

[CONTRIBUTION FROM THE PATHOLOGICAL DIVISION, BUREAU OF ANIMAL INDUSTRY]

Lupine Studies. XIV.¹ The Isolation of Anagyrine from *Lupinus laxiflorus* var. *silvicola* C. P. Smith

BY JAMES FITTON COUCH

The leguminous plant *Lupinus laxiflorus* var. *silvicola* C. P. Smith ranges from Mariposa County, Calif., northward to southern Oregon. It has long been of interest among the poisonous lupines because of the atypical symptoms which it produces in livestock. Instead of showing the ordinary and characteristic symptoms of lupine poisoning, animals affected by this species exhibit symptoms closely resembling those caused by the larkspurs, notably *Delphinium barbeyi* Huth.² This had resulted in some confusion in diagnosing spontaneous range poisoning.

It has now been found that *Lupinus laxiflorus* var. *silvicola* contains anagyrine, not hitherto known to exist in the genus *Lupinus*. The close relationship of anagyrine to the lupine alkaloids has been shown by Ing.³ Its occurrence in the genus *Lupinus* is further evidence of the close chemical similarity between the papilionaceous genera.

Anagyrine isolated from this lupine is indistinguishable from the alkaloid isolated from *Anagyris foetida*. It constitutes the major portion if not the whole of the alkaloidal material present. The related alkaloids often associated with anagyrine, cytisine, methylcytisine and sparteine, were not found in *L. laxiflorus* var. *silvicola*.

Anagyrine hydrochloride apparently crystallizes with varying quantities of water. Ing³ prepared it with one molecule, and Hardy and Galois⁴ reported a salt with four molecules. In this paper there are reported preparations with one-half and with three molecules of water.

Anagyrine behaves characteristically in the modified Grant's test for sparteine. When a strip of filter paper is moistened with a chloroform solution of anagyrine, dried and exposed to bromine vapor, the usual orange-yellow color develops. If the strip be now suspended in ammonia vapor, instead of bleaching it develops a pale blue color. On heating the strip the color changes to brown instead of scarlet as in the case of sparteine.

This behavior appears to be unique with anagyrine.

The alkaloid and its salts have been compared with monolupine previously reported from *Lupinus caudatus*⁵ and have been found to be different. Monolupine is not anagyrine. It differs in optical activity, does not form a very insoluble perchlorate, and mixed melting points of similar salts are markedly depressed and indefinite.

Experimental

Material.—Two lots of plant were examined. The first lot was collected by W. W. Eggleston in the Plumas National Forest, California, in July of 1926 and 1927. The second lot was collected by Forest Ranger Ben Beard at the same place in June and July, 1931. The species was identified by Mr. Eggleston.⁵ No chemical differences were found between the two lots. They contained 11.94 and 10.62% of moisture, respectively.

Extraction of the Alkaloids.—The coarsely-powdered dried plants were extracted by alcohol and the alkaloids were recovered by the process already described.⁷ The first lot, 3.96 kg., yielded 33.6 g. or 0.97% of crude alkaloid; the second, 14.76 kg., yielded 94.5 g. or 0.71% calculated to moisture-free plant.

Anagyrine Hydrochloride.—The crude alkaloid (10 g.) was dissolved in water, filtered and acidified with hydrochloric acid. The solution was concentrated and allowed to crystallize. The crystals were recrystallized from a mixture of hot alcohol and ethyl acetate. White needles, melting at 235–236° (uncor.) after desiccator-drying, were obtained.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot HCl \cdot 3H_2O$ (334.7): H_2O , 16.14; Cl, 10.61; N, 8.37. Found: H_2O , 15.62, 15.48; Cl, 10.84; N, 8.34, 8.35.

To 35.5 g. of crude alkaloid in 100 cc. of acetone was added a mixture of 20 cc. of hydrochloric acid and 20 cc. of alcohol and then enough alcohol, 10 cc., to redissolve the oily precipitate. On cooling, anagyrine hydrochloride crystallized out. The salt was recrystallized from a mixture of 2 parts alcohol and 1 part acetone. After several recrystallizations and drying in a desiccator the salt melted at 284.5–285.5°⁸ and did not depress the melting point of authentic anagyrine hydrochloride from *Anagyris foetida*.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot HCl \cdot 0.5H_2O$ (289.7): H_2O , 3.11; Cl, 12.25. Found: H_2O , 3.31; Cl, 12.43, 12.42. In water $[\alpha]^{25}_D -124.2^\circ$, $l = 2$, $c = 1.7316$, $a = 4.3^\circ$.

(1) Previous paper, *THIS JOURNAL*, **61**, 1523 (1939).(2) A. B. Clawson, *The Official Record*, Feb. 26, 1931, p. 31.(3) H. R. Ing, *J. Chem. Soc.*, 504–510 (1933).(4) Hardy and Galois, *Compt. rend.*, **107**, 247 (1888).(5) J. F. Couch, *THIS JOURNAL*, **58**, 686–687 (1936).(6) This species was described as *Lupinus silvicola* by Heller *Muhlenbergia*, **6**, 81 (1910).(7) J. F. Couch, *THIS JOURNAL*, **56**, 2434 (1934).

(8) Melting points are corrected unless otherwise indicated.

The anhydrous hydrochloride melted at 295–297°.

