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SHORT COMMUNICATION

ALKALOIDS OF *LUPINUS DIFFUSUS* NUTT.[1,2]

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Abstract—In addition to two trace alkaloids, sparteine and multiflorine have been isolated from *Lupinus diffusus* Nutt.

THE species *Lupinus diffusus* Nutt. belongs to the very large and economically important Leguminosae family. Lawrence[4] classifies (known locally as the "sandhill lupine") the genus *Lupinus* as a member of the sub-family Lotoideae (= Papilionaceae).

While many species of the genus *Lupinus* have been investigated for their alkaloid content,[5] to the best of our knowledge there is only one reference in which the plant of the present study has been studied. It was included in the very ambitious plant survey conducted by Wall and coworkers,[6] but apart from noting the presence of alkaloids Wall *et al.* did not carry out an investigation of the identity of the bases elaborated by the plant.

The plant material used in the present investigation was collected[7] during late April, 1961, near Gaston, S. C. At that time they were in full bloom, and the entire plant, including flowers and tap root, was taken. No attempt was made to study the alkaloidal distribution among the plant parts; rather, the whole plant, after air-drying for 2 weeks, was milled and used for extraction.

The extraction procedure consisted of a room temperature, batch-wise, chloroform treatment of the milled plant material, which was previously soaked in dilute aqueous ammonium hydroxide solution and contained in a bleached muslin sack. The basic material present in the combined chloroform extracts was then separated from the plant pigments through successive extractions with dilute aqueous hydrochloric acid. The total crude alkaloids were liberated from their hydrochloride salts, extracted into ether, and finally obtained as a red–brown-colored, viscous oil. The average yield of total crude alkaloids, based upon the weight of moisture-free plant material, was 1·3 per cent.

Paper chromatographic examination of the crude mixture indicated the presence of two major alkaloids, subsequently shown to be sparteine [(I), 0·88 per cent] and multiflorine [(II),

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[2] Taken in part from the dissertation submitted by R. F. M. in partial fulfillment of the Graduate School requirements for the Ph.D. in chemistry, University of South Carolina.

[3] C. Jules Seideman Memorial Fellow, 1961–1963. Grateful acknowledgement is made to Columbia Organic Chemicals, Inc., for providing this fellowship.

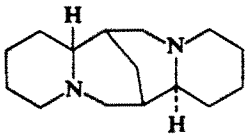
[4] G. H. M. LAWRENCE, *Taxonomy of Vascular Plants*, p. 545. Macmillan, New York (1951).

[5] N. J. LEONARD, In *The Alkaloids* (Edited by R H. F. MANSKE and H. L. HOLMES), Vols. III and VII. Academic Press, New York (1953) and (1960).

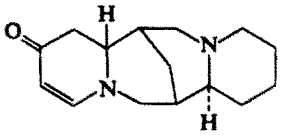
[6] M. E. WALL, C. S. FENSKE, H. E. KENNEY, J. J. WILLAMAN, D. S. CORRELL, B. G. SCHUBERT and H. S. GENTRY, *Am. J. Pharm.* **46**, 653 (1957).

[7] We are indebted to Dr. W. T. Batson, Department of Biology, University of South Carolina, for the expert identification of *L. diffusus*.

0·14 per cent]; and, two other bases, subsequently estimated to have constituted a total of about 0·02 per cent of the dry plant material.



(I)



(II)

Fractionation of the total crude alkaloid mixture was partially achieved through chromatography on alumina. Thus, sparteine (I) was eluted with ether, and then further purified by means of molecular distillation. The two minor alkaloidal constituents, along with trace amounts of sparteine (I) and multiflorine (II), were found in fractions obtained by continued elution with ether containing increasing amounts of ethyl acetate. Multiflorine (II) was then found to be the only basic material present in subsequent fractions. Molecular distillation provided pure multiflorine, which was then recrystallized from *n*-hexane.

The ubiquitous sparteine was readily identified through direct comparison of its properties both as the free base and as two salts, with authentic material. Multiflorine was carefully characterized and quite a bit of structural information was obtained before we were struck by the similarity of properties we observed and those reported by Crow[8] and by Comin and Deulofeu.[9] Admixture melting of our material with a sample of multiflorine, generously furnished by Professor Crow, did not provide a clear-cut basis for establishing the identity; however, this was clearly shown by the congruity of the X-ray powder patterns determined from each sample.

