

42. *The Alkaloids of the Seeds of Delphinium elatum, L.*

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The alkaloids from the seeds of *Delphinium elatum* consist chiefly of methyl-lycaconitine, with small quantities of two crystalline bases to which it is proposed to assign the names *delpheline* and *delatine*. Methyl-lycaconitine yields as hydrolytic products, methylsuccinic and anthranilic acids and lycoctonine, a base obtained by hydrolysis of the alkaloid lycaconitine from the roots of *Aconitum lycoctonum*, the presence of the same alkaloid in these two genera of the plant family *Ranunculaceæ* thus being established for the first time.

DELPHINIUM alkaloids are of special interest in two ways. *Delphinium*, like *Aconitum*, is a genus of the natural order *Ranunculaceæ*. Both genera yield alkaloids of high and low toxicity and there is some evidence of similarity in pharmacological activity between certain alkaloids of both groups, but although identity, or at least similarity in chemical structure, between some members of the two groups has long been suspected, there has so far been no evidence to confirm this suspicion.

The other point has topical interest arising out of the present emergency. The oil of *Delphinium Staphisagria* (Stavesacre oil) was at one time used as a pediculicide and its insecticidal action was no doubt due to the alkaloids partly extracted in the oil. *D. Staphisagria* seeds are produced in Southern Europe and so stavesacre oil is no longer available. Some years ago a firm of seedsmen in London placed at the disposal of these laboratories a considerable supply of the seeds of a horticultural species, *D. elatum*, and it seemed worth while to examine the alkaloids from this species and if possible to ascertain whether the oil had the insecticidal character of stavesacre oil.

D. elatum has been little studied. Keller (*Arch. Pharm.*, 1925, 263, 275) records the isolation of a crystalline base, $C_{33}H_{51}O_8N$, m. p. 218°. In the present investigation two crystalline alkaloids, *delpheline*, $C_{22}H_{33}O_5N$, and *delatine*, $C_{19}H_{25}O_3N$, have been isolated and methyl-lycaconitine, $C_{37}H_{48}O_{10}N_2$, which, although it forms a crystalline *hydriodide*, has not been obtained crystalline.

Delpheline melts at 227° (sinters at 222°) and were it not that it contains more nitrogen than Keller (*loc. cit.*) records for his crystalline base it would appear that the two alkaloids are identical. Delatine does not appear to have been previously isolated from a delphinium. Methyl-lycaconitine is so named, because, just as lycaconitine, an alkaloid from the roots of *Aconitum lycoctonum*, gives as final hydrolytic products, succinic acid, anthranilic acid and lycoctonine (Schulze and Bierling, *Arch. Pharm.*, 1913, 251, 30, 37, 41, 46), so under similar conditions methyl-lycaconitine yields methylsuccinic acid, anthranilic acid and lycoctonine.

There is little doubt that Manske (*Canadian J. Res.*, 1938, 16, B, 57) isolated crude methyl-lycaconitine from the aerial portion of *D. Brownii*, for from the products of alkaline hydrolysis of his base, he isolated anthranilic acid, methylsuccinic acid and a crystalline base. Although he said this crystalline base was similar to, if not identical with, lycoctonine, he suggested the formula $C_{22}H_{37}O_7N$ or $C_{22}H_{39}O_7N$ and added that the formula

$C_{20}H_{33}O_6N$ was not entirely excluded. It seems certain that the substance he analysed was lycocotinine, $C_{25}H_{39}O_7N, H_2O$, for the figures found were: C, 62.12, 62.14; H, 8.62, 8.86; OMe, 26.10, 29.26, 23.51, 25.23 and are in good agreement with those required for the hydrated base, namely, C, 62.07; H, 8.55; 4MeO, 25.57. Further, the optical activity of his substance, $[\alpha]_D^{20} + 52.2^\circ$ ($c = 0.8$ in methyl alcohol), is not very different from $[\alpha]_D^{20} + 49.64^\circ$ ($c = 9$ in dry alcohol) given by Schulze and Bierling (*loc. cit.*, p. 32) for lycocotinine or from $[\alpha]_D^{15} + 53.7^\circ$ ($c = 0.8$ in methyl alcohol) and $[\alpha]_D^{20} + 53.2^\circ$ ($c = 2$ in dry alcohol) now found for that from methyl-lycaconitine.

The present author is now able to confirm Manske's suggestion and for the first time to establish the occurrence of the same alkaloid in species of *Aconitum* and *Delphinium*. Unfortunately there is not at hand a specimen of lycocotinine from *Aconitum lycocotinum* to compare directly with that from *Delphinium elatum*, but there can be little doubt of their identity.

EXPERIMENTAL.

The m. p.'s are corrected.

