acid; those less soluble in water are an olefin alcohol ($C_{10}H_{18}O$, constituting 90% of the non-water-soluble constituents), an amyl (probably *iso*-amyl) alcohol, phenylethyl alcohol, esters of formic, acetic and caprylic acids. Geraniol and terpineol were indicated but not positively identified.

San Dimas, California

[Contribution from the Pathological Division, Bureau of Animal Industry, United States Department of Agriculture]

LUPINE STUDIES. IV. ISOLATION OF *d*-LUPANINE FROM LUPINUS KINGII (S. WATSON)

By James Fitton Couch

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This paper reports an investigation of the alkaloidal constituents of an American species of lupine, *Lupinus kingii*, not hitherto examined chemically, and the isolation from it of an alkaloid that has been identified as *d*-lupanine. This alkaloid has been isolated from several European species[1] of the genus *Lupinus*, and from a single American species, the perennial *L. polyphyllus*.[2]

The plant material used in this investigation was collected on July 28 and August 1, 1922, on the summit of Flat Top Mountain, in the Wasatch Range, Utah.[3] At that time the plants were well grown; some were still in flower while others were in fruit. The collected material was carefully dried in the shade, bagged and shipped into Washington where the chemical study was made.

The plant yielded a total of 0.83% of crude alkaloid. All of this material consisted of *d*-lupanine; a careful search was made for *dl*-lupanine and for hydroxylupanine, both of which alkaloids sometimes accompany *d*-lupanine in species of this genus, but no evidence of their presence was obtained. The hydrochloride prepared from this alkaloid melted at 127°. When this salt was mixed with an authentic sample of *d*-lupanine hydro-

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[1] Hagen, *Dissertation, Halle*, **1885**. Siebert, *Arch. Pharm.*, **229**, 531 (1891). Soldaini, *ibid.*, **231**, 321 (1893). Davis, *ibid.*, **235**, 199, 218 (1897). Gerhard, *ibid.*, **235**, 355 (1897). Callsen, *ibid.*, **237**, 566 (1899). Bergh, *ibid.*, **242**, 416 (1904).

[2] Schmidt [*ibid.*, **235**, 192 (1897)] has reported the isolation of *d*-lupanine from *L. perennis* by his pupils, Gerhard and Bergh. Both of these latter, however, term the plant with which they worked *L. polyphyllus*, a perennial species that is a commercial article in Germany. I am informed by Mr. W. W. Eggleston that it is very unlikely that Schmidt and his pupils could have obtained the American species *L. perennis* in the quantities reported in their memoirs and that, undoubtedly, the plant they studied was the American perennial, *L. polyphyllus*. Investigation of authentic specimens of *L. perennis* is now in progress in this Laboratory.

[3] The plant material was identified by Mr. W. W. Eggleston, Botanist at the Bureau of Plant Industry, United States Department of Agriculture, to whom the writer expresses his gratitude.

chloride that had been prepared from *L. albus* and which also melted at 127°, there was no alteration of the melting point. The properties of the alkaloid from *L. kingii* are compared in Table I with those reported from *d*-lupanine by various observers. The similarity leaves no doubt of the identity of the former.

Crystals of the hydrochlorides of *d*-lupanine and of the alkaloid from *L. kingii* were submitted for study and comparison to Mr. W. H. Fry, Petrographer at the Bureau of Soils, United States Department of Agriculture, who has kindly furnished the following report.

"Both crystals (A and B) are optically biaxial with a large axial angle, only one emergence of an optic axis appearing in the field of the microscope at one time. The double refraction is moderately high. The interference figure is so nearly a straight line as to make the determination of the optical character doubtful, but in a few exceptionally distinct figures the very slight curvature made it possible to determine the optical character as positive, provisionally. Freshly crushed grains with sharp angles soon became rounded in the immersion media available, and the solvent action thus indicated would of course preclude an accurate determination of the refractive indices. As measured under these conditions, however, the mean refractive index is about 1.55+. Under the microscope, the crushed crystals have a marked tendency to lie in such a position as shows the emergence of an optic axis. So far as the microscopic examination went, no distinction could be found between the samples A and B."

