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(−)-(*trans*-4′-β-D-Glucopyranosyloxycinnamoyl)lupinine, a New Lupin Alkaloid in *Lupinus* Seedlings

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A new glucosidic lupin-alkaloid, (−)-(*trans*-4′-β-D-glucopyranosyloxycinnamoyl)-lupinine (**5**), was newly isolated from the fresh seedlings of *Lupinus luteus*. Its structure has been confirmed by direct comparison with a synthetic material. The concentration of **5** in the immature and mature seeds of *L. luteus* was negligibly low, but its concentration increased rapidly during the first 3—10 day's growth of seedlings and became a minor component again in the later stages of the plant's growth.

Keywords——Leguminosae; *Lupinus luteus*; alkaloid; hydroxycinnamic acid; (−)-(*trans*-4′-hydroxycinnamoyl)lupinine; (−)-(*trans*-4′-α-L-rhamnosyloxycinnamoyl)-lupinine; (−)-(*trans*-4′-hydroxy-3′-methoxycinnamoyl)lupinine; (−)-(*trans*-4′-α-L-rhamnosyloxy-3′-methoxycinnamoyl)lupinine; (−)-(*trans*-4′-β-D-glucopyranosyloxycinnamoyl)lupinine; glycoside

During the course of a study of the lupin alkaloids in Leguminosae,[2–14] the fresh seedlings of *Lupinus luteus* were found to contain four derivatives (**1**—**4**) of 4-hydroxycinnamic acid bonded to (−)-lupinine along with other lupin alkaloids. The concentrations of these alkaloids (**1**—**4**) in the mature seeds are negligibly low, but their concentrations increased rapidly during the first 3—10 day's growth of seedlings at 28° both in the dark and under the daylight. In the later stages of the plant's growth, the concentration of these alkaloids diminished and they again became minor components.

It has also been confirmed that (−)-(*trans*-4′-hydroxycinnamoyl)lupinine (**1**) is formed from (−)-lupinine (**6**) and 4-hydroxycinnamic acid (**7**) by two enzymatic systems, one a ligase catalyzing the formation of the CoA thioester of **7** and other a transferase catalyzing the formation of **1** from the CoA thioester.[13]

In the present work we have further demonstrated the existence of (−)-(*trans*-4′-β-D-glucopyranosyloxycinnamoyl)lupinine (**5**) in the fresh seedlings of *Lupinus luteus*. The present paper deals with its structure determination as **5**.

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Results and Discussion

From the *n*-hexane-insoluble portion of the total crude alkaloid, obtained from the 75% EtOH extract of the freshly harvested 10-day-old seedlings, a new glucosidic lupin alkaloid (**5**) was further isolated in a yield of 9.2×10^{-4} % of the fresh weight as a colourless amorphous solid, $[\alpha]_D^{22}$ −58.9°: $[\alpha]_{436}^{22}$ −134°; ultraviolet (UV) spectrum λ_{max}^{EtOH} nm (log ε) 225 (4.13), 298 (4.35), 306 (4.35).

On thin-layer chromatography (TLC) it gave characteristic colour reactions with *p*-anisidine–HCl or *p*-anisaldehyde–H_2SO_4 reagents for the reducing sugars and a reddish bright yellow reaction with Dragendorff's reagent, suggesting a glucoside of **1**.

The sugar obtained on controlled hydrolysis of **5** with 3.5% HCl was identified as D-glucose by co-chromatography on paper chromatography (PC) and TLC, and by a specific β-D-glucose oxidase system.[16-18] The aglycon was also confirmed as **1** by direct comparison with an authentic sample[4] in all measurable respects (MS, TLC and HPLC) and by chemical hydrolysis of **1** into (−)-lupinine and 4-hydroxycinnamic acid as described in a previous paper.[4]

In the MS spectrum of **5**, a parent ion was not observed but the predominant fragment ions corresponding to loss of glucosyl moiety below the ions at *m/e* 315 (11%) were very similar to those of **1**.

The 100 MHz–nuclear magnetic resonance (NMR) spectrum of **5** showed signals at δ 6.34 and 7.63 (6/7 H each, *trans*), δ 5.86 and 6.88 (1/7 H each, *cis*) for a –CO–CH=CH– grouping; δ 4.98 (1H) for an anomeric proton and the expected signals for the *p*-substituted benzene moiety between δ 6.9—7.6.

