

The published data for glycine^{12,13} illustrate this quite satisfactorily, and we have obtained similar data for ammonia and veronal. On the basis of these results the nature of the activation by these simple organic compounds appears to differ from that caused by proteins, but the parathyroid hormone, even in the high concentrations employed (as much as 75 units per ml. of digest solution) has no individual and characteristic effect of its own.

Experimental

A concentrate of "alkaline" phosphatase was prepared from beef kidney cortex by the method of Albers and Albers.¹⁴ The resulting solution was evaporated to dryness in the frozen state; such preparations are stable for months at 5°.

The digest solutions were 0.025 *M* in borate buffer of

(12) Bodansky, *J. Biol. Chem.*, **115**, 101 (1936).

(13) Williams and Watson, *ibid.*, **135**, 337 (1940).

(14) Albers and Albers, *Z. physiol. Chem.*, **232**, 189 (1935).

pH 9.75, 0.0005 *M* in magnesium chloride, and 0.015 *M* in sodium glycerophosphate (52% β); the enzyme was present in a concentration of 0.004%. The thoroughly dialyzed protein solutions were adjusted to pH 9.75 immediately before incorporation in the above mixture. The total volume was 10 ml.; hydrolysis was allowed to proceed at 30° for sixty minutes. The final values of pH were 9.55 \pm 0.05 unit. Inorganic phosphate liberated during the digestion was estimated by the method of Fiske and Subbarow.¹⁵ Control experiments in the presence of each of the three proteins demonstrated that no inorganic phosphate appeared if enzyme or substrate was omitted.

Summary

The parathyroid hormone, like two other typical proteins, egg and serum albumins, accelerates the liberation of phosphate by kidney phosphatase. It has no peculiar effect which can be attributed to its function as a hormone.

(15) Fiske and Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).

BOSTON AND CAMBRIDGE, MASSACHUSETTS

RECEIVED SEPTEMBER 1, 1942

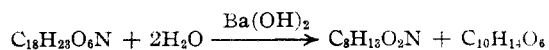
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Structure of Riddelline, the Alkaloid in *Senecio Riddellii*. I

BY ROGER ADAMS, K. E. HAMLIN, JR., C. F. JELINEK AND R. F. PHILLIPS

The similarity of the alkaloids occurring in the plant genera *Senecio*, *Trichodesma*, *Heliotropium*, and *Crotalaria* has previously been discussed.¹ As was pointed out, these alkaloids are esters which on alkaline hydrolysis yield an acid and an alkanolamine, retronecine or some other closely related bicyclic base.

As a result of investigations on the alkaloid Riddelline, obtained by ethanolic extraction of *Senecio Riddellii*, it can be stated that this compound possesses the properties characteristic of the group. Riddelline was first isolated from the plant by Manske,² who reported a molecular formula of C₁₈H₂₃O₆N. His directions for extraction and isolation were followed and as a result of analytical data on the pure riddelline, its hydrochloride and methiodide, Manske's formula was confirmed. Hydrolysis of riddelline indicated that this alkaloid was typical of the *Senecio* group. Alkaline cleavage yielded a basic product, which proved to be retronecine, and a crystalline acid, C₁₀H₁₄O₆, designated as riddelic acid.



(1) Adams and Rogers, *THIS JOURNAL*, **61**, 2815 (1939).

(2) Manske, *Can. J. Res.*, **B17**, 1 (1939).

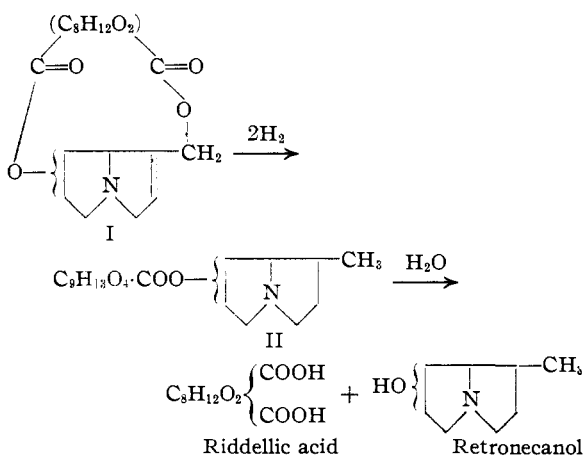
From the above equation it is to be noted that in common with most of the other *Senecio* alkaloids, water enters into the reaction with no loss of carbon dioxide and that the acid product contains ten carbon atoms. The retronecine, which was isolated in a nearly quantitative yield, was identified by comparison with an authentic sample.