Total Alkaloids.—The hydrochlorides of the total alkaloids obtained from 500 g. of the seeds by the method later described for the isolation of the alkaloids weighed 8.56 g. = 1.71% of the seeds.

Isolation of Alkaloids.—The crushed seeds (17.7 kg.) were percolated with 90% alcohol (118 l.). The percolate on concentration separated into two layers. The aqueous alcoholic layer was concentrated until most of the alcohol had been removed. The oily layer and aqueous solution were mixed with an equal volume of light petroleum (b. p. 60—80°) and shaken with excess of 10% hydrochloric acid. The aqueous layer was separated, and the petroleum layer extracted three times with 50 c.c. of 10% hydrochloric acid. The united aqueous layers were treated with excess of sodium bicarbonate and extracted twice with an equal volume of chloroform. The filtered chloroform solution was shaken with sufficient *N*-hydrochloric acid (464 c.c.) to render the mixture slightly acid to Congo-paper. Both the aqueous layer (A) and the chloroform layer (B) contained hydrochlorides of weak bases.

The original aqueous alkaline solution after extraction with chloroform was treated with excess of 50% sodium hydroxide solution and extracted four times with an equal volume of chloroform. The filtered chloroform solution was shaken with sufficient *N*-hydrochloric acid to render the mixture slightly acid to Congo-paper. The aqueous layer on concentration gave 7.81 g. of crude crystalline delatine hydrochloride (0.044% of the seeds) and 22.3 g. of amorphous hydrochlorides (0.125% of the seeds).

The aqueous solution (A) containing hydrochlorides of weak bases was treated with excess of sodium bicarbonate and extracted with ether. The ethereal solution on concentration gave 15.25 g. of crude crystalline delpheline (0.086% of the seeds); the residue in the mother-liquor on neutralisation with *N*-sulphuric acid yielded 46.2 g. of sulphates (0.261% of the seeds).

The chloroform layer (B) was treated with water, and the chloroform removed by distillation. The tar which separated was extracted with a little 1% hydrochloric acid, and the united aqueous solution and washings treated with excess of sodium bicarbonate and extracted with ether and then with chloroform (C). The ethereal solution on concentration gave 16.3 g. of crude crystalline delpheline (0.092% of the seeds); the bases remaining in the mother-liquors yielded on neutralisation with *N*-sulphuric acid 164 g. of sulphates (0.927% of the seeds). These sulphates were fractionated by regeneration of the bases and extraction of their ethereal or chloroformic solutions with small quantities of *N*-sulphuric acid. The bases regenerated from the fractions of sulphates having rotations between $[\alpha]_D + 17.4^\circ$ and $+20.9^\circ$ were dissolved in about twice the quantity of alcohol, and their solution made very faintly acid to Congo-paper with approximately *N*-hydriodic acid. Crude crystalline methyl-lycaconitine hydriodide gradually separated, amounting to 67 g.

The bases in the chloroform extract (C) on neutralisation with dilute sulphuric acid yielded 73 g. of sulphates (0.412% of the seeds), having $[\alpha]_D^{20} + 20.4^\circ$, which on conversion into hydriodides as previously described yielded 45 g. of crude crystalline methyl-lycaconitine hydriodide. The total crude methyl-lycaconitine hydriodide obtained amounted to 0.791% of the seeds, the total crude delpheline to 0.178%, and the total crude delatine hydrochloride to 0.125%. So far neither the other bases nor their salts have been obtained crystalline.

Delpheline. The base, purified by crystallisation from 70% alcohol, forms anhydrous prisms, m. p. 227° (sinters at 222°), $[\alpha]_D^{15} - 25.8^\circ$ ($c = 2$ in chloroform) (Found: C, 67.4; H, 8.6; N, 3.4; OMe, 21.0; NMe, 8.1. $C_{22}H_{33}O_5N$ requires C, 67.5; H, 8.5; N, 3.6; 3OMe, 23.8; NMe, 7.4%). It is a weak base; its salts tend to lose acid on drying. The hydrochloride crystallises from dilute acetone in prisms containing one molecule of water, m. p. 219° with frothing (sinters at 215°), $[\alpha]_D^{20} - 42.8^\circ$ ($c = 2$ in water) (Found: C, 59.5; H, 8.3; N, 3.1; Cl, 7.1; OMe, 18.6; NMe, 5.7. $C_{22}H_{33}O_5N, HCl, H_2O$ requires C, 59.2; H, 8.1; N, 3.1; Cl, 7.9; 3OMe, 20.9; NMe, 6.5%). The nitrate crystallises from dilute acetone in anhydrous prisms, m. p. 191—193°, $[\alpha]_D^{20} - 41.2^\circ$ ($c = 2$ in water) (Found: C, 58.7; H, 7.8; N, 5.7; OMe, 19.8; NMe, 5.0. $C_{22}H_{33}O_5N, HNO_3$ requires C, 58.1; H, 7.6; N, 6.2; 3OMe, 20.5; NMe, 6.4%).