TABLE I

PROPERTIES OF ALKALOID FROM *L. kingii* COMPARED WITH THOSE OF *d*-LUPANINE

	Alkaloid from *L. kingii*	d-Lupanine
Hydrochloride, m. p.	127°	127°
Specific rotation	+51.37°[a]	+55.37°[b]
Chlorine content, %	11.44 11.23	11.06 (calcd.)
Gold chloride, m. p.	200°	199–200°
Gold content, %	33.50	33.52 (calcd.)
Hydriodide, m. p.	183–4°	183–5°
Iodine content, %	33.70	33.74 (calcd.)
Methyl iodide, m. p.	239–240°	239–241°[c]
Iodine content, %	32.54	32.53 (calcd.)
Methyl-lupanine gold chloride, m. p.	204–5°	205–6°
Picrate, m. p.	180°	180°[d]

[a] For crystalline salt.

[b] For anhydrous salt, reported by Davis.

[c] Siebert reported the melting point of this compound as 248–249°. This value is evidently a corrected melting point. Applying the usual correction on our determination the value obtained is 247–248°, in good agreement with that of Siebert.

[d] Reported by Thoms and Bergerhoff, *Arch. Pharm.*, **263**, 1 (1925).

Experimental Part

Moisture Content of the Air-dried Plant.—Two samples were heated to constant weight at 120°. Subs., 1.4438, 1.2956 g.: loss, 0.1514, 0.1368. Moisture, 10.49, 10.56.

Isolation of the Alkaloid.—12.18 kg. of coarsely ground, air-dried, whole plant was extracted by intermittent percolation with alcohol containing acetic acid. The solvent was removed from the percolate; the residue was mixed with water and precipitated with aqueous lead acetate. The filtrate was freed from lead with hydrogen sulfide concentrated to one liter, made alkaline with potassium hydroxide and extracted with chloroform until the major portion of the alkaloid was extracted. Extraction with chloroform was continued to recover the small amount of alkaloid still remaining in the solution and this second lot (Alkaloid C) was kept separate from the first. The solvent was removed from the first lot of chloroform extract by heat, leaving a sirupy, red-brown mass weighing about 100 g. This was digested with hot benzene, which dissolved the greater portion of it. When cooled, the benzene deposited a thin film which was added to the portion of the original sirup not dissolved by benzene, the mixture being set aside as Alkaloid B for later study.

The benzene was allowed to evaporate spontaneously from the solution of Alkaloid A, when a thick, reddish sirup of characteristic, crude lupine-alkaloid odor was obtained. This weighed 74.5 g. and constituted 0.61% of the plant used. This material was identified as *d*-lupanine by the following procedure.

A portion of the sirupy mass was dissolved in water; the aqueous solution was alkaline to litmus and became cloudy when heated, through separation of the alkaloid, which is more soluble in cold than in hot water. A portion of the solution, acidified with hydrochloric acid and polarized, rotated the plane to the right. This solution responded to the usual tests for alkaloids.

d-**Lupanine Gold Chloride.**—Two g. of the alkaloid was dissolved in dil. hydrochloric acid, the solution filtered through diatomaceous earth and treated with an excess of solution of gold chloride ($HAuCl_4$). The voluminous yellow precipitate formed was collected on a filter, washed with acid water and recrystallized from boiling water. A quantity of clumped, yellow needles thus obtained was dried in a vacuum; m. p., 200°, with decomposition.

Anal. Subs., 0.2672 (dried at 110°): residue, 0.0895. Calcd. for $C_{15}H_{24}ON_2$: $HAuCl_4$, 33.52. Found: Au, 33.50.

d-**Lupanine Picrate.**—About 1 g. of the free alkaloid was dissolved in warm alcohol and the solution added to a warm alcoholic solution of the calculated quantity of picric acid. The clear mixture was allowed to cool slowly, when small, yellow needles separated. These were collected and recrystallized from water. The crystals, dried in a desiccator for one week, melted sharply at 180°, the proper melting point.

d-**Lupanine Hydrochloride.**—An aqueous solution of 10 g. of the alkaloid was neutralized with dil. hydrochloric acid, decolorized with animal charcoal, evaporated to a sirup and allowed to crystallize in a vacuum. Colorless, prismatic needles were obtained; m. p., 127°. These contained water of crystallization and showed a tendency to effloresce slightly. When they were mixed with an authentic sample of *d*-lupanine hydrochloride from *Lupinus albus* the mixture melted at 127°.

Anal. Subs., 0.1193, 0.1848: AgCl, 0.0552, 0.0839. Calcd. for $B.HCl.2H_2O$: Cl, 11.06. Found: 11.44, 11.23.