Riddelic acid was obtained in both an anhydrous and hydrated form. It is optically active and is shown by direct titration to be dibasic. However, by addition of excess alkali and back titration, evidence for a third carboxyl was found which indicates the probability of the presence of a lactone linkage. The acid formed a dimethyl ester on treatment with diazomethane. Molecular weight determinations in dioxane and benzene indicate that dimethyl riddellate is a monomer, although in the latter solvent apparently one molecule of benzene associates with a molecule of solute. Preliminary hydrogenation experiments show that with a platinum oxide catalyst, two moles of hydrogen are absorbed to give a product which as yet has not been obtained in a pure state. Significantly, when the dimethyl ester of riddelic acid was hydrogenated with platinum oxide, only

one mole equivalent of hydrogen was absorbed to give a nearly quantitative yield of dimethyl dihydorriddellate.

On alkaloids analogous to riddelliine catalytic hydrogenation has also been employed as a degradative procedure. In the case of monocrotaline,¹ hydrogenolysis takes place upon absorption of one molecule of hydrogen followed by absorption of a second molecule and the reduction of the double bond in the basic portion of the molecule. Thus, monocrotalic acid and retronecanol are isolated.

Reduction of riddelliine using Raney nickel resulted in the absorption of two molecules of hydrogen. A single product was isolated in excellent yield, which indicated that no cleavage had occurred. This tetrahydorriddelliine (II), ($C_{18}H_{27}O_6N$), is optically active and possesses the physical properties of an amino acid. Hydrolysis by means of barium hydroxide yielded an acid identical with riddelic acid and a base identified as retronecanol. On the basis of this evidence riddelliine exists as a cyclic diester (I), one molecule of the dibasic riddelic acid being linked to the two hydroxyls of a molecule of retronecine. Thus it is an alkaloid similar in general structure to grantianine.³ The first molecule of hydrogen apparently attacks the labile ester linkage and the second reduces the double bond of the basic moiety. Using the formulas recently proposed for retronecine and retronecanol,⁴ the following equations may be written



If hydrogenation is carried out using a platinum oxide catalyst, somewhat different results are obtained. In this case, four mole equivalents of

hydrogen are rapidly absorbed. The octahydro-riddelliine has not yet been obtained in a pure state. However, alkaline hydrolysis of the crude reduction product has yielded an acid and the base retronecanol. Although the acid has not been purified, the additional two moles of hydrogen must have reacted with the acid portion of the molecule, a fact which agrees with the hydrogenation experiment on riddelic acid itself.

Experimental

Extraction of Riddelliine from *Senecio Riddellii*.—About 1.41 kg. of dried, finely ground *Senecio Riddellii* (entire plant) was extracted by percolation with 95% ethanol over a forty-eight hour period. Most of the solvent was removed *in vacuo* and the concentrate was poured into a large evaporating dish. After acidification to congo red with citric acid, the extract was mixed with water to precipitate the resins and the remaining ethanol was boiled off over a steam-cone. The mixture was then allowed to stand at room temperature overnight to permit caking of the resinous material. The solution was decanted from the resin and extracted with successive portions of ether until the ether extracts were colorless, then with two 50-cc. portions of chloroform. The aqueous solution was now made alkaline to litmus with aqueous ammonia and extracted with successive portions of chloroform until evaporation of the extract left no residue. After distilling off the solvent, 11.3 g. of crude, crystalline riddelliine was obtained. Recrystallization from absolute ethanol yielded 6.4 g. (0.45%) of product, m. p. 193–195° (cor.) with decomposition in an evacuated tube. It was noted that for several experiments, the yield of pure alkaloid varied from 0 to 0.70% depending on the plant material. Further purification from absolute ethanol produced white prisms, m. p. 197–198° (cor.) with decomposition in an evacuated tube.

Anal. Calcd. for $C_{18}H_{23}O_6N$: C, 61.92; H, 6.64; N, 4.01. Found: C, 61.87; H, 6.69; N, 4.00.

Rotation. 0.0889 g. made up to 5 cc. with chloroform at 25° gave $\alpha_D -3.90$; $l, 1$; $[\alpha]^{25}_D -109.5^\circ$.

These results are in satisfactory agreement with the work of Manske² who has reported 196° (cor.) as the melting point of the alkaloid and an analysis corresponding to the formula, $C_{18}H_{23}O_6N$.