It is interesting that multiflorine, a rather recent addition to the lupin alkaloid group, has essentially a world-wide distribution: South America (*L. multiflorus* Lam.);[9] Australia (*L. digitatus* Forsk.);[8] Europe (*L. albus*);[10] and now, North America (*L. diffusus*).[11]

Both sparteine and multiflorine exhibited slight depressant activity as indicated by the results of preliminary physiological (central nervous system) tests.[12] Similar examination of a sample containing a high concentration of the two trace alkaloids suggested a much higher level of activity. In an attempt to obtain suitable working quantities of the two trace alkaloids, a large collection of *L. diffusus* was carried out in April of 1965. These plants were gathered from the same location as were the plants initially obtained in April, 1961, but chromatographic examination of the total crude alkaloids extracted from the former revealed only the presence of sparteine and multiflorine. This enigma was further compounded by the fact that examination of the alkaloidal content obtained from another batch of *L. diffusus*, gathered[13] in April, 1964, showed the presence of all four alkaloids.

[8] W. D. CROW, *Australian J. Chem.* **12**, 474 (1959).
[9] J. COMIN and V. DEULOFEU, *Australian J. Chem.* **12**, 468 (1959).
[10] M. WIEWIOROWSKI and J. WOLINSKA-MOCYDLARZ, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.* **11**, 709 (1961).
[11] Multiflorine has also been established as a major alkaloidal constituent of *L. westianus* Small, collected near Panama City, Florida [M. S. SAHLI, *Doctoral Dissertation*, University of South Carolina, Columbia, South Carolina (1966)].
[12] These tests were initially carried out by Hazleton Laboratories of Hazleton, Virginia, under the Pharmacologic Testing Program of the Psychopharmacology Service Center, National Institute of Mental Health. Essentially the same preliminary indications were subsequently obtained by Professor W. J. Kinnard, School of Pharmacy, University of Pittsburgh, who very generously agreed to take on the biological testing when the Pharmacologic Testing Program was interrupted.
[13] The authors thank Mr. Max Schreiber for carrying out this collection.

EXPERIMENTAL[14]

Alkaloids of Lupinus diffusus *Nutt.* Full-bloomed, whole plants (including roots) were gathered near Gaston, S. C., during late April of 1961.[7] The material was air-dried during 2 weeks and then milled to about 80 mesh. The average moisture content of the milled, air-dried, whole plant was determined to be about 8 per cent. While several extractions were carried out during the course of this work, each essentially followed the same procedure, and the details of one typical room temperature extraction are as follows.

One kg of milled, whole plant material was first thoroughly moistened with 2 N aqueous ammonia solution and then loosely packed into a bleached muslin sack. The securely tied sack and contents were totally immersed in chloroform and allowed to remain with periodic agitation during 2 days. At that time the chloroform extract was removed (the sack squeezed as dry as possible), and the sack reimmersed in fresh chloroform (*c*, 3 l.). The plant material was allowed to steep in the fresh chloroform for another 2-day period and then changed again. In this manner, three successive room-temperature extractions were carried out.[16] All three chloroform extracts (*c*, 9 l.) were combined, filtered free of plant fines, and washed with four successive, 500-ml portions of 1 N HCl solution. The combined aqueous acid washes were then made strongly basic with 40% NaOM solution. The resulting cloudy mixture was extracted with five successive, 300-ml portions of ether. The combined, yellow-colored, ethereal extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated to leave 12·5 g of total crude alkaloids as a viscous, red–brown-colored oil.

Paper chromatographic examination of the total crude alkaloids revealed the presence of four spots: two intense spots [R_f 0·52, shown later to be sparteine (I) and R_f 0·22, shown later to be multiflorine (II)] and two faint spots: R_f 0·46 and R_f 0·37, unknown minor alkaloids.

Column elution chromatography on Woelm neutral alumina (Brockmann Activity Grade I) of the total crude alkaloids gave rise to separation of the major alkaloidal constituents, subsequently identified as sparteine and multiflorine. Thus, elution with ether provided sparteine essentially free of the other basic components. Continued elution with ether containing increasing amounts of ethyl acetate led to a number of fractions containing all four bases, but subsequent fractions were found to contain only multiflorine.

