and the ability of gliotoxin to form salts. The high negative specific rotation of gliotoxin (Table IV) showed very interesting changes in alkaline media. These changes must be connected with the observed instability of gliotoxin toward alkali.

The data concerning the action of gliotoxin on bacteria, fungi and higher animals (Tables V, VI and VII) showed variations which depended on the method of testing. Each set of data appeared consistent within itself, and in terms of a standard, such as mercuric chloride, could be related to other sets. The fungicidal action showed more variability than is usually encountered.

Summary

The production of gliotoxin, the powerful bacteriostatic agent of *Gliocladium fimbriatum*, has been described. The empirical formula and molecular weight have been revised. The crystal form, solubility, ultraviolet absorption curve, and optical activity have been determined. It has shown a high degree of bacteriostatic action on a variety of animal and plant pathogenic organisms. Its action on higher animals has been recorded.

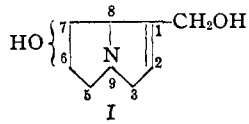
ITHACA, N. Y. RECEIVED JUNE 30, 1943

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

Structure of Monocrotaline. IX. Proof of the Position of the Double Bond in Retronecine[1]

BY ROGER ADAMS AND J. E. MAHAN

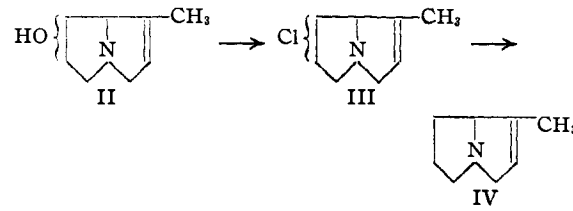
Retronecine, the base obtained along with monocrotic acid by the alkaline hydrolysis of monocrotaline has been postulated[1,2] as having structure I. The presence of a $-CH_2OH$ group



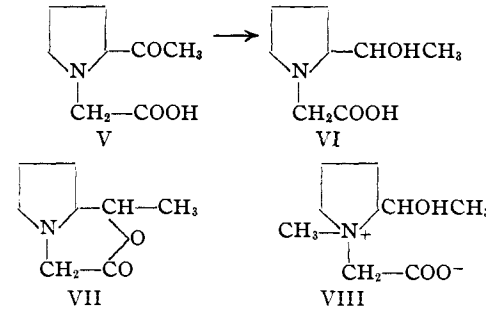
I

in the 1-position[1] of the pyrrolizidine nucleus has already been established by direct chemical evidence. The double bond was assigned to the 1,2 position in order to provide an allylic system which would explain the ease of hydrogenolysis of the primary hydroxyl. An alternative position for the double bond would be between the 1,8 carbons but this is less likely since it would then be situated at a bridge-head carbon atom.[3] This communication describes experiments which prove conclusively that the double bond is in the 1,2 position.

Desoxyretronecine (II), a reduction product of retronecine, was converted by thionyl chloride to compound III, herein designated as chloroisoheliotridene. This chlorinated product was reduced with chromous chloride to the unsaturated base, isoheliotridene (IV), which in turn was reduced catalytically with hydrogen to heliotridane,[4,5] the parent base of retronecine and its partially reduced derivatives. The hydrochloride of isoheliotridene (IV) was subjected to ozonolysis in aqueous solution. A product was obtained which was postulated as the hydrochloride of 2-acetyl-1-pyrrolidineacetic acid (V).



II III IV



V VI VII VIII

The free amino acid (V) proved to be unstable and no procedure was found for isolating it in a pure state. The hydrochloride was, therefore, used in all subsequent studies. The substance was characterized as a ketone by the preparation of a

(1) For previous paper see Adams and Hamlin, THIS JOURNAL, **64**, 2593 (1942).

(2) Adams, Carmack and Mahan, *ibid.*, **64**, 2593 (1942).

(3) Bredt, *Ann.*, **437**, 1 (1924).

(4) Menshikov, *Ber.*, **66**, 875 (1933).

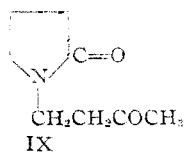
(5) Konovalova and Drekhov, *Bull. soc. chim.*, [5] **4** 1285 (1937).

2,4-dinitrophenylhydrazone and, further, as a methyl ketone by the formation of a copious precipitate of iodoform upon addition of sodium hypoiodite in aqueous solution.

