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The Alkaloids of Crotalaria juncea

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From *Crotalaria juncea* seed by ethanolic extraction, riddelline, seneciphylline and senecionine have been isolated and identified. By subsequent methanolic extraction, a new alkaloid designated as junceine was obtained together with trichodesmine, previously reported as the alkaloid in *Trichodesma incanum*. An optically inactive amino acid also was present, which was identified as β-hydroxy-N-methyl-DL-norvaline A.

Ethanolic extraction of seed of *Crotalaria juncea* gave a 0.003% yield of alkaloidal material. A preliminary investigation of the mixture by paper chromatography indicated the presence of three components, R_f 0.40, 0.58 and 0.62, respectively. Since separation of Senecio alkaloids having R_f values comparable to these has been achieved previously in this Laboratory[1] by means of partition chromatography, the same procedure was used on the present mixture. Three alkaloids were separated and proved to be riddelline, seneciphylline and senecionine, respectively.

When the plant material, already exhausted with ethanol, was extracted with methanol, two alkaloids were indicated by a paper chromatogram, R_f 0.38, 0.54. The infrared spectrum of the crude methanolic extract showed the presence of a carboxyl (zwitterion) band. The crude material was therefore extracted with water in order to separate the compound having salt-like character from the other components of the mixture. A compound was thus isolated whose presence had not been indicated by developing with iodine a preliminary papergram of the total methanolic extract. This compound proved to be an optically inactive amino acid, $C_6H_{13}NO_3$. The methanol-soluble material from which the amino acid had been extracted was chromatographed on Florisil. Two products were separated. The empirical formulas, the yields and the physical properties of the three products present in this methanolic extract are given in Table I.

TABLE I

METHANOLIC EXTRACTS OF *Crotalaria juncea*

Compd.	Empirical formula	M.p., °C.	$[\alpha]_D$	R_f (BuOH–AcOH)[c]	Wt., g.[d]
A	$C_{18}H_{27}NO_7$	191–192	−3.0°[a]	0.38	0.9
B	$C_{18}H_{27}NO_6$	160–161	+38.2[b]	.54	.7
C	$C_6H_{13}NO_3$	227–228	0	..	.2

[a] Pyridine. [b] 95% ethanol. [c] The solvent consists of the upper layer obtained after shaking equal volumes of *n*-butyl alcohol with 5% aqueous acetic acid. [d] From 200 lb. of seed.

From an ethanolic extraction of a second batch of *Crotalaria juncea* seed, only a very small amount of compound A was obtained and no riddelline, seneciphylline, senecionine or compounds B or C could be detected. However, methanolic extraction of the material already exhausted with ethanol yielded compound A accompanied by compound C. The variation in the results from the two batches of seed may be due to the stage of growth, season and location of collection.[2–5]

Compound A.—An alkaloid, jacoline, m.p. 221°, $[\alpha]_D$ +48° ($CHCl_3$), R_f 0.26, with the same empirical formula as that of compound A, has been isolated from *Senecio jacobea* L.[6] Since jacoline is soluble in chloroform and compound A when pure is not, and since the melting points and optical rotations of the two products are different, it may be deduced that they are not identical. The comparison of the R_f values of compound A and jacoline, with the R_f value of the alkaloid jacobine as a standard (Table II), is further evidence that compound A and jacoline are isomers.

TABLE II

R VALUES OF ALKALOIDS[a]

Alkaloids	Empirical formula	$Rf^{(1)}$	$Rf^{(2)}$	$Rm^{(1)}$	$Rm^{(2)}$
Jacoline	$C_{18}H_{27}NO_7$	0.26	..	0.67	..
Compound A	$C_{18}H_{27}NO_7$	..	0.38	..	0.86
Jacobine	$C_{18}H_{25}NO_6$	.39	.44	..	..

[a] $Rf^{(1)} = R_f$ as determined by Bradbury and Culvenor,[6] $Rf^{(2)} = R_f$ as determined during the present investigation, $Rm^{(1)} = Rf^{(1)}$ of jacoline/$Rf^{(1)}$ of jacobine, $Rm^{(2)} = Rf^{(2)}$ of compound A/$Rf^{(2)}$ of jacobine. The R_f values determined during this investigation were measured at 24 ± 1° using the descending technique and butanol–acetic acid as the solvent. Bradbury and Culvenor used the ascending technique and the same solvent system. The values obtained by the technique used in this Laboratory are consistently a little higher than those reported by the Australian workers.

