

Studies on Orchidaceae Alkaloids

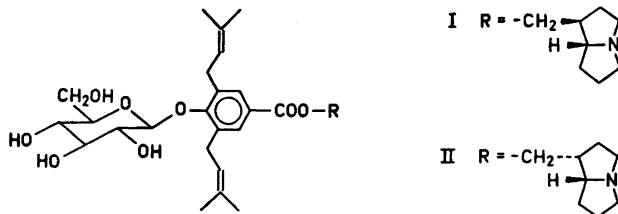
XXIII.* Alkaloids from *Liparis loeselii* (L.) L. C. Rich.
and *Hammarbya paludosa* (L.) O. K.

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One alkaloid, possessing a constitution identical with that postulated for auriculin,² has been isolated from *Liparis loeselii*. From *Hammarbya paludosa* two further alkaloids of the same class, *i.e.* amino ester glucosides of alkylated *p*-hydroxybenzoic acids, have been isolated.

In *Liparis loeselii* (L.) L. C. Rich. we have found one alkaloid (I) which was obtained as an amorphous solid. Upon alkaline hydrolysis, nervogenic acid² and laburnine were obtained. Acid hydrolysis gave D-glucose which was identified by optical rotation, paper chromatography and GLC-MS of the derived hexa-*O*-acetyl-D-glucitol.³ Exhaustive methylation of the alkaloid followed by acid hydrolysis, reduction and acetylation gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, identified by GLC-MS.⁴ Laburnine shows $[\alpha]_D^{25} + 15.5^\circ$,⁵ and the low optical rotation of the glucoside, $[\alpha]_D^{23} - 14^\circ$, together with the fact that it was degraded by alkali (4 M NaOH, 90°, 15 h),⁶ indicate that I is a β -D-glucoside. We therefore suggest the structure I for the alkaloid.



From *Hammarbya paludosa* (L.) O. K. two alkaloids (II and III) have been isolated. For II we suggest the name paludosine. Alkaline hydrolysis of

* For paper XXII, see Ref. 1.

paludosine afforded nervogenic acid and lindelofidine. Acid hydrolysis gave glucose identified as hexa-*O*-acetyl-D-glucitol. Methylation analysis gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. The low optical rotation of paludosine $[\alpha]_D^{25} + 9^\circ$, compared with the rotation of lindelofidine $[\alpha]_D + 79^\circ$ and the ease of alkaline hydrolysis (4 M NaOH, 90°, 12 h) indicate that II is also a β -D-glucoside. Hence we suggest the structure II for paludosine.

The second alkaloid (III) isolated from *Hammarbya paludosa* shows a resemblance to II, but is still the subject of investigation.

EXPERIMENTAL

Mass spectra were recorded on an LKB 9000 spectrometer and with a Perkin-Elmer 270 mass spectrometer. IR spectra were recorded on a Perkin-Elmer 257 instrument, the UV spectra on a Beckman DK 2 instrument, and the NMR spectra on a Varian A-60 A spectrometer.

Isolation and characterization of alkaloid (I) from Liparis loeselii. Fresh plants* of *Liparis loeselii* (80 g) were extracted with methanol (2 \times 300 ml). The filtered solution was concentrated *in vacuo* to 100 ml, acidified (pH 2), and washed with chloroform (5 \times 80 ml). The aqueous solution was made alkaline (pH 10) with sodium hydroxide and was then extracted with a mixture of chloroform-ethanol (3 : 1, 6 \times 50 ml). The combined chloroform-ethanol solutions were dried (Na₂SO₄) and the solvents evaporated. The residue was dissolved in a few drops of methanol, water was added, and the alkaloid precipitated as its *reinecke salt*. (Found: C 48.2; H 5.96; Cr 6.01; N 10.9. Calc. for C₃₅H₁₅CrN₇O₈S₄: C 47.8; H 5.96; Cr 5.91; N 11.2.) The *reinecke salt* was dissolved in water-acetone (1 : 1) and the solution filtered through a column of Dowex 1-X4 (Cl⁻). Evaporation of the solvents yielded the amorphous *hydrochloride of I* (290 mg). An aqueous solution of the hydrochloride was made alkaline and extracted with chloroform (5 \times 30 ml). The chloroform solution upon evaporation yielded the base as an amorphous solid $[\alpha]_D^{25} - 14^\circ$ (c 0.56, ethanol). UV spectrum: λ_{\max} (ethanol) 247 nm (log ϵ 4.15). IR spectrum: σ_{\max} (KBr) 1718 cm⁻¹. Mass spectrum, *m/e* (rel. intensity): M⁺559 (0.1), 397 (10), 230 (58), 175 (84), 159 (97), 124 (100), 83 (87). The alkaloid gives a positive Molisch reaction.

