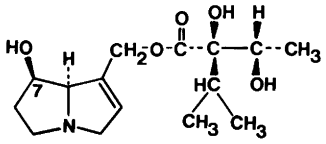
Alkaloids of *Anchusa officinalis* L. Identification of the Pyrrolizidine Alkaloid Lycopsamine

ÅSTA I. BROCH-DUE and
ARNE J. AASEN *

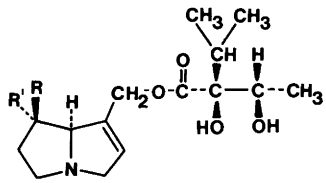
Department of Pharmacy, University of Oslo, Blindern, Oslo 3, Norway

Pyrrolizidine alkaloids possessing a 1,2-double bond are considered to exhibit carcinogenic and hepatotoxic activity[1,2] and a number of cases of human poisoning have been attributed to these alkaloids. A large number of people in India[3] and Afghanistan[4] recently contracted severe liver disease and many died after consuming cereals contaminated with seeds of *Crotalaria* sp. and *Heliotropium* sp., respectively. *Anchusa officinalis* L. has been used in folk medicine as a stimulant and as a drug against a number of illnesses, such as heart and lung disease.[5-7] The present communication describes the isolation and identification of the major pyrrolizidine alkaloid in this plant.

GC/MS of the fractions obtained by chromatography of a Zn-reduced methanol extract revealed, as stated by Pedersen,[8] that the mass spectrum of the major alkaloid was nearly identical to that of indicine (*1*). Since only five [1] [indicine (*1*), echinatine (*2*), lycopsamine (*3*), rinderine, and intermedine] of the sixteen possible stereoisomers are known, identification solely based on spectral data and optical activity is hazardous. When the base is an oil and authentic alkaloids are unavailable, separate examination of the basic and acidic moieties obtained on hydrolysis or hydrogenolysis is essential.



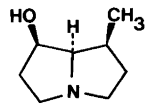
1; Indicine



2; R=H, R'=OH; Echinatine

3; R=OH, R'=H; Lycopsamine

Catalytic hydrogenation of 1,2-unsaturated pyrrolizidine alkaloids esterified at C-9 gives, in addition to hydrogenolysis of the allylic ester grouping, stereospecific saturation of the double bond to yield a methyl group *trans* to the C-8 proton.[1] Hydrogenation of a small amount of the major alkaloid of *Anchusa officinalis* L. gave a base which was indistinguishable from retronecanol (*4*), and a



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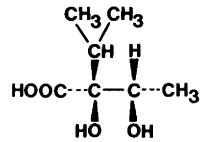

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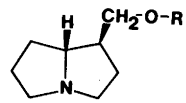
carboxylic acid which was identical to (2*S*,3*S*)-viridifloric acid (*5*)[9] obtained in the same way from echinatine (*2*). The major alkaloid of *A. officinalis* L. is thus lycopsamine (*3*). This and its stereoisomer, intermedine, have previously been isolated from other members of *Boraginaceae: Symphytum* × *uplandicum* Nyman,[10] *Amsinckia intermedia* Fisch. & Mey[11] *Amsinckia hispida* (Ruiz. & Pav.) Johnst.[11] and *Amsinckia lycopsoides* Lehm.[11]

Three minor alkaloids were also present, but in amounts too small to allow isolation and structure determination. As noted by Pedersen,[8] one of them exhibited a mass spectrum which indicated it being lycopsamine, or one of its diastereoisomers, carrying an acetoxy group instead of a hydroxy group at C-7; the base peak was found at *m/e* 180 compared to 138 for lycopsamine. The other two basic constituents could be laburnine (*6*) which was only detected in the seeds, and laburnine acetate (*7*), or diastereoisomers of *6* and *7*. This was deduced from their mass spectra (GC/MS) which agreed well with published data.[12,13]



6; R=H; Laburnine

7; R=Ac

Experimental. Melting points (uncorrected), rotations and GC/MS were recorded on Reichert, Perkin-Elmer 241, and Jeol JMS-D300 instruments, respectively. ^{1}H NMR spectra were obtained with Jeol FX 90Q, Varian XL 100 and Bruker WP 200 instruments. Analytical TLC and column chromatography were performed on silica gel: F_{254} plates and Merck Kieselgel 60 (0.040 – 0.063 mm), respectively. Analytical GLC was performed on a glass column (2 m × 1.9 mm) packed with 2.5 % OV-1 on

* Present address: Department of Chemistry, University of Tromsø, N-9001 Tromsø, Norway.

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Varaport 30 using a Varian 1400 instrument equipped with a flame ionization detector.

Extraction of alkaloids. The roots (352 g), seeds (4 g) and green parts (1269 g) of dried *A. officinalis* L. which had been collected in the flowering state at Hurum, near Oslo, were milled and extracted with methanol in a Soxhlet extractor for 9 h, 6 h and 33 h, respectively. The methanol was removed under reduced pressure and the residue suspended in 0.25 M H_2SO_4 which was then extracted with chloroform. The acidic phase was stirred with zinc dust for 5 h at 0 °C to reduce *N*-oxides. After filtration, the pH was adjusted to 9 – 10 with concentrated NH_3 and the alkaloids were extracted with chloroform (6 times). The chloroform solution was dried with sodium sulfate and evaporated *in vacuo* yielding 272 mg, 1.2 mg and 371 mg crude alkaloids from the roots, seeds and green parts, respectively. The extracts, except that from the seeds which was examined by GC/MS only, were eluted from silica gel columns with chloroform – methanol (1:1 and 1:3) and methanol. The ratio between lycopsamine, 7-acetyllycopsamine (or a stereoisomer) and laburnine acetate (or a stereoisomer) in the roots and green parts was approximately 80:15:5 as judged from TLC and GLC. The major alkaloid (>90 %) in the seeds was laburnine, or a stereoisomer, and only traces of lycopsamine could be detected by TLC and GC/MS.

