[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Crotalaria Alkaloids: the Structure of Junceine

BY ROGER ADAMS AND MAURIZIO GIANTURCO

RECEIVED DECEMBER 23, 1955

Junceine, a new alkaloid isolated from the seed of *Crotalaria juncea* is hydrogenated in the presence of a catalyst to tetrahydrojunceine. This product is a salt of retronecanol and a monobasic acid, junceic acid. The infrared absorption spectra of junceine, tetrahydrojunceine and junceic acid resemble closely those of trichodesmine, tetrahydrotrichodesmine and trichodesmic acid, respectively. By alkaline degradation junceine, like trichodesmine, gives methyl isobutyl ketone Junceine absorbs two mole-equivalents of periodic acid in contrast to trichodesmine which absorbs only one. In this oxidation of junceine, formaldehyde is formed thus indicating the presence of a CH_2OH group. The chemical and physical properties of junceine and its degradation products lead to the conclusion that junceine is an hydroxyl derivative of trichodesmine.

The infrared absorption spectrum of junceine, $C_{18}H_{27}NO_7$, m.p. 191–192°, $[\alpha]D$ − 3° (pyridine), the new alkaloid isolated from the seed of *Crotalaria juncea*,[1,2] shows ester bands at 1720 cm.$^{-1}$, alcoholic hydroxyl bands at 3520 cm.$^{-1}$ and a band at 830 cm.$^{-1}$, probably due to a RR'C=CHR'' grouping (Table I). On hydrogenation with palladium-on-strontium carbonate or platinum oxide as a catalyst, two mole-equivalents of hydrogen were absorbed and the tetrahydro derivative was readily isolated. The infrared absorption spectrum of this product showed absence of ester bands and carbon–carbon double bond bands. The band at 1615 cm.$^{-1}$, not found in the spectrum of junceine, can be accounted for best as a carboxyl (zwitterion) absorption and the band at 2500 cm.$^{-1}$ as indicative of a salt-like structure. There is also a band in the hydroxyl stretching region and one at 1765 cm.$^{-1}$ characteristic of a γ-lactone.

The salt-like character of tetrahydrojunceine was demonstrated by treatment with cold aqueous hydrochloric acid. From the acidic aqueous solution by extraction with ether, a new acid, $C_{10}H_{16}O_6$, was isolated; after making the solution alkaline with concentrated sodium hydroxide and again extracting with ether, the base, retronecanol, was obtained.

The acid, called junceic acid, m.p. 180–182°, is monobasic.[3] The infrared spectrum showed a band at 1742 cm.$^{-1}$ (Nujol mull) or at 1780 cm.$^{-1}$ (tetrahydrofuran)[4,5] indicative of a 5-membered lactone; a band at 1710 cm.$^{-1}$ for a carboxyl carbonyl; bands at 3480 and 3340 cm.$^{-1}$ for alcoholic hydroxyls; no bands for carbon–carbon double bonds.

Junceine was oxidized readily by periodic acid; one mole-equivalent of reagent was consumed in two minutes or less and a total of two mole-equivalents in 20 minutes, but no more reagent was used during a period of three hours. As judged by the behavior of other alkaloids of known structure toward periodic acid,[2] junceine has three hydroxyls on adjacent carbons and one hydroxyl is primary in nature. By distilling the oxidized reaction mixture, formaldehyde was obtained, thus definitely establishing the presence of a $-CH_2OH$ group.

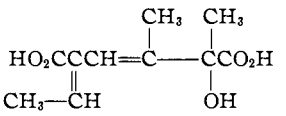
TABLE I

INFRARED ABSORPTION FREQUENCIES[a] (CM.$^{-1}$) OF JUNCEINE AND REACTION PRODUCTS

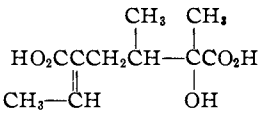
Compound	Junceine	Tetrahydro-junceine	Junceic acid
Alcohol hydroxyl	3520(s)	3220(s), 3340(s)	3340(s), 3480(m)
Salt structure	.....	2300–2600(broad)	.....
Ester C=O	1720(s)	.....	.....
Acid C=O	.....	.....	1710(s)
Lactone C=O	.....	1765(s)	1740(s), 1725(v.w.)
C=C	830(w)	.....	.....
CO_2^-	.....	1615(s)	.....