De Waal[7] has also reported the isolation from *Senecio sceleratus* of an alkaloid, $C_{18}H_{27}NO_7$, m.p. 178°, $[\alpha]^{21}D$ ±54.0° (EtOH), which he called sceleratine.[8] It is different from compound A, as indicated by melting point and rotation. Moreover, it is reported to be extremely soluble in organic solvents whereas compound A has a low solubility.

Since compound A appears to be a new alkaloid, the name junceine is suggested for it.[9]

(1) R. Adams and M. Gianturco, THIS JOURNAL, **78**, 398 (1956).

(2) R. H. F. Manske, *Can. J. Res.*, **14B**, 6 (1936).

(3) G. Barger and J. J. Blackie, *J. Chem. Soc.*, 584 (1937).

(4) N. F. Richardson and F. L. Warren, *ibid.*, 452 (1943).

(5) G. Barger and J. J. Blackie, *ibid.*, 743 (1936).

(6) R. B. Bradbury and C. C. J. Culvenor, *Australian J. Chem.*, **7**, 378 (1954).

(7) H. L. De Waal and T. P. Pretorius, *Onderstepoort J. Vet. Sci. Ani. Ind.*, **17**, 181 (1941).

(8) The purity of the product is somewhat questionable if judged by the analyses. Calcd. for $C_{18}H_{27}NO_7$: C, 58.52; H, 7.37; N, 3.79. Found by De Waal: C, 58.02, 58.14; H, 7.25, 7.03; N, 4.8.

(9) The term "tricrotaline" might also have been selected, were it not for the fact that H. L. De Waal and A. Crous, *J. South African Chem. Inst.*, **I**, 29 (1948), have indicated that Marais and Smit were to use the name tricrotalic acid for a substance that obviously must be derived from an alkaloid, tricrotaline.

Compound B.—A comparison of the melting point and rotation of compound B and the melting point of its methiodide with those of trichodesmine and its methiodide, respectively, indicated the probable identity of the two alkaloids. Trichodesmine was isolated by Menshikov and Rubinstein from *Trichodesma incanum*,[10] but the structure of the alkaloid was not determined. An investigation now being conducted in this Laboratory has demonstrated trichodesmine to be a derivative of glutaric acid, thus establishing its resemblance to the alkaloids obtained from other Crotalaria species.[11] Riddellic acid (from riddelline), seneciphyllic acid (from seneciphylline) and senecic acid (from senecionine) are substituted adipic acids. Acids of this latter type are common in the alkaloids obtained from plants of the Senecio genus. The finding in *Crotalaria juncea* of alkaloids with acid moieties derived from both adipic and glutaric acids is a matter of interest. Results of the investigations on the structures of trichodesmine and junceine will be reported shortly.

Compound C.—This compound is an amino acid as deduced from its analysis, physical properties and the presence in its infrared spectrum of bands at 2380 and 1620 cm.$^{-1}$. No bands were found in the 1600–1500 cm.$^{-1}$ region. If an NH_3^+ group were present in the product, bands would be expected in this region, but if an NH_2^+ were present no such bands would be found.[12] It may thus be deduced that there is a substituent present on the amino group in compound C. This fact was confirmed by a positive test for a secondary amine.[13] An alcoholic hydroxyl group was demonstrated by the presence of a band at 3450 cm.$^{-1}$.[14]

Compound C was subjected to treatment with two rather specific reagents. A quantitative oxidation with periodic acid showed a slow reaction between one mole of reagent and one mole of compound C.[15] This slow rate might be expected of a compound containing a secondary, rather than a primary, amino group and a hydroxyl group on adjacent carbon atoms. In a quantitative oxidation with lead tetraacetate in acetic acid, the consumption of two moles of reagent was observed, thus suggesting that one hydroxyl and one primary or secondary amino group are attached to successive carbon atoms adjacent to a carbonyl function.

