[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND PHARMACEUTICAL CHEMISTRY OF THE UNIVERSITY OF MARYLAND]

Alkaloids of *Achillea millefolium* L. I. Isolation and Characterization of Achilleine[1,2]

BY F. M. MILLER AND LEE MING CHOW[3]

RECEIVED OCTOBER 21, 1953

From *Achillea millefolium* L., a crystalline alkaloid, achilleine, $C_{14}H_{26}N_2O_6$, has been isolated and characterized, and has been found to be active as a hemostatic.

Of the several medicinal uses reported for the yarrow plant, *Achillea millefolium* L., one of the most interesting is as a hemostatic.[4] In the only phytochemical investigation of this plant, two ill-defined materials, achilleine[5] and moschatine[6] were obtained from *A. millefolium* and *A. moschata*, respectively, and were designated as glyco-alkaloids. The purpose of this investigation was to isolate and characterize the alkaloids more completely, and to determine their hemostatic activity.

From the leaves of *A. millefolium* it has been possible to obtain in crystalline form an alkaloid, $C_{14}H_{26}N_2O_6$, m.p. 247–248°, $[\alpha]_D$ −14.3° (water, *c* 10), for which the name achilleine is retained. The alkaloid is weakly basic, gives positive, but non-reproducible, results in the N-methyl determination, and contains no methoxyl group. It is very soluble in water, slightly soluble in alcohol, but almost completely insoluble in less polar solvents.

Achilleine gives a positive test with the Molisch reagent, is saturated toward potassium permanganate and forms a pink color in the pine splinter test. Since it does not absorb light in the 220–300 mμ region, the pine splinter test is presumed to indicate a pyrrolidine or piperidine[7] nucleus. The infrared spectrum of achilleine contains only one identifiable band, that of an amide carbonyl group at 6.1 μ.

Achilleine proved to be highly resistant to hydrolysis. Refluxing for 30 hours with 5% sulfuric acid produced little change, and a portion of the alkaloid was recovered unchanged after five hours heating with 2% sulfuric acid in a sealed tube at 150–160°. From the hydrolysis mixture it has been possible to obtain ammonia and a small amount of a yet uncharacterized material which behaves as the sodium salt of an amino acid. This latter material gives the same pine splinter test as the parent alkaloid, but appears to be unsaturated from its behavior toward potassium permanganate. It was not possible to isolate a sugar by hydrolysis under these conditions, although a positive furfural test with aniline acetate paper indicated the presence of a sugar derivative.

On the basis of these observations, achilleine may be tentatively formulated a glyco-alkaloid containing a pyrrolidine or piperidine nucleus bearing an N-methyl group and a carboxamide function. Further, the inference might be drawn from the resistance of achilleine to hydrolysis that the basic nitrogen atom and the carboxamide group are in close proximity, since acid hydrolysis would be retarded by a neighboring ammonium ion. Additional studies on the nature of the alkaloid are in progress.

Pharmacology.—In a preliminary pharmacological investigation, achilleine was found to reduce the clotting time of blood in rabbits as determined by the Sabraze method.[8] Following an intravenous dose of 0.5 mg. per kg. body weight, the normal mean clotting time of 170 ± 31 seconds was shortened to 117 ± 26 seconds, a 32% decrease. The action was found to last 45 minutes with no observable toxic effect.

Acknowledgments.—The authors wish to acknowledge the assistance of Dr. C. T. Ichniowski in the pharmacological testing. The infrared data were determined under the direction of Dr. Robert Spurr. Sir Robert Robinson (Oxford) generously donated a sample of carpaine.

Experimental[9]

Extraction.—A continuous extraction apparatus was packed with one kilogram of the powdered leaf of *Achillea millefolium*[10] and the material extracted with 5 l. of alcohol for ten days until the extracts obtained were very lightly colored. This solution was concentrated and the last traces of alcohol removed under reduced pressure, leaving a green residue weighing 120 g. The residue was extracted with several portions of water until the extracts were colorless, giving a total volume of one liter of an orange-red solution having a definite acid reaction. Fifty grams of freshly prepared lead hydroxide was added with stirring and the mixture allowed to stand overnight to complete the neutralization. The yellow lead salts were filtered by suction, leaving a solution with a sweetly aromatic odor, which was saturated with hydrogen sulfide to precipitate the excess lead. The solution was boiled to drive off excess hydrogen sulfide and then filtered. The light yellow filtrate was evaporated to a small volume on a water-bath, and on cooling a semi-solid residue remained. This dark brown mass was extracted with 95% alcohol (800 ml.) until the extracts were only slightly yellow. The alcohol was removed by distillation, and the residue extracted with 1.2 liters of absolute alcohol. This solution was distilled to dryness and the last traces of alcohol removed under reduced pressure to give a reddish-brown mass. On dissolving this material in 600 ml. of water a brown resin precipitated and was filtered off. On evaporation to dryness a sticky semi-solid material (20 g.) remained. This crude material corresponded quite