[a] All spectra were determined in Nujol mull.

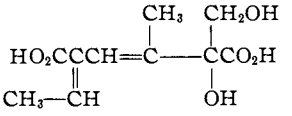
Accompanying junceine in *Crotalaria juncea* are several alkaloids whose acid moieties are substituted adipic or glutaric acids (I, II, III, IV).



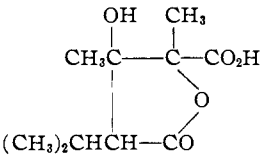
I, seneciphyllic acid[6]



II, senecic acid[7a]



III, riddellic acid[7b]



IV, trichodesmic acid[2]

The alkaloid, trichodesmine, shown in V as a derivative of the open form of trichodesmic acid, gives methyl isobutyl ketone on treatment with alkali.[2,8] Methyl ethyl ketone is obtained in a similar manner from monocrotaline.[2] Treatment of junceine with alkali under similar conditions gave methyl isobutyl ketone, thus establishing junceine as closely related to trichodesmine with which it occurs in the same plant. The most probable structure of junceine which accounts for the results of the alkaline degradation, the periodic acid oxidation and the spectral data is shown in VI.[9]

(1) R. Adams and M. Gianturco, THIS JOURNAL, **78**, 1919 (1956).

(2) R. Adams and M. Gianturco, *ibid.*, **78**, 1922 (1956).

(3) The titration method used was a modification of that described by J. Radell and E. T. Donahue, *Anal. Chem.*, **27**, 590 (1954).

(4) R. Adams, P. R. Shafer and B. H. Braun, THIS JOURNAL, **74**, 5612 (1952) found that the 5-membered lactone carbonyl absorption in the epimeric monocrotalic acids is abnormally low (1740–1750 cm.$^{-1}$) when the spectrum is determined in Nujol mull, but in the normal range (1760–1780 cm.$^{-1}$) when determined in solution. Similar results were found with trichodesmic acid.

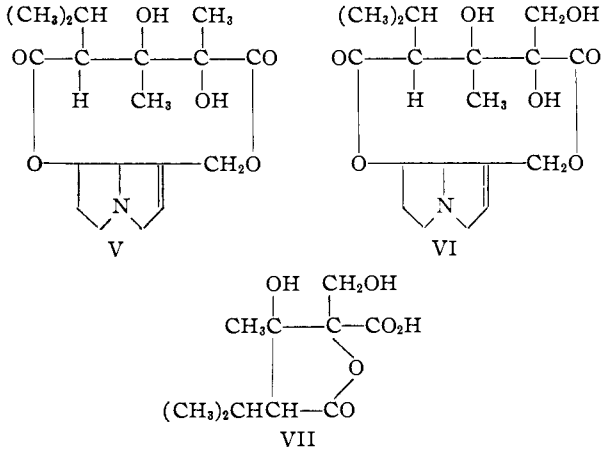
(5) A weak absorption at 1725 cm.$^{-1}$ (Nujol mull) or at 1750 cm.$^{-1}$ (tetrahydrofuran) probably indicates the presence of a small amount of 6-membered lactone.

(6) R. Adams, T. R. Govindachari, J. H. Looker and J. D. Edwards, THIS JOURNAL, **74**, 700 (1952).

(7) (a) M. Kropman and D. L. Warren, *J. Chem. Soc.*, 2852 (1949); 700 (1950); (b) R. Adams and B. L. Van Duuren, THIS JOURNAL, **75**, 4638 (1953).

(8) G. M. Menshikov and N. Rubinstein, *Ber.*, **68**, 2039 (1935).

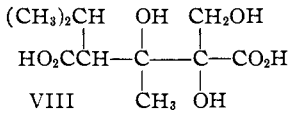
(9) Another possible structure analogous to that discussed and rejected for trichodesmine (ref. 2, footnote 10a), has not been considered because it does not obey the isoprene rule.