Riddelliine Hydrochloride.—A solution of 0.5 g. of riddelliine in a slight excess of 0.1 N hydrochloric acid was evaporated under reduced pressure with gentle heating on the water-bath. The residue was recrystallized from absolute ethanol, m. p. 225–226° (cor.) with decomposition in an evacuated tube.

Anal. Calcd. for $C_{18}H_{24}O_6NCl$: C, 56.03; H, 6.27; N, 3.63. Found: C, 55.86; H, 6.40; N, 3.62.

Rotation. 0.0900 g. made up to 5 cc. with water at 25° gave $\alpha_D -1.45$; $l, 1$; $[\alpha]^{25}_D -80.6^\circ$.

Riddelliine Methiodide.—To a solution of 1 g. of riddelliine in 4 cc. of chloroform and 3 cc. of methanol was added 1.5 cc. of methyl iodide. After warming a few minutes, the mixture was cooled and filtered; yield 1.2 g. After recrystallization from water, the methiodide melts with decomposition at 260–262° (cor.) after beginning to

(3) Adams, Carmack and Rogers, *THIS JOURNAL*, **64**, 571 (1942).

(4) Adams, Carmack and Mahan, *ibid.*, **64**, 2593 (1942); Adams and Hamlin, *ibid.*, **64**, 2597 (1942).

darken at 235°. Manske² has reported the melting point of riddelliine methiodide to be 259° (cor.) with decomposition.

Anal. Calcd. for $C_{19}H_{29}O_4NI$: C, 46.44; H, 5.29; N, 2.85. Found: C, 46.67; H, 5.55; N, 2.82.

Alkaline Hydrolysis of Riddelliine

A. Riddelic Acid ($C_{10}H_{14}O_6$).—A mixture of 5 g. of riddelliine, 10 g. of barium hydroxide octahydrate and 70 cc. of water was heated under reflux for one hour and cooled. The solution was saturated with carbon dioxide and the precipitated barium carbonate filtered off. After acidification to congo with hydrochloric acid, the solution was extracted continuously with ether for seventy-two hours. The ether extract, which contained an appreciable aqueous layer, was evaporated to dryness *in vacuo*. The residue was a viscous orange glass which began to crystallize after standing in a vacuum desiccator at refrigerator temperature. After recrystallization from ether-petroleum ether (b. p. 30–60°), the product was obtained as colorless needles, m. p. 62° (cor.); yield, 3.02 g. (85%).

Anal. Calcd. for $C_{10}H_{14}O_6 \cdot H_2O$: C, 48.40; H, 6.50. Found: C, 48.52; H, 6.63.

Rotation. 0.2038 g. made up to 5 cc. with absolute ethanol at 31° gave $\alpha_D -0.08$; $l, 1$; $[\alpha]^{31D} -1.96^\circ$.

The anhydrous form of riddelic acid resulted if the ether extracts were withdrawn periodically from the continuous extraction, were dried over anhydrous magnesium sulfate and the ether removed *in vacuo*. Also the monohydrate was converted readily into anhydrous riddelic acid by boiling with benzene or by fractional crystallization from ether-petroleum ether. Recrystallization from ether-petroleum ether gave colorless prisms, m. p. 102–103° (cor.).

Anal. Calcd. for $C_{10}H_{14}O_6$: C, 52.17; H, 6.08. Found: C, 52.11; H, 6.21.

Rotation. 0.2644 g. made up to 5 cc. with absolute ethanol at 32° gave $\alpha_D -0.14$; $l, 1$; $[\alpha]^{32D} -2.65^\circ$.

The neutral equivalents for both the hydrated and anhydrous forms of riddelic acid always ran about 5% high for a dibasic acid. However, the values for the hydrate checked satisfactorily those for the anhydrous form.

Hydrogenation of Riddelic Acid (PtO₂ Catalyst).—A solution of 1 g. of riddelic acid monohydrate in 25 cc. of absolute ethanol was hydrogenated at 2–3 atmospheres pressure using 0.05 g. of platinum oxide. Within fifteen minutes, hydrogenation was complete, a total of two mole equivalents being absorbed. After filtering the catalyst and removing the solvent *in vacuo*, a reddish glass remained. This product has not been obtained in a pure state.