Compound V, as the hydrochloride, was reduced readily at room temperature by hydrogen and platinum catalyst to the corresponding carbinol, 2 - (1 - hydroxyethyl) - 1 - pyrrolidineacetic acid (VI) which was stable both as the hydrochloride and free amino acid. A by-product was always present which was a basic oil characterized by means of the methiodide and picrate. It proved to have an empirical formula the same as the carbinol (VI) except for the elimination of a molecule of water. Its basicity and its formula indicated that it is the lactone (VII) of the carbinol (VI). This was confirmed by direct conversion of the carbinol to the lactone by means of acetic anhydride.

The presence of the carboxyl group in the carbinol (VI) was established by treatment of the molecule in moist ether with diazomethane.[6] A crystalline betaine (VIII) was thus formed which on account of its hygroscopicity was analyzed as the hydrochloride.

The basic nucleus of retronecine as 1-methylpyrrolizidine has been established beyond dispute.[7] Deductions with respect to the position of the double bond in isoheliotridene (IV), which is in the same position as in retronecine, may be considered on the basis of the experiments just described. The product (V) of ozonolysis contains a $-COCH_3$ group as shown by the iodoform reaction, and the double bond in isoheliotridene can therefore be only in the 1,2 or 1,8 position. In the 1,2 position it would yield an amino acid derivative, in the 1,8 position a pyrrolidone of structure IX. Since the product has a carboxyl



IX

group in it as shown by lactone and betaine formation from the corresponding carbinol of the ketone, the 1,8 position for the double bond is eliminated. The ease of formation of the lactone (VII) indicates the adjacency of the carbinol and carboxyl groups which is explicable only when the double bond is in the 1,2 position in isoheliotridene.

(6) Kuhn and Brydowna, *Ber.*, **70B**, 1333 (1937).

(7) Adams and Rogers, THIS JOURNAL, **63**, 228 (1941).

Experimental

Chloroisoheliotridene.—A solution of 25 g. of desoxyretronecine hydrochloride[8] in 60 cc. of purified thionyl chloride was refluxed on the steam cone for three and one-half hours. The cooled, dark colored solution was poured slowly onto 400 g. of ice and the gummy material which separated was removed by filtration. The filtrate was concentrated to 150 cc. and again filtered to remove some insoluble residue. The aqueous solution was made strongly alkaline and extracted with several portions of ether (until ethereal picric acid would cause no precipitation of a picrate). After drying over anhydrous magnesium sulfate and removal of ether the product was distilled; b. p. 59.5–60.5° (4.5 mm.); $n^{20}D$ 1.5030; d^{20}_4 1.098; yield 18.7 g. (83%).

Anal. Calcd. for $C_8H_{12}NCl$: C, 60.95; H, 7.67; N, 8.89. Found: C, 61.16; H, 7.70; N, 9.17.

Rotation. Pure liquid gave $\alpha^{32}D$ +54.70; *l*, 1; $[\alpha]^{32}D$ +50.10°.

Chloroisoheliotridene Picrate.—This product was prepared by treating an ethanol solution of chloroisoheliotridene with a slight excess of picric acid, and purified by recrystallization from 95% ethanol; yellow, fine, elongated prisms, m. p. 179.5–180° (cor.) with decomposition.

Anal. Calcd. for $C_{14}H_{15}N_4O_7Cl$: C, 43.47; H, 3.91; N, 14.49. Found: C, 43.59; H, 4.21; N, 14.79.

Isoheliotridene.—A solution of chromous chloride was prepared as follows. Two hundred grams of chromic chloride hexahydrate dissolved in 600 cc. of concentrated hydrochloric acid was reduced under an atmosphere of nitrogen with 200 g. of amalgamated zinc. When the reduction was complete as evidenced by a change in color from green to a clear blue, the solution was siphoned from the residual zinc and mercury into a flask containing 18.7 g. of chloroisoheliotridene. The resulting solution was refluxed under nitrogen for three and one-half hours, cooled by adding ice, made strongly basic with 50% sodium hydroxide and steam distilled. The clear steam distillate (300–400 cc.) was made more strongly alkaline with sodium hydroxide and the isoheliotridene extracted with ether. After drying over anhydrous sodium carbonate and removal of ether the product was distilled; b. p. 73° (30 mm.); $n^{20}D$ 1.4836; d^{20}_4 0.9306; yield 12.9 g. (88%). Isoheliotridene is a colorless liquid which turns slightly yellow on standing for several weeks.

Anal. Calcd. for $C_8H_{13}N$: C, 77.99; H, 10.63; N, 11.37. Found: C, 78.01; H, 10.55; N, 11.29.

Rotation. Pure liquid gave $\alpha^{28}D$ −42.27°; *l*, 1; $[\alpha]^{28}D$ −45.79.