V VI

VII

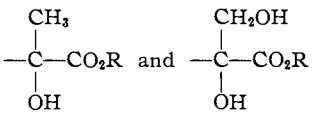
On the basis of structure VI, junceic acid must be very similar to trichodesmic acid (IV) and hence would have structure VII. The presence of a trace of 6-membered ring lactone in junceic acid, as indicated by the infrared spectrum of junceic acid, could arise from lactone formation in the open chain acid VIII, if the carboxyl at the left lactonized with the primary hydroxyl group.



VIII

The 5-membered lactone VII, however, would be the more favored in a molecule of this kind.

It is thus obvious that trichodesmine and junceine form another pair of alkaloids differing from each other only by the two groupings



Other parts which have the same relationship are represented by seneciphylline[6] and riddelline[7b] and by senecionine[7a] and retrorsine.[10] The R_f values of the two alkaloids in each pair differ from each other by a practically constant quantity. This difference is due to the contribution of the primary hydroxyl group to the partition coefficient of the alkaloids between stationary and mobile phase.

The stereochemistry of the three asymmetric carbons in the acid moiety of junceine (VII) has not been determined. Moreover, the two carboxyls might be interchanged in their ester formation with the two hydroxyls of retronecine. The ester linkages in VI, however, conform to those found in other Senecio alkaloids of which the constitution has been established.[7a,b] In these, the carboxyl group that has the stronger ionization constant undoubtedly esterifies the primary hydroxyl of the necine.

Acknowledgment.—The authors are indebted to Mr. J. Nemeth and Mrs. M. Benassi for the microanalyses and to Mr. J. Brader for the infrared spectra.

(10) E. L. Leisegang and F. L. Warren, *J. Chem. Soc.*, 702 (1950).

Experimental

All melting points are corrected.

Tetrahydrojunceine.—A solution of 0.138 g. of junceine in 39.5 ml. of 90% ethanol was hydrogenated at room temperature and atmospheric pressure in the presence of 0.05 g. of palladium-on-strontium carbonate catalyst (6%). Two moles of hydrogen were absorbed in one hour. The solution was filtered, the catalyst washed with ethanol and the combined ethanol solutions evaporated to dryness in a vacuum. The oily residue after treatment with a little dry acetone, solidified. It was crystallized from dry acetone, m.p. 150°, yield 0.110 g. (80%). Rotation: 0.058 g. made up to 1.5 ml. with 66% ethanol at 28° gave α_D −0.18°, *l* 1; $[\alpha]^{28}D$ −4.7°.

Anal. Calcd. for $C_{18}H_{31}NO_7$: C, 57.89; H, 8.37; N, 3.75. Found: C, 58.04; H, 8.41; N, 3.60.

The same product was obtained when the hydrogenation was conducted with a platinum catalyst. A solution of 0.20 g. of junceine in 30 ml. of ethanol and 2 ml. of acetic acid over 0.50 g. of platinum oxide catalyst adsorbed two mole equivalents of hydrogen in ten minutes.

Separation of the Basic and Acid Constituents of Tetrahydrojunceine.—An aqueous solution of tetrahydrojunceine was treated with one mole-equivalent of 0.1 *N* hydrochloric acid and the solution was continuously extracted with ether in a semi-micro extraction apparatus, built according to Wayman and Wright.[11] Evaporation of the ether left a white crystalline material mixed with reddish impurities. Crystallization from tetrahydrofuran and petroleum ether (b.p. 30–60°) yielded an oil, which crystallized with difficulty. Recrystallization yielded white crystals, m.p. 182° dec. with sintering at 180°.

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.69; H, 6.99.

Microtitration of Junceic Acid.—A solution of 16 mg. of junceic acid in 10 ml. of methanol–benzene (1:3) was titrated with a 0.5 *N* solution of sodium methoxide in the same solvent mixture, with blue thymol as an indicator; calcd. amount of 0.01885 *N* $NaOCH_3$ for one carboxyl group, 3.72 ml., found 3.89 ml.