Dimethyl Riddellate.—A suspension of 5 g. of riddelic acid monohydrate in 30 cc. of chloroform was treated with an excess of an ethereal solution of diazomethane. During the addition of the diazomethane, the mixture was shaken continuously and the temperature maintained below 10°. The solvents were distilled *in vacuo* leaving a yellow oil which readily distilled at 144–145° (1 mm.); yield, 5.10 g. (98%). This product was redistilled at 0.075 mm. to yield a pale yellow, viscous oil; d_{20}^4 1.171; n_{20}^{20D} 1.4870.

Anal. Calcd. for $C_{12}H_{18}O_6$: C, 55.80; H, 7.03. Found: C, 55.96; H, 6.86.

Rotation. 0.4931 g. made up to 5 cc. with absolute ethanol at 32° gave $\alpha_D -0.28$; $l, 1$; $[\alpha]^{32D} -2.84^\circ$.

Molecular weight determinations by the cryoscopic method in dioxane gave a value of 265; theoretical for dimethyl riddellate is 258. Using benzene as the solvent, a value of 340 was obtained. Assuming a molecule of benzene to be associated, the actual molecular weight would be 262.

Dimethyl Dihydroriddellate.—A solution of 5.1 g. of dimethyl riddellate in 50 cc. of absolute ethanol was hydrogenated at 2–3 atmospheres pressure using 0.1 g. of platinum oxide catalyst. Exactly one mole equivalent of hydrogen was absorbed within one hour and although shaking was continued one-half hour no additional hydrogenation occurred. The catalyst was filtered and the solvent removed *in vacuo*. The pale yellow oil remaining was distilled, b. p. 146–147° (1 mm.). The product was a colorless viscous oil; yield 5.0 g. (97%); n_{20}^{20D} 1.4682; d_{20}^4 1.153.

Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.37; H, 7.84.

Rotation. 0.4577 g. made up to 5 cc. with absolute ethanol at 32° gave $\alpha_D -1.40$; $l, 1$; $[\alpha]^{32D} -15.3^\circ$.

B. Retronecine Hydrochloride.—The aqueous solution, remaining after ether extraction of the riddelic acid, was evaporated *in vacuo* to dryness. The crystalline residue was extracted with boiling absolute ethanol and the extracts concentrated. The crystalline product after recrystallization from absolute ethanol melted at 160–161° (cor.). A mixed melting point with an authentic sample of retronecine hydrochloride showed no depression; yield, 2.5 g. (91%).

Tetrahydroriddelliine.—A solution of 5 g. of riddelliine in 150 cc. of ethanol and 25 cc. of water⁵ was hydrogenated at 2–3 atmospheres in the presence of 3 g. of Raney nickel. After three hours, two mole equivalents of hydrogen had been absorbed and the reduction was complete. The catalyst was filtered off and the solvent removed *in vacuo*. A white crystalline solid remained which was purified by dissolving in a little hot glacial acetic acid and adding absolute ethanol until the solution became cloudy. White microscopic prisms were obtained, m. p. 205° (cor.) with decomposition; yield 4.4 g. (87%).

Anal. Calcd. for $C_{18}H_{27}O_6N$: C, 61.17; H, 7.70; N, 3.96. Found: C, 60.75; H, 7.55; N, 4.05.

Rotation. 0.0944 g. made up to 5 cc. with absolute ethanol at 31° gave $\alpha_D -0.18$; $l, 1$; $[\alpha]^{31D} -9.5^\circ$.

Tetrahydroriddelliine is fairly soluble in water, easily soluble in glacial acetic acid but practically insoluble in ethanol.

When first prepared by hydrogenation of riddelliine in absolute ethanol, the tetrahydroriddelliine was an amorphous solid, very hygroscopic and easily soluble in ethanol. However, after once obtaining the crystalline product, the original experiment could not be duplicated.

Alkaline Hydrolysis of Tetrahydroriddelliine

A. Riddelic Acid.—A mixture of 1 g. of tetrahydroriddelliine, 2 g. of barium hydroxide octahydrate and 15 cc. of water was refluxed for one hour and cooled. After

(5) It is necessary to have sufficient water present to dissolve the tetrahydroriddelliine formed during the reaction. If this precaution is not taken the precipitate formed inactivates the catalyst thus stopping hydrogenation.

saturating with carbon dioxide, the solution was filtered and made acid to congo. Continuous ether extraction for twenty-four hours and removal of the solvent *in vacuo*, yielded an oil which crystallized after seeding with riddelic acid. Recrystallization from ether-petroleum ether (b. p. 30–60°) gave 0.35 g. of a white crystalline solid melting at 61–62° (cor.). A mixed melting point with riddelic acid monohydrate (m. p. 62°) gave no depression.