Alkaline hydrolysis of I. The alkaloid (53 mg) was dissolved in aqueous sodium hydroxide (4 M) and the solution kept at 90° overnight. The reaction mixture was then acidified with hydrochloric acid and extracted with chloroform (4 \times 10 ml). The combined chloroform solutions were dried, concentrated, and filtered through silica gel. Evaporation of the solvent afforded 4-hydroxy-3,5-bis(3-methyl-2-butenyl) benzoic acid, nervogenic acid (9.2 mg). The UV, IR, NMR, and mass spectra of this product agreed well with those reported for nervogenic acid.³

The aqueous solution was made alkaline with sodium hydroxide and extracted with chloroform (4 \times 20 ml) in a mechanical shaker, each time for 2 h. The combined chloroform solutions, on evaporation, yielded laburnine (6.7 mg) which was acetylated with ketene. The laburnine acetate was purified by preparative GLC and was indistinguishable from an authentic sample⁸ (GLC, optical rotation, MS, and m.p. 138° of the *picrate*).

Acid hydrolysis of I. The alkaloid (10 mg) was dissolved in sulphuric acid (0.25 M, 5 ml) and the solution kept at 90° for 2 h. The solution was neutralized with barium carbonate and the aromatic amino ester removed by precipitation with *reinecke salt*. The excess reagent was removed by passing the solution through a column of Dowex 1-X4 (Cl⁻). The solution showed a positive optical rotation. Paper chromatographic analysis of this solution (Whatman 1, ethyl acetate-pyridine-water, 8 : 2 : 1) showed a spot with the same *R_F* value as glucose. The presence of glucose was further verified by reduction to D-glucitol and acetylation to the hexaacetate which was identified by combined GLC-MS.³

Methylation and acid hydrolysis of I. The alkaloid (5 mg) was fully methylated with methyl iodide and methyl sulphanyl sodium in methyl sulphoxide according to Hakomori.⁹

* Collected near Stockholm, Sweden.

The methylated alkaloid was hydrolyzed in sulphuric acid (0.25 M, 2 ml) at 90° for 2 h. The reaction mixture was neutralized with barium carbonate and the methylated sugar was reduced with sodium borohydride and acetylated, giving 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, as demonstrated by GLC-MS.⁴

Isolation and characterization of alkaloids from Hammarbya paludosa. Fresh plants* (50 g) were extracted with methanol (2 × 200 ml). The extract was concentrated to 100 ml, acidified and washed with chloroform (5 × 80 ml). The aqueous solution was made alkaline (pH 10) with sodium hydroxide and extracted with chloroform-ethanol (3 : 1, 4 × 50 ml). The combined chloroform ethanol solutions were dried (Na₂SO₄) and the solvents evaporated. The crude alkaloid fraction was further purified *via* the reinecke salt. The alkaloids were separated by preparative TLC (cellulose, Merck microcrystalline, butanol, saturated with water), giving II (55 mg, *R_F* 0.3), [α]_D²³ + 9° (c 0.56, ethanol), and III (8 mg, *R_F* 0.05), [α]_D²³ + 9° (c 0.31, ethanol). The alkaloids were obtained as amorphous solids. Their IR and UV spectra are almost superimposable on those of I. The MS of II is identical with that of I.

Alkaline hydrolysis of II. The alkaloid II (50 mg) was hydrolyzed in sodium hydroxide (3 ml, 4 M) at 90° for 12 h. The separation of the reactants into acidic and basic components was made as for I. The acidic portion consisted of nervogenic acid² (9 mg) which was identified as above. The basic component (4.7 mg) was identified as lindelofidine [α]_D²¹ + 70°. Acetylation with ketene produced an acetate indistinguishable from authentic lindelofidine acetate⁸ (GLC, m.p. of the *picrate* 106–107°; authentic lindelofidine acetate *picrate* m.p. 106–107°).

Acid hydrolysis of II. The alkaloid II (5 mg) was hydrolyzed in sulphuric acid (0.25 M, 3 ml) at 90° for 2 h. The reaction mixture was treated as for I. Reduction with sodium borohydride and subsequent acetylation gave hexa-*O*-acetyl-D-glucitol, which was identified by combined GLC-MS. Methylation and acid hydrolysis, followed by reduction with sodium borohydride and acetylation, produced 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol which was identified by GLC-MS.

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* Collected near Stockholm, Sweden.