Anagryne perchlorate was prepared according to Ing as long needles soluble in hot water and crystallizing readily from cold, decomposing without melting at a high temperature.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot HClO_4$ (344.7): C, 52.22; H, 6.15; N, 8.12. Found⁹: C, 52.13, 52.30; H, 6.37, 6.31; N, 8.10, 8.13.

Anagryne Gold Chloride.—An aqueous solution of the hydrochloride was treated with excess of gold chloride and the resulting precipitate was recrystallized from boiling dilute hydrochloric acid after filtering from the gold precipitated by reduction. The double salt crystallized in long golden needles melting at 167–168° (uncor.) after desiccator-drying. Analysis indicated somewhat more than the theoretical gold content, possibly due to reduced gold.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot HAuCl_4$ (584.2): Au, 33.75. Found: Au, 34.04, 34.09.

Anagryne Picrate.—The hydrochloride in water treated with alcoholic picric acid gave a bright yellow crystalline precipitate which was collected and dried. It melted at 169.5° and did not depress the melting point of authentic anagryne picrate. Attempts to recrystallize these picrates from water resulted only in crystals of picric acid.

Anagryne Base.—Ten grams of the hydrochloride was alkalinized with sodium hydroxide and the free alkaloid shaken out with ether. After removal of the solvent the

(9) The analyses were made by the Arlington Laboratories, V. A. Conard, Director.

base was distilled under reduced pressure. At 12 mm. the major portion distilled between 260 and 270°. The distillate was a light yellow oil that solidified to a yellow glass. In water solution it gave a red color with ferric chloride and in acid solution readily reduced potassium permanganate. It was levorotatory in alcohol, $[\alpha]_{25}^{20}D = -168^\circ$, $l = 1$, $c = 1.0596$, $a = 1.78^\circ$.

Anagryne Methiodide.—The base, 2 g., in 15 cc. of acetone and 2 cc. of methyl iodide evolved heat and began to deposit crystals within thirty minutes. The crystals were recrystallized from methanol and dried at 110°. They melted at 262–263°.

Anal. Calcd. for $C_{15}H_{20}ON_2 \cdot CH_3I$: I, 32.86. Found: I, 32.77.

Other Bases.—The mother liquors from the hydrochloride were alkalinized with sodium hydroxide in water and the liberated bases were shaken out with chloroform. A portion of the residue was shown to be anagryne. No other base could be identified in the remainder. Cytisine, methylcytisine and sparteine were proved absent by appropriate tests.

Summary

Dry *Lupinus laxiflorus* var. *silvicola* C. P. Smith contains 0.7 to 1% of alkaloids, principally anagryne. Cytisine, methylcytisine, and sparteine were not found in the plant. Anagryne gives a characteristic blue color in the modified Grant test for sparteine.

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A Method for the Preparation of α,β -Diglycerides of Fatty Acids

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The preparation and identification of α,β -diglycerides of the fatty acids has been difficult, due principally to the migration of acyl groups from the β - to the α' -position, as shown by Fischer.¹ Fairbourne and associates^{2,3,4} have shown that the unsymmetrical diglycerides reported by earlier investigators^{5,6,7,8,9} were probably the symmetrical isomers or mixtures in every case. The methods reported by Humnicki and Lunkiewicz,¹⁰ Weizman and Haskelberg¹¹ and Delaby and Dubois¹² also involved reaction conditions that

would almost certainly yield products containing little if any of the α,β -diglycerides.

The methods of Abderhalden and Eichwald¹³ and Bergmann and associates,¹⁴ using propylamine, and the trityl ether method of Helferich and Sieber,¹⁵ have proved to be satisfactory for the preparation of aromatic α,β -diglycerides, but in no case have α,β -diglycerides of the fatty acids been prepared and characterized as pure compounds. Recently Verkade and associates¹⁶ have outlined a general method for the preparation of α,β -diglycerides that should be satisfactory, involving the catalytic detritylation of α,β -diacyl- α' -trityl glycerol, but their work has not been reported in detail.

(13) Abderhalden and Eichwald, *Ber.*, **49**, 2005 (1916).

(14) Bergmann, *et al.*, *ibid.*, **54**, 936 (1921); *Z. physiol. Chem.*, **137**, 27, 47 (1924).

(15) Helferich and Sieber, *ibid.*, **170**, 31 (1927); **175**, 311 (1928).

(16) Verkade, van der Lee, de Quant and Zuydewijn, *Proc. Acad. Sci. Amsterdam*, **40**, 580 (1937).

- (1) Fischer, *Be.*, **53**, 1621 (1920).
- (2) Fairbourne and Cowdrey, *J. Chem. Soc.*, 129 (1929).
- (3) Fairbourne, *ibid.*, 389, 372 (1930).
- (4) Fairbourne, Gibson and Stephens, *ibid.*, 445 (1931).
- (5) Guth, *Z. Biol.*, **44**, 78 (1903).
- (6) Grun and Theimer, *Ber.*, **40**, 1792 (1907).
- (7) Renshaw, *This Journal*, **36**, 537 (1914).
- (8) Thomson, *Trans. Roy. Soc. Canada*, **20**, 445 (1926).
- (9) Heiduschka and Schuster, *J. prakt. Chem.*, **120**, 145 (1928).
- (10) Humnicki and Lunkiewicz, *Bull. soc. chim.*, **40**, 422 (1929).
- (11) Weizman and Haskelberg, *Compt. rend.*, **189**, 104 (1929).
- (12) Delaby and Dubois, *ibid.*, **187**, 767, 949 (1928).