Lycopsamine. The alkaloid could not be induced to crystallize; Culvenor *et al.*[11] have reported that no crystalline derivative of lycopsamine could be obtained. On co-chromatography (TLC and GLC) with indicine (*1*) and echinatine (*2*), separation was only observed from the latter, which has an αOH-group at C-7. Its mass spectrum was virtually identical to those of indicine (*1*) and echinatine (*2*) (*cf.* Ref. 14 for the spectrum of the latter compound). $[\alpha]^{20}$ +2.9° (589 nm), +3.3 (578 nm), +4.9 (546 nm) (*c* 1.1 in EtOH); lit.[11] +3.3° (589 nm) (EtOH). ^{1}H NMR ($CDCl_3$, 200 MHz): In addition to complex, overlapping multiplets at δ *ca.* 2.1, 2.8, 3.5 and 4.2, resolved signals appeared at δ 0.90 (3H, d, *J* 7 Hz), 0.96 (3H, d, *J* 7 Hz), 1.31 (3H, d, *J* 6.5 Hz), 2.19 (1H, heptet, *J* 7 Hz), 4.03 (1H, q, *J* 6.5 Hz), 4.80 and 4.88 (2H, AB-system, *J* 14 Hz), 5.96 (1H, broad singlet). The spin-spin couplings between the signals at δ 0.90, 0.96 and 2.19 and at δ 1.31 and 4.03 were confirmed by decoupling experiments.

Hydrogenation of lycopsamine. The major alkaloid (53 mg) dissolved in methanol (1 ml) and 0.5 N HCl (0.5 ml) was hydrogenated in the presence of PtO_2 (22 mg) at room temperature and atmospheric pressure. The solution was filtered through celite and evaporated *in vacuo*. The residue was dissolved in H_2O (1.5 ml) and 0.5 N HCl (0.5 ml) and extracted with ether continuously for 22 h. The extract was dried with sodium sulfate and concentrated to dryness yielding (2*S*,3*S*)-viridifloric acid (*5*) (18 mg) which was recrystallized from pentane/ether. M.p. 123 – 131 °C; 124 – 132 °C when mixed with an authentic specimen similarly obtained from echinatine (*2*); lit.[15] 123 – 127 °C. $[\alpha]^{20}$ −2.3° (589 nm), −2.4° (578 nm), −2.6° (546 nm), −4.0° (436 nm), −6.0° (365 nm) (*c* 0.7 in H_2O); corresponding values for authentic acid: −3.3°, −3.3°, −3.3°, −4.6°, −6.5° (*c* 0.2 in H_2O); lit.[9] −2.0° (589 nm) (H_2O). ^{1}H NMR (D_2O, 90 MHz): δ 0.92 (3H, d, *J* 6.8 Hz), 0.95 (3H, d, *J* 6.6 Hz), 1.23 (3H, d, *J* 6.8 Hz), 2.1 (1H, m), 4.07 (1H, q, *J* 6.8 Hz). *m/e* (%): 118 (85), 103 (100), 85 (34), 57 (38), 56 (35), 45 (52), 43 (97). ν_{max} (KBr): 3450 (s), 1706 (s), 1259 (s), 1169 (s), 1005 (s).

The aqueous phase was made alkaline with concentrated NH_3 and extracted with chloroform (8 × 10 ml). The extract was dried with sodium sulfate and concentrated *in vacuo* to an oil (14 mg) which crystallized after several weeks at −18 °C. On co-chromatography (GLC) with authentic retronecanol (*4*) and 7α-hydroxy-1β-methylpyrrolizidine similarly obtained from echinatine (*2*), separation was only observed from the latter compound. $[\alpha]^{20}$ −81.9° (589 nm), −85.4° (578 nm), −96.3° (546 nm), −156.8° (436 nm) (*c* 0.9 in EtOH); lit.[16] for retronecanol: −103° (589 nm) (EtOH). The mass spectrum (GC/MS) was identical to that of retronecanol (*cf.* Ref. 13 for spectrum of retronecanol).

7-Acetyllycopsamine (or a stereoisomer). GC/MS, *m/e* (%): 181 (36), 180 (100), 136 (77), 120 (56), 93 (71), 43 (39). The gross structure was supported by signals in the ^{1}H NMR spectrum of an impure sample: δ 0.89 (3H, d, *J* 7 Hz), 0.94 (3H, d, *J* 7 Hz), 1.25 (3H, d, *J* 6.5 Hz), 2.03 (3H, s), *ca.* 4.7 and 4.8 (*ca.* 2H, AB-system ?), 5.34 (1H, m), 5.85 (1H, broad s).

Laburnine acetate (or a stereoisomer). GC/MS, *m/e* (%): 183 (M^+, 23), 124 (100), 83 (53), 82 (29), 55 (27), 43 (30); lit.[12] 183 (27), 124 (100), 83 (54), 55 (22).

Laburnine (or a stereoisomer). The mass spectrum obtained by GC/MS was nearly identical to that published by Neuner-Jehle *et al.*[13]

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