The acid solution was filtered from reddish impurities, made alkaline with sodium hydroxide and continuously extracted with ether in the same semimicro extraction apparatus. The ethereal extract was diluted with more ether, dried over sodium sulfate, and the ether was evaporated. The oily residue crystallized after drying in a stream of nitrogen. A micro-distillation in vacuum afforded a white crystalline product, m.p. 95–96°, which proved to be retronecanol.

Anal. Calcd. for $C_8H_{15}NO$: C, 68.04; H, 10.71. Found: C, 67.89; H, 10.52.

Periodic Acid Oxidation of Junceine.—The procedure for the oxidation with periodic acid was the same as that outlined in a previous paper.[2] Junceine absorbed one mole of reagent in less than 2 minutes, two moles in 20 minutes and 2.1 moles in 24 hours.

Formaldehyde from the Periodic Acid Oxidation of Junceine.—A solution of 0.200 g. of junceine in 7 ml. of 0.02 *M* periodic acid was prepared in a distillation flask. The solvent was distilled through a Holzman column and the distillate was collected in a saturated aqueous solution of dimedone. After a 12-hour standing at room temperature in a stoppered flask, the solution was filtered and the white solid, m.p. 188–189°, identified as the dimedone derivative of formaldehyde.

Methanolic Alkaline Hydrolysis of Junceine.—A mixture of 0.403 g. of junceine and a solution of 0.6 g. of potassium hydroxide in 3.8 ml. of methanol was heated under reflux until all the alkaloid was in solution. Soon potassium carbonate began to separate. The suspension was filtered and the solvent evaporated to dryness in a vacuum. The residue was extracted with anhydrous ether, the ether was evaporated and the residue was crystallized from acetone. The product was retronecine as shown by melting point, analysis and comparison with an authentic sample. Attempts to obtain a necic acid failed.

Anal. Calcd. for $C_8H_{15}NO_2$: C, 61.93; H, 8.38; N, 9.03. Found: C, 61.75; H, 8.69; N, 9.14.

(11) M. Wayman and G. F. Wright, *Ind. Eng. Chem., Anal. Ed.*, **17**, 55 (1945).

Methyl Isobutyl Ketone, a Product of Alkaline Hydrolysis of Junceine.—A solution of 0.20 g. of junceine in 5 ml. of 10% aqueous sodium hydroxide was heated under reflux for one hour. The solvent was boiled off and the distillate collected in four 1.2-ml. fractions. The first three fractions gave a positive sodium nitroprusside test for methyl ketones.[12] They were reunited and dinitrophenylhydrazine reagent[13] was added. After 15-minutes standing at room temperature, the liquid was carefully pipetted off and the residue was crystallized from ethanol; yellow crystals, m.p. 95°. The yield of crude material was 0.085 g. (56%).

Anal. Calcd. for $C_{12}H_{18}N_4O_4$: C, 51.42; H, 5.75. Found: C, 51.34; H, 5.60.

No depression of melting point was observed on admixture with an authentic sample of the 2,4-dinitrophenylhydrazone of methyl isobutyl ketone. The infrared spectra of the two products were also identical.

R_f Value Determinations.—The R_f values of several Senecio alkaloids have been determined before in this Laboratory using butanol–5% acetic acid as the mobile phase.[1,14] However, since the value for retrorsine had not been previously determined, the R_f values of all the alkaloids referred to in this communication were redetermined at the same time. The results are shown in Table II.

TABLE II

Alkaloid	Formula	R_f	ΔR_f
Senecionine	$C_{18}H_{25}NO_5$	0.62	0.18
Retrorsine	$C_{18}H_{25}NO_6$	.44	
Seneciphylline	$C_{18}H_{23}NO_5$	.58	.18
Riddelline	$C_{18}H_{23}NO_6$	.40	
Trichodesmine	$C_{18}H_{27}NO_6$	.54	.16
Junceine	$C_{18}H_{27}NO_7$	.38	

(12) F. Feigl, "Spot Tests"; Elsevier Publishing Co., New York, N. Y., 1954, p. 160.

(13) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171.