A 25cc. (25°) aqueous solution of 0.3796 g., examined polariscopically in a 200mm. tube by sodium light at 25°, gave as a mean of ten observations a rotation to the right of 1.56°, whence $[\alpha]^{25}_{D} = +51.37°$.

d-**Lupanine Hydriodide.**—About 2.5 g. of the sirupy alkaloid was dissolved in 10 cc. of 60% alcohol, and 2 cc. of concd. hydriodic acid was added. The solution was allowed to evaporate in the air, and deposited a mass of nearly colorless prisms within three hours. These were collected and recrystallized from hot water. Dried in air, the crystals effloresced slightly. Dried at 120°, the substance melted at 183–184°.

Anal. Subs., 0.3319: AgI, 0.2069. Calcd. for B.HI: I, 33.74. Found: 33.70.

***d*-Lupanine Methyl Iodide.**—Two g. of the alkaloid and 2 g. of methyl iodide were weighed into a pressure bottle, mixed and the cover was tightly screwed on. Crystals began to form in 35 minutes in the cold, and at the end of three hours the mixture was solid. It was then heated in a water-bath for 105 minutes to complete the reaction, cooled and allowed to stand for 36 hours; the reaction product was crystallized from hot alcohol and yielded a mass of nearly white leaflets. Dried in a desiccator over sulfuric acid, these crystals melted at 239–240°.

Anal. Subs., 0.5210: AgI, 0.3136. Calcd. for $B.CH_3I$: I, 32.53. Found: 32.54.

Methyl-lupanine Gold Chloride.—Two different lots of this substance were prepared by the same process. Samples of *d*-lupanine methyl iodide were freed from iodide with silver nitrate, filtered from the precipitated silver iodide and then freed from silver with a small excess of hydrochloric acid. Gold chloride solution was added to the filtrate. Upon concentration to one-half volume and cooling, the double salt crystallized in fine needles, m. p. (dried in a vacuum) 204–205°, in good agreement with the temperature reported by Bergh, but somewhat higher than that reported by Davis. Analysis, however, showed that both of these preparations contained more gold than the addition of one molecular equivalent of gold chloride demands, and less than would be present if two molecular equivalents of gold chloride had reacted with one of methyl-lupanine. This is in accord with the experience of Hagen. Subsequent investigators, however, do not report any difficulty in preparing the normal compound containing one equivalent of gold chloride.

Anal. Subs., 0.2334, 0.1722 (I); 0.2607 (II): Au, 0.0886, 0.0653 (I); 0.0866 (II). Calcd. for $B.CH_3.HAuCl$: Au, 32.69; calcd. for $B.CH_3.2HAuCl_4$: 41.81; calcd. for $B.CH_3.1.5HAuCl_4$: 38.26. Found: 37.96, 37.91 (I); 33.22 (II).

Alkaloid B.—The brown mass insoluble in benzene weighed 8.1 g. It was completely soluble in alcohol. To purify the alkaloid which it contained it was extracted with dil. hydrochloric acid, which left behind the major portion of the mixture as an insoluble resin which weighed 3.3 g. The solution of the hydrochloride was decolorized by boiling with animal charcoal, and the filtrate was concentrated and set aside to crystallize in a vacuum. This solution was dextrorotatory. Only a small amount of crystals was obtained; these were mixed with the sirupy solution of the salt and were thus unfit for melting-point determination. They were converted into the gold chloride compound and this was analyzed.

Anal. Subs., 0.1950: Au, 0.0652. Calcd. for $C_{15}H_{24}ON_2.HAuCl_4$: Au, 33.52. Found: 33.44.

Alkaloid C.—The material extracted from the original alkaline solution amounted to less than 1 g. It was converted into hydrochloride, purified and then precipitated as the gold double salt for identification.

Anal. Subs., 0.1223: Au, 0.0405. Calcd. for $C_{15}H_{24}ON_2.HAuCl_4$: Au, 33.52; for $C_{15}H_{24}O_2N_2.HAuCl_4$: Au, 32.60. Found: 33.12.

Summary

An American species of lupine, *Lupinus kingii*, yields 0.83% of alkaloid, calculated on a basis of dry weight of plant. The alkaloid agrees with the characters of *d*-lupanine, an alkaloid not hitherto found in a native American annual lupine.

WASHINGTON, D. C.