B. Retronecanol.—The aqueous solution remaining after ether extraction of the acid just described was made alkaline with 10% aqueous sodium hydroxide and extracted with ether. After drying the ether extracts over anhydrous magnesium sulfate and distilling the ether, a solid remained. This product, recrystallized from petroleum ether (b. p. 30–60°), melted at 95–96° (cor.) which is identical with the melting point of retronecanol.

A picrate was prepared from water which melted at 211° with decomposition. A mixed melting point with retronecanol picrate (m. p. 211°) showed no depression.

Hydrogenation of Riddelliine (PtO₂ Catalyst).—A solution of 5 g. of riddelliine in 150 cc. of ethanol and 25 cc. of water was hydrogenated at 2–3 atmospheres pressure using 0.1 g. of platinum oxide catalyst. A total of four mole equivalents of hydrogen was absorbed within one hour. The catalyst was filtered off and the solvent removed *in vacuo*. A white amorphous solid material remained which was very hygroscopic and soluble in ethanol. Attempts to obtain a crystalline product failed.

One gram of this material was taken up in 5 cc. of water and 10 cc. of 50% aqueous sodium hydroxide added. After refluxing one hour and cooling, the mixture was extracted with ether. The ether extract was dried over anhydrous magnesium sulfate and the ether distilled. A solid product remained which after one recrystallization from petroleum ether melted at 95–96°. Retronecanol melts at 95–96°. A picrate of the above base was prepared in ethanol and melted at 213°. A mixed melting point with authentic retronecanol picrate showed no depression.

Acidification and continuous ether extraction of the

aqueous solution remaining after removal of the retronecanol yielded an acid fraction as a brown viscous oil. This has not been obtained in a pure state.

Summary

1. Riddelliine, the alkaloid of *Senecio Riddelli*, has been isolated and shown to have the molecular formula reported by Manske, C₁₈H₂₈O₆N.

2. Upon saponification, riddelliine gives a molecule of retronecine and one molecule of a crystalline acid, C₁₀H₁₄O₆, designated as riddelic acid. It is dibasic and with diazomethane gives a dimethyl ester.

3. Riddelic acid, upon reduction with hydrogen and platinum oxide, absorbs two moles of hydrogen but no pure product was isolated. Dimethyl riddellate under similar conditions absorbs only one mole of hydrogen to give dimethyl dihydorriddellate.

4. Riddelliine, upon reduction with hydrogen and Raney nickel, absorbs two moles of hydrogen to form tetrahydorriddelliine which has the properties of an amino acid and can be hydrolyzed to retronecanol and riddelic acid. With platinum oxide as a catalyst, four moles of hydrogen are absorbed but the product has not been obtained in a pure state. On saponification the crude octahydorriddelliine yields retronecanol.

5. Riddelliine is thus shown to be an ester from one mole of the dibasic acid, riddelic acid, and one mole of retronecine, each of the two hydroxyls in the molecule being utilized.

URBANA, ILLINOIS

RECEIVED SEPTEMBER 18, 1942

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CALCO CHEMICAL DIVISION OF THE AMERICAN CYANAMID COMPANY]

Sulfanilamide Derivatives. VIII. Sulfanilylamidines¹

By E. H. NORTHEY, ALAN E. PIERCE AND D. J. KERTESZ

In view of the fact that most of the recently developed chemotherapeutic agents of high potency, including sulfapyridine, sulfathiazole and sulfadiazine, have the structure of sulfanilyl cyclic amidines, 4-(NH₂)C₆H₄SO₂NHC(=N) it was thought desirable to prepare an analogous series of amidine derivatives of the type 4-(NH₂)-C₆H₄SO₂NHC(R)=NR'. A number of such compounds have been made, however, the structure appears to be better represented by

(1) Presented in part before the Division of Medicinal Chemistry, A. C. S., Buffalo, N. Y., September, 1942.

the formula 4-(NH₂)C₆H₄SO₂N=C(R)NHR', because the monosulfanilylamidines did not form alkali salts corresponding to the well known sodium salts of the above sulfanilamido heterocycles. This would indicate lack of ionizable hydrogen associated with the amide nitrogen. Also, while sulfapyridine is stable to alkaline hydrolysis and is cleaved by mineral acids to sulfanilic acid and 2-aminopyridine,² the sulfanilylamidines are cleaved to sulfanilamide by either

(2) Crossley, Northey and Hultquist, THIS JOURNAL, 62, 372 (1940).