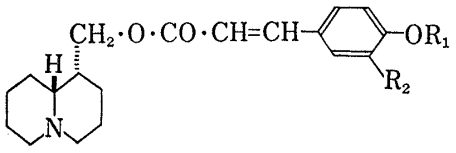
The β-configuration of glucosidic linkage was established by means of enzymatic hydrolysis with β-D-glucosidase (from almond), which gave equimolecular amount of **1** and D-glucose.

From the above results, it can therefore be presumed that the structure of a new glucosidic alkaloid is (−)-(*trans*-4′-β-D-glucopyranosyloxycinnamoyl)lupinine (**5**). In addition, from the NMR spectrum of **5**, it was revealed that **5** was a mixture of *trans* and *cis*-isomers at the ratio of *ca.* 6 : 1, respectively: during a treatment of the *trans*-cinnamic acid derivatives found in nature under the daylight, the transformation into the *cis*-isomers is unavoidable as described in previous papers.[4-6,13,19]

Finally, **5** was identical in all measurable respects with the product obtained by a modified Koenigs–Knorr reaction of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosylbromide with **1** followed by a hydrolysis with 2.5% NH_4OH.

No detectable amount of **5** was found in the immature and mature *Lupinus* seeds. However, its concentration increased rapidly during the first 3—10 day's growth of seedlings as those for **1**, **2**, **3** and **4**. In the later stages of the plant's growth, the concentration of **5** diminished and it again became a minor component.

A study on the biosynthetic correlation for the formation of these alkaloids (**1**—**5**) is currently under investigation in our laboratory.



1: R_1=H, R_2=H
2: R_1=α-L-rhamnosyl, R_2=H
3: R_1=H, R_2=OCH_3[15]
4: R_1=α-L-rhamnosyl, R_2=OCH_3
5: R_1=β-D-glucosyl, R_2=H

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Experimental

General Methods——NMR spectra were determined at 100 MHz in $CDCl_3$ containing 5% CD_3OD. Chemical shifts are quoted in δ units relative to TMS.

The following solvent systems were used throughout for both silica gel TLC and PC: 1, CH_2Cl_2–MeOH–28%NH_4OH (60: 39: 1, v/v); 2, CH_2Cl_2–MeOH–28%NH_4OH (90: 9: 1, v/v): 3, 7%MeOH·CH_2Cl_2–28%NH_4-OH (500: 5, v/v): 4, CH_2Cl_2–MeOH (6: 4, v/v); 5, AcOEt–pyridine–H_2O (2: 1: 2, v/v). HPLC was carried out with solvent 6, 15%MeOH·ether–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.

Extraction and Isolation of 5——The fresh seedlings (*ca.* 1.4 kg) of *Lupinus luteus*, grown in the dark or under the daylight for 9—10 days at 28°, were homogenized in EtOH and the total crude alkaloid (2.1 g) was divided into *n*-hexane-soluble and insoluble portions. The *n*-hexane-insoluble portion (0.8 g) was chromatographed on a silica gel column (Merck, Type 60, 150 g) using CH_2Cl_2–MeOH–28%NH_4OH–H_2O (60: 39: 1: 5, v/v), 20 ml fractions being collected. **5** mainly appeared in fractions 21—23. **5**-Rich fractions were further purified by HPLC: **5** was clearly isolated from other contaminants in a position of the retention time 27 min, whilst **1**, **2**, **3**, **4** and (−)-lupinine appeared in the positions of 5, 10, 7, 12, and 18 min, respectively. **5** exhibited on TLC a reddish bright yellow immediately after splaying with Dragendorff's reagent, a pale yellow and a grayish blue colour with *p*-anisidine–HCl and *p*-anisaldehyde–H_2SO_4 reagents, respectively, for the reducing sugars.

The *Rf* values on silica gel TLC of **5** obtained in solvents 1, 2 and 3 were 0.48, 0.02 and 0.00, whilst **1** exhibited the following *Rf* data: 0.64, 0.42 and 0.58 in solvents 1, 2 and 3, respectively. Under the same conditions, (−)-lupinine moved at *Rf*'s of 0.26, 0.18 and 0.33, respectively.