Delatine. The base crystallises in prisms containing one molecule of water from aqueous solution of its hydrochloride on the addition of sodium hydroxide solution. The hydrated base has m. p. 148° (sinters at 141°), $[\alpha]_D^{20} + 13.5^\circ$ ($c = 2$ in *N*/5-hydrochloric acid) (Found: loss at 105° in a vacuum, 5.4. $C_{19}H_{25}O_3N, H_2O$ requires H_2O , 5.4%). The anhydrous base has m. p. 261—264° (Found: C, 72.6; H, 8.2; N, 4.9; OMe, nil. $C_{18}H_{25}O_3N$ requires C, 72.3; H, 8.0; N, 4.5%). The hydrochloride, purified by crystallisation from water, gave anhydrous crystals, m. p. 274—277°, $[\alpha]_D^{15} + 13.4^\circ$ ($c = 2$ in water) (Found: C, 64.6; H, 7.6; N, 4.3; Cl, 8.4; OMe, nil. $C_{19}H_{25}O_3N, HCl$ requires C, 64.8; H, 7.5; N, 4.0; Cl, 8.3%).

Methyl-lycaconitine hydriodide. The hydriodide, purified by recrystallisation from 70% alcohol, gave crystals, m. p. 201° (decomp.). The salt being sparingly soluble in water or alcohol, its optical rotation was observed in alcoholic potassium hydroxide solution; the $[\alpha]_D$ was found to vary with the strength of the potassium hydroxide solution used. The following figures were obtained: $[\alpha]_D^{20} + 18.5^\circ$ ($c = 2$ in *N*-alcoholic potassium hydroxide), $[\alpha]_D^{25} + 24.4^\circ$ ($c = 2$ in *N*/5-solution), $[\alpha]_D^{25} + 31.2^\circ$ ($c = 2$ in 0.024*N* solution) (Found in air-dried salt; loss at 105° in a vacuum, 4.2. $C_{27}H_{48}O_{10}N_2, HI, 2H_2O$ requires H_2O , 4.3%). Found in anhydrous salt: C, 54.6; H, 6.5; N, 3.8; I, 14.9; OMe, 15.9; NMe, 2.6. $C_{27}H_{48}O_{10}N_2, HI$ requires C, 54.9; H, 6.1; N, 3.5; I, 15.7; OMe, 15.4; NMe, 3.6%).

Methyl-lycaconitine. The free base was obtained as a nearly colourless, friable mass by shaking a suspension of the hydriodide in water with excess of sodium bicarbonate in the presence of chloroform, and removing the chloroform from the filtered chloroform layer by distillation. The residue, after first drying at 70° and then at 90° and finally at 105° in a vacuum, had m. p. 128° (sinters at 119°) and $[\alpha]_D^{20} + 49.1^\circ$ ($c = 2$ in dry alcohol). The base was not obtained crystalline.

Alkaline Hydrolysis of Methyl-lycaconitine.—Methyl-lycaconitine from the hydriodide (6 g.) was dissolved in alcohol (40 c.c.) and heated on the water-bath with 15 c.c. of *N*-sodium hydroxide until most of the alcohol had evaporated.

On addition of water (25 c.c.), 2.58 g. of crude crystalline lycoctonine, m. p. 139° with frothing (sinters at 135°), separated. The filtrate was extracted with chloroform. On hydrolysis the extract gave a further 0.5 g. of lycoctonine. The filtrate was concentrated to low bulk, *n*-hydrochloric acid (7.5 c.c.) added until a faint turbidity was produced, and the liquid filtered. On addition of *n*-hydrochloric acid (7.5 c.c.) to the filtrate, 1.49 g. of crude methylsuccinylanthranilic acid separated as an oil passing rapidly into crystals, m. p. 146°. The ethereal extract of the filtrate yielded a further small quantity of this acid.

Lycoctonine. The crude substance, purified by crystallisation from dilute alcohol, gave needles with one molecule of water, m. p. 143° (sinters at 138°), $[\alpha]_D^{20} + 53.2^\circ$ ($c = 2$ in dry alcohol), $[\alpha]_D^{25} + 53.7^\circ$ ($c = 0.8$ in methyl alcohol) (Found: C, 62.0; H, 8.6; N, 3.4; OMe, 23.4; NMe, 6.9; equiv., by titration with *n*/5-hydrochloric acid, with methyl-red as indicator, 485. Calc. for $C_{25}H_{39}O_7N.H_2O$: C, 62.1; H, 8.6; N, 2.9; 4OMe, 25.6; NMe, 6.0%; equiv., 483). As pointed out by Schulze and Bierling (*loc. cit.*, p. 32), the hydrated substance on drying at 100°/40 mm. loses water corresponding to one molecule and becomes an amorphous glassy mass. This has been found to sinter at 119°, melt at 126°, and froth at 143° (Schulze and Bierling record sintering at 120°, transparent at 127°, melts at 131–133°, and evolves gas at 137°) (Found for dried substance: C, 64.3; H, 8.7; N, 3.3; OMe, 23.9; NMe, 6.8. Calc. for $C_{25}H_{39}O_7N$: C, 64.5; H, 8.5; N, 3.0; 4OMe, 26.6; NMe, 6.2%).