Compound C melts at 227–228° dec. and forms a copper salt, m.p. 219–220°. These constants are identical with those of β-hydroxy-N-methyl-DL-norvaline A which has been reported.[16] Moreover, the ratio of the R_f value of compound C to that of β-hydroxy-DL-norvaline A was identical with the ratio of the R_f value of β-hydroxy-N-methyl-DL-norvaline A, $CH_3CH_2CHOHCH(NHCH_3)CO_2H$, to that of β-hydroxy-DL-norvaline A as calculated from the data obtained by Izumiya.[17]

A direct comparison of compound C with β-hydroxy-N-methyl-DL-norvaline A was made possible through the kindness of Dr. Izumiya who provided us with a sample from his laboratory. The infrared spectra of the two compounds were identical and a melting point of the mixture showed no depression.

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Experimental

Extraction and Isolation of the Alkaloids.—Two lots of seed of *Crotalaria juncea* (designated as Lot A and Lot B) were extracted in a Brighton solid–liquid extractor. All the samples were given a preliminary treatment with light petroleum ether. No basic material was present in the petroleum ether.

Ethanolic Extraction of a Sample of Lot A.—Ground seed (100 lb.) was placed in the percolation unit of the solid–liquid extractor, covered with 95% ethanol, and allowed to stand for four days. Solvent was then added to the solvent boiler and the reflux started. Cold solvent was allowed to percolate through the plant material for 18 hours. The liquid extract was then distilled until about 8 l. remained. This was removed from the solvent boiler, and concentrated further under reduced pressure to a small volume. After acidification to congo red with a 2 *N* solution of citric acid, it was subjected to steam distillation to remove the adhering solvent. The aqueous solution (about 1–2 l.) remaining in the steam distillation flask contained much suspended matter and was therefore diluted with three times its volume of water and set aside for 48 hours. The clarified solution was then decanted from the separated resins, which were thoroughly washed with 2 *N* aqueous citric acid. The aqueous solutions were reunited, continuously extracted with ether for 24 hours to remove any traces of resin, and then brought successively to *p*H 7.5, 8.5 and 10 with aqueous ammonia. Papergrams of samples from the chloroform extracts of the aqueous solution at each of the different *p*H values indicated the presence in all three of them of the same products (R_f of 0.40, 0.58 and 0.62) in approximately the same ratios. The chloroform extracts were reunited and the solvent was removed under reduced pressure. The residue, weighing 1.35 g., was treated with 10 ml. of absolute methanol and the solvent was eliminated again to remove all traces of chloroform. Washing the residue with a little absolute methanol now caused crystals to appear. These weighed 0.85 g. (R_f 0.40, 0.58 and 0.62) and were separated from the colored solution by filtration. The liquid was evaporated to dryness under reduced pressure; the residue was dissolved in 15 ml. of chloroform and stirred for a few minutes with 1.5 g. of alumina (Merck, suitable for chromatographic adsorption). The alumina was allowed to settle and the chloroform was decanted through a filter paper. The alumina was then stirred with two more 15-ml. fractions of chloroform. Finally one treatment was performed with 40 ml. of chloroform containing 30% of its volume of propanol. This chloroform–propanol extract was evaporated separately and contained merely a very small amount of resinous material.

Evaporation of the chloroform extracts yielded 0.2 g. of a white powder (R_f 0.40, 0.58 and 0.62) which was added to the 0.85 g. previously separated.

Separation of Compounds of R_f 0.40, 0.58 and 0.62.—A chromatographic column was prepared by the usual procedure[1] using 80 g. of Celite 545. A solution of 1.0 g. of the alkaloidal mixture in a few milliliters of chloroform was poured over 2 g. of Pyrex glass powder and the solvent evaporated. The glass powder was packed on top of the

(10) G. P. Menshikov and N. Rubinstein, *Ber.*, **68**, 2039 (1935).

(11) R. Adams, P. R. Shafer and B. H. Braun, THIS JOURNAL, **74**, 5612 (1952); R. Adams and B. L. Van Duuren, *ibid.*, **75**, 2377 (1953).

(12) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen Co., London; Ltd., John Wiley and Sons, Inc., New York, N. Y., 1954.

(13) F. Feigl, "Spot Tests," Vol. II, Elsevier Publishing Co., New York, N. Y., 1954, p. 189.