5: Colourless amorphous solid, $[\alpha]_D^{22}$ −58.9°, $[\alpha]_{436}^{22}$ −134° (c=0.31, EtOH); UV λ_{max}^{EtOH} nm (log ε) 225 (4.13), 298 (4.35), 306 (4.35); MS significant peaks at *m/e* 315 (11%, M$^+$−glucosyl moiety), 168 (10), 152 (100), 111 (21); NMR: δ 4.98 (1H, m, anomeric H), δ 6.34 and 7.63 (6/7H each, two doublets, J=16 Hz, –CO–CH=CH– (*trans*)), δ 5.86 and 6.88 (1/7H each, two doublets, J=13 Hz, –CO–CH=CH– (*cis*)), δ 6.9—7.6 (4H, multiplet, *p*-substituted aromatic H). It was proved from the NMR data that **5** was a mixture of *cis*- and *trans*-isomers at the ratio of *ca.* 1: 6, respectively.

Hydrolysis of 5 into 1 and D-Glucose——1) With 3.5% HCl at 60° for 3.5 hr, the glucosidic alkaloid (**5**, 2 mg) was hydrolyzed into **1** and D-glucose: the aq. solution after evaporation of the solvent to dryness *in vacuo* at 40° was made alkaline with 2.5%NH_4OH and then extracted with CH_2Cl_2. The basic product obtained from the CH_2Cl_2 extracts was purified by TLC or HPLC and confirmed to be completely identical with those of the natural **1**[4] in all measureable respects (TLC, HPLC and MS).

The mother liquor after removal of the base **1** was adjusted to pH 5.0—5.5 by dil. HCl and passed through a column of IR-120 (H^+ form). The passed solution, giving a characteristic grayish blue colour with *p*-anisaldehyde-H_2SO_4 reagent, was evaporated to dryness. The resulting residue was confirmed to be D-glucose by silica gel co-TLC, impregnated with 0.02 M Na-acetate, in solvent 4 (*Rf* 0.40), cellulose co-TLC in solvent 5 (*Rf* 0.19) and by a specific β-D-glucose oxidase system which was coupled to N,N-diethylaniline and 4-aminoantipyrine as chromogenic reagents (IatroSet Glu-E, Iatron Laboratories, Inc.: colour maximum at 630 nm).[17]

2) By the β-D-glucosidase from sweet almonds (Miles), the glucoside (**5**) also could be degraded stoichiometrically to **1** and D-glucose (75% yield in 30 min at 28°): standard reaction mixtures to demonstrate the enzymatic hydrolysis of **5** into **1** and D-glucose contained **5** (0.25 mg) and β-D-glucosidase (1.0 mg) in a final volume of 0.8 ml. Reaction mixtures were normally maintained at pH 5.5 by 0.1 M K-acetate buffer and incubated at 28° for 30 min. The alkaline reaction mixtures (pH 10) terminated with 10% NH_4OH were pretreated as described in the above, and then **1** and glucose fractions were subjected to HPLC and a specific β-D-glucose oxidase system, respectively, which allowed, rapid, quantitative determinations to be made.

Synthesis of 5 from 1——**5** was synthesized in 42% yield from 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-bromide (**8**) and **1**, prepared from (−)-lupinine as described in a previous paper,[4] by a modified Koenigs–Knorr reaction: a solution of equimolecular amounts of **1** (10.2 mg), **8** (13 mg) and 0.1 N KOH (0.32 ml) in acetone (1 ml) was allowed to stand in the dark at 15—20° for 45 hr. For deacetylation of the product, involving the acetyl-derivative of **5** as intermediate, after removal of the solvent *in vacuo*, the resulting residue was permitted to stand further in a few drops of 2.5% NH_4OH for 1.5 hr at 60° and then extracted with CH_2Cl_2 at about pH 11.0. The crude **5** obtained from the CH_2Cl_2 extracts was purified by HPLC as described in the isolation section.

The natural product was shown to be completely identical with **5**, obtained in the synthetic sequence towards **1**, by its MS, HPLC and co-TLC.

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