 5.52 (1H, *bs*).

From these results, the structure of **3** can be represented as (−)-(*trans*-4′-rhamnosyloxycinnamyl) lupinine (**3**), which has not been found in nature. It was subsequently inferred from its large negative optical rotation ($[\alpha]_D^{22}$ −105°; **2**: −13.4°) that the configuration at the anomeric centre of the rhamnosyl unit in **3** involves an α-L-rhamnosidic linkage. With regard to the configuration of the glycosidic linkage, it is also a general observation that D-sugars occur with β-glycosidic linkage and L-sugars with α-glycosidic linkages [3, 4].

Furthermore, the NMR spectrum of **3** showed two pairs of AB-doublets due to *cis* and *trans* olefinic protons of the cinnamyl moiety: from the data of chemical shifts,

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coupling constants and absorption intensities, it was proved that 3 was a mixture of *cis* and *trans* isomers at the ratio of *ca* 1:2, respectively. The transformation of *trans*-cinnamic acid derivatives into the *cis*-isomers during a treatment of the samples in daylight is unavoidable [1].

The concentration of 3 in dry *Lupinus* seeds is very low, but its concentration increased rapidly along with that of 2 during the first 5–9 days growth of seedlings at 28°.

EXPERIMENTAL

General methods. TLC was performed with (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), 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. NMR spectra were measured at 100 MHz in $CDCl_3$ containing 5% CD_3OD using TMS as an int. stand. MS were taken with a direct inlet system at 70 eV and the optical rotations in MeOH.

Isolation of (−)-(trans-*4'-rhamnosyloxycinnamyl*) *lupinine* (3). The basic fraction (5 g) obtained from the 75% EtOH extracts of the fresh seedlings of *Lupinus luteus* (1.2 kg), grown in the dark or under daylight for 7–9 days at 25–28°, was chromatographed on a Si gel column (Merck, type 60, 500 g) using first solvent 1 and then, after recovery of the 3-containing eluates between lupinine (1) and spartein rich fraction. with solvent 2. A 3-rich fraction (0.37 g), appeared in the early eluates with solvent 2, which was still contaminated with other bases, was further purified by Si gel chromatography (Merck, type 60, 230–400 mesh, 2 × 43 cm) as before using 20% MeOH.Et_2O–H_2O–28% NH_4OH (500:25:0.5), 20 ml fractions being collected. 3 (0.103 g) was obtained from fractions 13–18, as a colourless amorphous solid, which produced one spot by analytical TLC on Si gel in solvent 1 and 2, and one peak by HPLC with solvent 7. 3 exhibited a reddish bright yellow immediately on TLC after spraying with Dragendorff's reagent. 3: $[\alpha]_D^{22} - 105°$ (*c* = 1.16, MeOH), MS. *m/e* 461 (M^+, 3%), significant peaks at *m/e* 315 (4), 168 (5), 164 (6), 152 (100), 147 (13), 119 (6), NMR (5% CD_3OD–$CDCl_3$): δ 1.26 (3H, *d*, *J* = 6 Hz, Me of rhamnosyl-moiety), 5.52 (1H, *bs*, anomeric H), 6.9–7.7 (4H, *m*, aromatic H), 5.85 and 6.87 (*ca* 1/3H each, two doublets, *J* = 13 Hz, —CO—CH=CH— (*cis*)), 6.32 and 7.62 (*ca* 2/3H each, two doublets, *J* = 16 Hz, —CO—CH=CH—(*trans*)), IR ν_{max}^{KBr} cm^{-1}: 3415 (OH), 2935 (CH), 1710 (ester), 1625 (—CH=CH—), 1240 and 1170 (ester). The R_f values on Si gel TLC for 3, 2 and 1 obtained in solvent 1 were 0.2, 0.5 and 0.3, repsectively, and in solvent 2 0.6, 0.7 and 0.3, respectively.

Hydrolysis of 3 *into* 2 *and rhamnose.* The rhamnoside (3, 4 mg) was easily hydrolysed into 2 and rhamnose with 3% HCl at 45–50° for 15 hr: the aq. soln after evapn of the solvent to dryness *in vacuo* at 40° was made alkaline with dil. NH_4OH and then extracted with CH_2Cl_2. The product obtained from the CH_2Cl_2-extracts was confirmed to be completely identical with those of the natural 2 [1] in all measurable respects (TLC, HPLC and MS). The aq. soln after removal of the base 2 was also concd *in vacuo* and passed through a column of Dowex-50 (H^+ form). The effluent, giving a characteristic yellow green colour with *p*-anisidine phthalate, was evapd to dryness. The resulting residue was identical with rhamnose by CO-TLC with a standard sample.

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