(14) No absorption in the usual NH stretching region, 3500–3300 cm.$^{-1}$, is shown by any amino acid.[12]

(15) Periodic acid oxidation is applicable to compounds having two hydroxyl groups or one hydroxyl and one primary or secondary amino-group attached to adjacent carbon atoms, but not to α-hydroxy or α-amino acids.

(16) N. Izumiya, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **72**, 702 (1951).

(17) N. Izumiya, *ibid.*, **72**, 784 (1951).

column and elution was started with a 7:3 mixture of carbon tetrachloride and chloroform. Fractions of 5 ml. were collected. The results obtained are indicated in Table III.

TABLE III

SEPARATION OF ALKALOIDS FROM *Crotalaria juncea*

Fractions	R_f value (BuOH–AcOH)[a]	Res. on evap., g.
38–43	0.58, 0.62	0.31
44–45	..	None
46–90	0.40	0.50

[a] Table I, note *c*.

Fractions 38–43 were combined and chromatographed again according to the procedure described[1] for the separation of the alkaloids in "hieracifoline." Senecionine and seneciphylline were obtained in approximately 1:1 ratio.

Anal. Calcd. for seneciphylline, $C_{18}H_{23}NO_5$: C, 64.85; H. 6.95; N, 4.20. Found: C, 64.71; H, 7.03; N, 4.40. Rotation: 0.0412 g. made up to 2 ml. in chloroform at 25° gave α_D −2.58°, *l* 1; $[\alpha]^{25}D$ −125.2°.

Anal. Calcd. for senecionine, $C_{18}H_{25}NO_5$: C, 64.46; H, 7.51; N, 4.18. Found: C, 64.60; H, 7.83; N, 4.44. Rotation: 0.070 g. made up to 2.0 ml. in chloroform at 25° gave α_D −1.95°, *l* 1; $[\alpha]^{25}D$ −55.7°.

Fractions 46–90 were united after infrared determinations on several separate fractions indicated that only one compound was present. Crystallization from ethanol yielded 0.4 g. of white crystals, m.p. 195–196°.

Anal. Calcd. for riddelline, $C_{18}H_{23}NO_6$: C, 61.92; H, 6.64; N, 4.01. Found: C, 61.73; H, 6.79; N, 4.11. Rotation: 0.0267 g. made up to 1.5 ml. in chloroform at 25° gave α_D −1.94°, *l* 1; $[\alpha]^{25}D$ −108.9°.

The identity of seneciphylline, senecionine and riddelline was proved by the determination of melting points, melting points of mixtures with authentic samples, optical rotations and R_f values. The infrared spectra were also identical with those of authentic samples.

Methanolic Extraction of a Sample of Lot A.—The material already extracted with ethanol was covered with methanol and the mixture was allowed to stand for 20 days with occasional circulation of fresh solvent through the percolation unit. The concentrated extract was worked up in a way similar to that used for the ethanolic extract and about 1 g. of a white powder (R_f 0.38 and 0.54) was obtained. A determination of the infrared spectrum of the mixture showed the presence in the material of a compound of salt-like character. Therefore, the white powder was washed with water and the water washings evaporated under reduced pressure. The resulting solid product (compound C) weighed 0.1 g. It was purified by crystallization from a mixture of methanol and ethanol; white crystals, m.p. 227–228°. It is optically inactive.

Anal. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52. Found: C, 49.11; H, 8.74; N, 9.54.

A paper chromatogram of the material after separation of the amino acid showed the presence of two compounds, R_f 0.38 and 0.54.

Separation of Compounds of R_f 0.38 and 0.54.—A solution of 0.9 g. of the white powder, obtained after separation of compound C from the methanolic extract, in 50 ml. of chloroform was poured over 10 g. of Pyrex glass powder. The solvent was eliminated and the glass powder was placed on top of a 2.2 × 50 cm. chromatographic column previously packed with Florisil. Petroleum ether (b.p. 30–60°) and a variety of combinations of petroleum ether and carbon tetrachloride, carbon tetrachloride and chloroform failed to elute any product. Chloroform was subsequently used as the eluent and white crystals (R_f 0.54) were obtained from four 50-ml. fractions. Subsequent fractions contained no material. Elution was then carried out with a 4:1 chloroform–propanol mixture. From the first four 50-ml. fractions white crystals (R_f 0.38) were obtained.