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(1) Abstracted from the thesis presented by L. M. C. to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Presented before the Division of Organic Chemistry at the 122nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1952.

(3) Li Foundation Fellow.

(4) W. Bohn, "Die Heilwerte heimischer Pflanzen." Quoted by L. Kroeber, *Pharm. Zentralhalle*, **67**, 98 (1926); E. Y. Shass and M. N. Varlakov, *Farmatsiya*, **7**, No. 4, 33 (1944), [C. A., **43**, 5541 (1949)].

(5) B. Zanon, *Ann.*, **58**, 21 (1846).

(6) A. von Planta-Reichenau, *ibid.*, **155**, 153 (1870).

(7) The color of the pine splinter test is identical with that given by carpaine, which has been demonstrated to contain a piperidine nucleus [H. Rapaport and H. D. Baldridge, THIS JOURNAL, **74**, 5365 (1952)], but markedly different from that given by typical indoles.

(8) J. A. Kolmer, E. H. Spaulding and H. W. Robinson, "Approved Laboratory Technic," Appleton-Century-Crofts, New York, 1951, p. 110.

(9) Melting points were observed on a Fisher-Johns block. Carbon, hydrogen and nitrogen analyses are by Mrs. Mary Aldridge and associates.

(10) Obtained from the Meer Corporation, New York, N. Y.

closely to the "achilleine" of von Planta.[6] The use of water for the extraction[5] gave a material which was much less tractable.

Isolation.—A solution of 20 g. of the crude extract in 100 ml. of water was washed with several 25-ml. portions of chloroform, until the chloroform layer was only slightly colored. The aqueous solution was then shaken with 150 ml. of 1-butanol–chloroform (1:4 by volume) in several portions until the washings became very light in color. The dark aqueous layer was then evaporated to dryness on a water-bath to give a residue weighing 16 g.

This residue was then dissolved in 100 ml. of water and added to a column of 300 g. of Amberlite IR-120[11] prepared according to Cassidy.[12] The addition was carried out at a rate of 45 drops a minute, and followed by 900 ml. of water at the same rate. The first 400 ml. of eluant was darkly colored and acid in reaction. The second 400-ml. portion was yellow and acid, and the third 100-ml. portion was colorless and neutral. Then 200 ml. of 2% ammonia was added to the column at the same rate, and followed by 800 ml. of water. The first 200 ml. of eluant was only slightly colored and neutral to litmus. The second 250-ml. portion was orange-red and strongly alkaline, and the third portion was light yellow and alkaline.

Each of the three latter portions of eluant was evaporated to dryness on a water-bath. The second fraction of dark residue, on extraction with boiling alcohol, gave a reddish-brown solution containing a yellowish brown flocculent precipitate which was filtered and washed with alcohol. The combined filtrates, about 80 ml., were cooled overnight, and the brown flocculent powder which separated was removed by filtration. More of this material was precipitated by the addition of an equal volume of ethyl acetate. The ethyl acetate–alcohol filtrate was evaporated to dryness and the residue dissolved in 25 ml. of hot alcohol. A double volume of ethyl acetate was added to precipitate a brown gummy material and, after standing, this was removed by filtration to give a light orange solution. On removal of the solvent, the residue began to crystallize at the edges, and on cooling and rubbing with 5 ml. of alcohol the crystals were freed of the brown adhering material, filtered and washed with alcohol. The white crystals melted at 244–245°. On dissolving the crystalline material in water and allowing the water to evaporate spontaneously, needle-like bundles of crystals melting at 247–248° were obtained. Recrystallization from alcohol, the crystals separating after removal of most of the alcohol, yielded granules of the same melting point. A second crop of the same material was obtained similarly from the third portion of eluant, but none could be obtained from the first fraction. The yield was 500 mg., 0.05% of the dry leaf.