Identification of (−)-*sparteine* (*I*). The yellow-colored, viscous oil obtained from evaporation of the ether eluants, and which gave rise to only one spot upon paper chromatographic examination, was molecularly distilled in a Späth bulb [90–115°/0·3 mm (air bath)] to provide pure (−)-sparteine (I) as a colorless, viscous oil; $[\alpha]_D^{28} - 16{\cdot}8 \pm 0{\cdot}3°$ (*c* 5·26, methanol), lit.,[5] $[\alpha] - 17{\cdot}0°$ (ethanol). An i.r. spectrum (liq. film) determined from the material was found to

[14] Temperature readings are uncorrected. Combustion analyses are by the Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Infrared spectra were determined with a Perkin-Elmer, Model 337, grating spectrometer. Ultraviolet spectra were recorded on a Cary, Model 14, spectrometer, and proton magnetic resonance spectra were measured near room temperature on a Varian, A-60, spectrometer. Chloroform-*d* solutions, containing about 4% (v/v) tetramethylsilane (TMS) as internal standard were used. Chemical shifts are reported under the δ convention in ppm relative to TMS (zero ppm). Sodium "d" line polarimeter measurements were carried out with an O. C. Rudolph & Sons, Inc., instrument, Model 80. Descending paper chromatography was carried out on Whatman No. 1 paper using a solvent system of *n*-butanol: acetic acid [10:1 (v/v)] that had been saturated with water. Chromatograms were developed until the solvent front had gone approximately 25 cm. Spots were visualized via application (spraying) of Dragendorff reagent.[15]

[15] *The Merck Index* (5th Ed.), p. 696. Merck, Rahway, New Jersey (1940).

[16] The decision to employ only three extractions was based on results obtained during preliminary extraction experiments. The amount of precipitate obtained from an aliquot of a fourth extract, when treated with Mayer's reagent,[17] was found to be hardly visible; which was indicative of the presence of a very low alkaloid concentration.

[17] Ref. 15, p. 831.

be superimposable upon that obtained from authentic (−)-sparteine.[18] Titration with methanolic perchloric acid (external indicator) provided (−)-sparteine monoperchlorate, which, after recrystallization from methanol, was observed to melt at 170–171°; lit.,[5] 172°. Another portion of the distillate was dissolved in methanol and titrated (external indicator) with hydriodic acid to yield (−)-sparteine monohydriodide, recrystallized from absolute ethanol, m.p. 229–233°; lit.,[5] 235–236°.

Identification of (−)-multiflorine (II). Evaporation of those ethyl acetate eluants, obtained from alumina chromatography of the total crude alkaloids, which, upon paper chromatographic examination, gave rise to essentially one spot, provided a yellow-colored oil. This material was taken up in warm *n*-hexane, and the resulting solution, upon cooling, deposited slightly yellow-colored crystals which were obtained white after several recrystallizations from *n*-hexane; m.p. 107·5–108·5°; $[\alpha]_D^{26} -310 \pm 1°$ (*c*, 5·03, methanol); i.r., $\nu_{max}^{CHCl_3}$: 1640 (conjugated amide C=O stretch)[19] and 1590 cm^{-1} (conjugated C=C stretch);[19] u.v., $\lambda_{max}^{ethanol}$: 248 and 325 mμ (ε 7800 and 12,400); PMR, δ^{CDCl_3}: 1 proton doublet at 6·89 (*J* 13 c/s) coupled with 1 proton doublet at 4·94 (*J* 13 c/s) arising from the AX system, —N—CH=CH—C(O)—.

Anal. Calcd. for $C_{15}H_{22}N_2O$: C, 73·17; H, 9·00; N, 11·37; M.W., 246. Found: C, 73·01; H, 9·05; N, 10·80; M.W. (m/e), 246.[20]

The alkaloid multiflorine, $C_{15}H_{22}N_2O$, is reported as melting as 108–109°[8] and 107–108°.[9] Its optical rotation is reported as $[\alpha]_D^{20} -314°$ (*c*, 0·6, methanol)[8] and $[\alpha]_D^{22} -317°$ (*c*, 0·4, methanol).[9]

A slightly impure sample of multiflorine (m.p. 101–103°), generously provided by Crow,[8] when melted in admixture with a pure sample of alkaloid from the present study (m.p. 107·5–108·5°), displayed a melting range of 104–107°. A more definitive basis for identity was obtained by determination of X-ray powder patterns of each sample.[21] The congruity of twenty-eight line spacings unequivocally established the identity of the two materials.

[18] Prepared from commercially available (Inland Alkaloid Co.) sparteine sulfate.

[19] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules* (2nd Ed.). John Wiley, New York (1958); K. NAKANISHI, *Infrared Absorption Spectroscopy*. Holden-Day, San Francisco (1962).

[20] We are indebted to Professor Thomas Kinstel, Iowa State College, for determination of the mass spectrum from which this value for the parent ion was taken.

[21] We are indebted to Dr. D. W. Sink of Lenoir-Rhyne College for determination of these X-ray powder patterns.