The material, R_f 0.54, was crystallized by gradual concentration of a methanolic solution. The product (compound B, trichodesmine) weighed 0.35 g. and formed white crystals, m.p. 160–161°. Rotation: 0.051 g. made up to 1.5 ml. in 95% ethanol at 32° gave α_D + 1.30°, *l* 1; $[\alpha]^{32}D$ +38.2°.

Anal. Calcd. for $C_{18}H_{27}NO_6$: C, 61.17; H, 7.70; N, 3.96. Found: C, 61.31; H, 7.78; N, 3.95.

Methiodide of Compound B.—A solution of 0.078 g. of the alkaloid and an excess of methyl iodide in 0.5 ml. of absolute methanol was heated under reflux for 30 minutes. On cooling, white crystals separated which were purified by crystallization from absolute ethanol, m.p. 202°.

Anal. Calcd. for $C_{18}H_{27}NO_6 \cdot CH_3I$: I, 25.63. Found: I, 25.68.

The material, R_f 0.38, was crystallized by adding ether to a solution in pyridine, The product (compound A, junceine) weighed 0.45 g. and formed white crystals, m.p. 191–192°. Rotation: 0.200 g. made up to 25 ml. in pyridine at 30° gave α_D −0.024°, *l* 1; $[\alpha]^{30}D$ −3°.

Anal. Calcd. for $C_{18}H_{27}NO_7$: C, 58.52; H, 7.37; N, 3.79. Found: C, 58.53; H, 7.38; N, 3.82.

Methanolic Extraction of a Sample of Lot B.—Ethanolic extraction of a sample of Lot B yielded only a very small amount of Compound A. Extraction with cold methanol for 18 days with occasional circulation of the solvent gave 3 g. of a dark resinous material. This was washed with water to remove the amino acid. From the aqueous solution, by the procedure previously described, 0.1 g. of pure β-hydroxy-N-methyl-DL-norvaline-A resulted. The water-insoluble material contaminated with resin was dissolved in 50 ml. of chloroform and poured over 8 g. of Pyrex glass powder. The solvent was eliminated and the glass powder was poured on top of a 2.2 × 40 cm. chromatographic column packed with Florisil. Elution with 600 ml. of a 4:1 mixture of carbon tetrachloride and petroleum ether (b.p. 30–60°) yielded only a slight amount of tarry material. By using a 4:1 mixture of chloroform–propanol as eluent, white crystals (R_f 0.38) were obtained from four 50-ml. fractions. Tarry material remained on top of the column. Crystallization of the white material from pyridine–ether afforded 1.5 g. of compound A (junceine), m.p. 191–192°, undepressed on admixture with a sample obtained from the methanolic extract of Lot A.

Periodic Acid Oxidation of Compound C.—In a 100-ml. erlenmeyer flask was placed 15 mg. (accurately weighed) of compound C. After addition of 7 ml. of *M* aqueous sodium bicarbonate, 50 ml. of 0.02 *M* aqueous periodic acid was run in from a buret. In 5 hours, the oxidation was complete, as indicated by the estimation of the excess of periodic acid by the arsenite method: 5.12 ml. of reagent was consumed; theory for one mole 5.09 ml.

Lead Tetraacetate Oxidation of Compound C.—A solution of 10.5 mg. of compound C in 50 ml. of a 0.01 *N* solution of lead tetraacetate in acetic acid was permitted to stand at room temperature for 12 hours. The oxidation was complete and no further reduction of the reagent was observed as determined by titrations of the excess of lead tetraacetate present in aliquots of the solution by the usual potassium iodide–sodium thiosulfate method: 28.70 ml. of reagent was consumed; theory for two moles, 28.55 ml.

Copper Salt of Compound C.—The salt was prepared by treatment of the hot aqueous solution of the amino acid with copper hydroxide and purified by crystallization from water, m.p. 219–220° dec. Prolonged drying was necessary before analysis.

Anal. Calcd. for $(C_6H_{12}NO_3)_2Cu$: N, 7.87. Found: N, 7.91.

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