Anal. Calcd. for $C_{14}H_{26}N_2O_6$: C, 52.80; H, 8.23; N, 8.80; mol. wt., 318. Found: C, 52.58, 52.30, 52.57; H, 8.29, 8.34, 8.32; N, 8.96, 8.70, 9.01; mol. wt. (cryoscopic water), 300; OCH_3 negative. Although methyl iodide was evolved easily in the alkimide determination, repeated analysis did not yield reproducible results.

Properties.—Achilleine is readily soluble in water or hot alcohol, slightly soluble in cold alcohol, and insoluble in benzene, ether, chloroform and carbon tetrachloride. It has a sweet taste, an amine-like odor and a weakly basic reaction to litmus. The base forms a yellow precipitate with phosphomolybdic acid, but has no reaction with Wagner's or Meyer's reagents. It dissolves in concentrated sulfuric acid without apparent change. Achilleine gives a violet color in the Molisch test for carbohydrates, the color developing rather slowly, but it does not reduce Fehling solution or Tollens reagent, nor does it decolorize potassium permanganate solution. The alkaloid gives a pink color in the pine splinter test when the vapors are allowed to come into contact with a splinter which has been dipped in concentrated hydrochloric acid. Negative results were obtained in both Simon's and the carbon disulfide tests for secondary amines.[13]

A 1×10^{-4} molar solution of achilleine in 95% alcohol did not absorb ultraviolet light in the range 220 to 300 mμ, measured with a Beckman model DU spectrophotometer. The infrared absorption of achilleine, determined on a Nujol mull with a Perkin–Elmer double beam spectrophotometer, possessed only a rather broad band at 6.1 μ, other than the bands due to the Nujol in the range 3.0 to 8.0 μ. This band was identified as the amide carbonyl stretching band.[14] The optical rotation of the material melting at 247–248° is $[\alpha]_D$ −14.3° (water, *c* 10).

Hydrolysis of Achilleine.—A solution of 100 mg. of achilleine dissolved in 10 ml. of 5% sulfuric acid was refluxed over a small flame for 30 hours. No apparent change occurred during this time, and no evidence of hydrolysis could be obtained. A majority of the alkaloid was recovered unchanged.

A solution of 500 mg. of achilleine dissolved in 4 ml. of water and 1 ml. of 10% sulfuric acid was sealed in a tube (0.5 × 10 cm.) and heated in an oil-bath at 150–160° for five hours. The solution remained clear, and on cooling the tube was opened and the contents made alkaline with dilute sodium hydroxide. The solution was then steam distilled and ammonia was detected in the distillate by Nessler reagent. In another experiment the distillate was collected in dilute hydrochloric acid from which ammonium chloride was obtained on evaporation. The crystalline material was washed with absolute alcohol to give a white scale-like powder decomposing at 170°. The hydrochloride gave a negative carbylamine test.

Anal. Calcd. for NH_4Cl: N, 26.18; H, 7.53. Found: N, 25.67; H, 7.40.

The vapors of the distillate gave a positive furfural test when allowed to come into contact with aniline acetate paper.[15]

The residue from the steam distillation was neutralized with dilute sulfuric acid and extracted with ether. Evaporation of the ether solution gave no tractable material. The aqueous layer was concentrated to a small volume and tested for sugar. It gave a negative test with Fehling solution, but the same violet color in the Molisch test as before hydrolysis. The aqueous solution was then evaporated to dryness and extracted with alcohol to separate ionic material. The residue obtained when the alcohol extracts were evaporated to dryness was crystallized from alcohol and found to be recovered achilleine.

During the recrystallization of the recovered achilleine, a much less alcohol-soluble material, m.p. 235–238°, was obtained. This substance, which depresses the melting point of achilleine, was found to contain sodium. It gives a positive pine splinter test and slowly reduces potassium permanganate.

BALTIMORE 1, MARYLAND

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[11] Obtained through the courtesy of Rohm and Hass Company, Philadelphia, Pa.

[12] C. S. Cleaver, R. A. Hardy and H. G. Cassidy, THIS JOURNAL, **67**, 1343 (1945).

(13) A. I. Vogel, "A Textbook of Practical Organic Chemistry," Longmans, Green, London, 1948, p. 413.

(14) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand, New York, N. Y., 1949.

(15) S. P. Mulliken, "A Method for the Identification of Pure Organic Compounds," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1904, p. 33.