1-Methylsuccinylanthranilic acid (methyl-lycoctinic acid). The crude acid was dissolved in *n*-sodium hydroxide to form the disodium salt, the solution filtered, and *n*-hydrochloric acid added to give the monosodium salt; filtration through charcoal and addition of the required quantity of *n*-hydrochloric acid drop by drop liberated the free acid in anhydrous crystals, m. p. 155° (sinters at 147°), $[\alpha]_D^{24} - 7.0^\circ$ ($c = 2$ in dry alcohol) [Found: C, 57.5; H, 5.2; N, 6.0; equiv., by titration with *n*/5-sodium hydroxide with phenolphthalein as indicator, 128.7. $C_{10}H_{11}ON(CO_2H)_2$ requires C, 57.3; H, 5.2; N, 5.6%; equiv., 125.6].

Hydrolysis of 1-Methylsuccinylanthranilic Acid.—The acid (1.55 g.) was boiled with 10% hydrochloric acid (20 c.c.) for 1½ hrs., and the solution diluted with 80 c.c. of water and extracted twelve times with 100 c.c. of ether, which removed 1.425 g. of a mixture of anthranilic and *l*-methylsuccinic acids. A further 0.23 g. of methylsuccinic acid was obtained by evaporation of the aqueous solution in a vacuum over solid potassium hydroxide. The anthranilic acid was separated from the mixed acids by crystallisation from water. It melted at 147° and had the properties of anthranilic acid (Found: C, 61.3; H, 5.4; N, 10.6. Calc.: C, 61.3; H, 5.1; N, 10.2%).

l-Methylsuccinic acid was separated from the residue left on evaporation of the mother-liquor from the anthranilic acid by extraction with ether. The crystals which separated from the concentrated ethereal solution were recrystallised from benzene, m. p. 114° (sintered at 111°), $[\alpha]_D^{23} - 8.8^\circ$ ($c = 2$ in water) (Found: C, 45.3; H, 6.0; equiv., by titration with *n*/10-sodium hydroxide, with phenolphthalein as indicator, 66.0. Calc.: C, 45.4; H, 6.1%; equiv., 66.0). The acid gave the characteristic fluorescein test with great brilliance when heated with resorcinol and sulphuric acid, etc.

Acid Hydrolysis of Methyl-lycaconitine.—Methyl-lycaconitine from the hydriodide (6 g.) was dissolved in 50 c.c. of 10% hydrochloric acid and kept in a closed vessel for 14 days. To the solution diluted with water (50 c.c.), excess of solid sodium carbonate was added, which precipitated 3.9 g. of crude anthranoyl-lycoctonine. The filtrate was extracted with ether, which removed a small quantity of material, then treated with excess of hydrochloric acid and extracted twelve times with equal volumes of ether. The ethereal extract gave 0.61 g. of crude *l*-methylsuccinic acid, which was purified by recrystallisation from benzene (for properties, see under *l*-methylsuccinic acid).

Anthranoyl-lycoctonine. The base, purified by recrystallisation from 70% alcohol, had m. p. 172° (sinters at 168°), $[\alpha]_D^{24} + 32.4^\circ$ ($c = 2$ in *n*/5-hydrochloric acid). Its solutions have a beautiful blue-violet fluorescence (Found for substance dried at 105° in a vacuum: C, 64.9; H, 7.9; N, 5.1; OMe, 21.3; NMe, 4.3. $C_{32}H_{44}O_8N_2$ requires C, 65.7; H, 7.6; N, 4.8; 4OMe, 21.2; NMe, 4.9%).

Hydrolysis of Anthranoyl-lycoctonine.—Anthranoyl-lycoctonine (0.545 g.) was heated on a water-bath with alcohol (5.5 c.c.) and 1.1 c.c. of *n*-sodium hydroxide until the alcohol had evaporated. The residue was treated with 5 c.c. of water; 0.395 g. of lycoctonine, m. p. 142°, separated. The filtrate was neutralised with *n*-hydrochloric acid, concentrated, and treated with hydrochloric acid to make the total acid added 1.1 c.c. of *n*-acid. Anthranilic acid (0.06 g.) separated; a further 0.04 g. was obtained by extracting the filtrate with ether.

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