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

URBANA, ILLINOIS

[CONTRIBUTION FROM THE KETTERING-MEYER LABORATORY, SOUTHERN RESEARCH INSTITUTE, AFFILIATED WITH SLOAN-KETTERING INSTITUTE]

Synthesis of Potential Anticancer Agents. I. Chloropurines[1]

BY JOHN A. MONTGOMERY

RECEIVED NOVEMBER 11, 1955

2-Chloro-4,5-diaminopyrimidine, 6-chloro-4,5-diaminopyrimidine and 2,6-dichloro-4,5-diaminopyrimidine each react readily with ethyl orthoformate–acetic anhydride to give a mixture of the corresponding chloropurine and the N-acetylchloropurine. Since the N-acetylchloropurines are readily hydrolyzed by base to the chloropurines, this unique procedure has preparative value. The infrared spectra of these compounds are discussed.

All the chloropurines which appear in the literature have been prepared by chlorination of purinones with phosphorus oxychloride with or without using a tertiary amine, such as dimethylaniline.[2] Not only do the procedures used and the yields obtained vary widely, but the desired chloropurines are not always obtained.[3]

Attempts to convert chloro-4,5-diaminopyrimidines to the corresponding chloropurines using conventional reagents and procedures, *i.e.*, anhydrous formic acid, formamide, etc., have all failed,[2d,4] due to the hydrolysis of the chlorine atoms to hydroxyl groups. Robins found that in the case of the monochloro-4,5-diaminopyrimidines and formic acid, this hydrolysis takes place during formylation of the amino group and in the case of the 2,6-dichloro-4,5-diaminopyrimidine during the cyclization step.[4b] In either case the other product of the over-all reaction, water, must be formed and, therefore, the hydrolysis cannot be prevented.

A new approach to the synthesis of chloropurines involves the use of ethyl orthoformate, a reagent which has found application in the formation of other heterocyclic systems.[5] Since this work was begun, Richter and Taylor[6] have synthesized hypoxanthine by treating aminomalonamamidine dihydrochloride with ethyl orthoformate–acetic anhydride, closing both the pyrimidine and imidazole ring in one step. Albert, Brown and Cheeseman[7] found ethyl orthoformate very effective when coupled with acetic anhydride, in the preparation of 4-hydroxypteridine.

It has now been found that although a chloro-4,5-diaminopyrimidine reacts very slowly with ethyl orthoformate alone, it reacts readily with ethyl orthoformate–acetic anhydride to form a mixture of the chloropurine and N-acetylchloropurine.

Although the N-acetylpurines may be recrystallized unchanged from organic solvents such as ethyl acetate, recrystallization of N-acetyl-2-chloropurine from boiling water resulted in a complex reaction giving approximately equal amounts of 2-chloropurine and another compound, identified tentatively as 2-hydroxy-4-amino-5-formylaminopyrimidine on the basis of its ultraviolet and infrared spectra. The effect of hot water on N-acetyl-2-chloropurine, N-acetyl-6-chloropurine and N-acetyl-2,6-dichloropurine was not investigated further since it was found that if the N-acetylpurines are dissolved in warm 10% sodium hydroxide solu-

(1) This work was supported in part by funds from the C. F. Kettering Foundation.

(2) (a) E. Fisher, *Ber.*, **30**, 549, 2226 (1897); **32**, 435 (1899); (b) R. R. Adams and F. C. Whitmore, THIS JOURNAL, **67**, 1271 (1945); (c) J. Davoll and B. A. Lowy, *ibid.*, **73**, 2936 (1951); (d) A. Bendich, P. J. Russell and J. J. Fox, *ibid.*, **76**, 6073 (1954).

(3) R. K. Robins and B. E. Christensen, *J. Org. Chem.*, **16**, 324 (1951); THIS JOURNAL, **74**, 3624 (1952).

(4) (a) R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, *ibid.*, **75**, 263 (1953); (b) R. K. Robins, K. J. Dille and B. E. Christensen, *J. Org. Chem.*, **19**, 930 (1954).

(5) E. Schipper and A. R. Day, THIS JOURNAL, **73**, 5672 (1951); **74**, 350 (1952).

(6) E. Richter and E. C. Taylor, *Angew. Chem.*, **67**, 303 (1955).

(7) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 475 (1951).