When isoheliotridene is hydrogenated over platinum oxide at room temperature and atmospheric pressure it rapidly absorbs quantitatively one mole of hydrogen to give heliotridane (characterized by melting point of its picrate and mixed melting point with an authentic sample of heliotridane picrate).

Isoheliotridene Picrate.—Prepared in and recrystallized from absolute ethanol, the picrate formed yellow, elongated prisms, m. p. 198.5–199.5° (cor.), unchanged on further crystallization from dry benzene.

(8) Adams and Rogers, *ibid.*, **63**, 537 (1941).

Anal. Calcd. for $C_{14}H_{16}N_4O_7$: N, 15.90. Found: N, 15.72.

2-Acetyl-1-pyrrolidineacetic Acid Hydrochloride.—A solution of 4.2 g. (0.034 mole) of isoheliotridene in 50 cc. of distilled water was neutralized with one equivalent of 1 *N* hydrochloric acid. The clear solution which resulted was subjected to ozonolysis by means of a stream of ozonized oxygen (7%) flowing at the rate of 100 cc. per minute. The absorption of ozone was not quantitative and the ozonolysis was continued (five to six hours) until a test portion produced no precipitate on addition of a saturated aqueous solution of picric acid (isoheliotridene picrate precipitates immediately on addition of an aqueous solution of picric acid to an aqueous solution of isoheliotridene hydrochloride). At the completion of ozonolysis the solvent was removed *in vacuo* at 30°.[9]

The viscous yellow oil which remained was taken up in dry methanol at room temperature and dry ether added to turbidity. On standing in the icebox crystallization ensued; further addition of ether from time to time greatly assisted crystallization; yield (in two crops) 4.1 g., m. p. 168–170°. Purification was accomplished by several crystallizations from absolute ethanol–ether; yield 3.0 g. (42%); m. p. 180–181° (cor.); unchanged in melting point on further crystallization from methanol–ether. The hydrochloride of 2-acetyl-1-pyrrolidineacetic acid crystallized in colorless prisms which are very soluble in water and methanol, moderately so in absolute ethanol, and insoluble in dioxane, ether, petroleum ether and benzene. The substance gave a good iodoform test in aqueous solution at room temperature.[10]

Anal. Calcd. for $C_8H_{14}O_3NCl$: C, 46.27; H, 6.80; N, 6.75. Found: C, 46.37; H, 6.96; N, 6.77.

Rotation. 0.3751 g. made up to 5 cc. with dry methanol at 27° gave $\alpha^{27}D$ −0.33; *l*, 1; $[\alpha]^{27}D$ −4.40°.

Treatment of 2-acetyl-1-pyrrolidineacetic acid hydrochloride in aqueous solution with silver carbonate liberated the free amino acid which was obtained crystalline after removal of solvent *in vacuo* and permitting to stand a few days in an evacuated desiccator. When exposed to air this substance gradually turned dark brown.

2,4-Dinitrophenylhydrazone.—This substance was prepared by refluxing a solution of 0.191 g. (0.92 millimole) of 2-acetyl-1-pyrrolidineacetic acid hydrochloride and 0.182 g. (0.92 millimole) of 2,4-dinitrophenylhydrazine in 14 cc. of 95% ethanol for ten minutes. After addition of 0.2 cc. of concentrated hydrochloric acid, the solution was refluxed thirty minutes longer. The solvent was removed *in vacuo* at 50° and the crystalline residue purified by crystallization from absolute ethanol; m. p. 199–201° (cor.) with decomposition; yield 0.25 g. (70%). The substance formed orange-colored prisms which were moderately soluble in water, methanol and ethanol; practically insoluble in ether and benzene.

Anal. Calcd. for $C_{14}H_{18}O_6N_5Cl$: C, 43.36; H, 4.68; N, 18.06. Found: C, 43.53; H, 4.90; N, 17.94.

Titration with standard alkali, employing a Beckman glass electrode *p*H meter (Industrial model), gave a neutral equivalent of 382 (calcd. 387.8). The nature of the titration curve indicated that the hydrazone was an amino acid hydrochloride.

2-(1-Hydroxyethyl)-1-pyrrolidineacetic Acid Hydrochloride.—A solution of 2 g. of 2-acetyl-1-pyrrolidineacetic acid hydrochloride dissolved in 75 cc. of absolute ethanol was hydrogenated at room temperature and atmospheric pressure employing 0.1 g. of platinum oxide as catalyst. After the absorption of about 1.1 moles of hydrogen the reduction was complete (one hour) and the catalyst was then removed by filtration. The colorless sirup which remained after the removal of solvent *in vacuo* was taken up in absolute ethanol and ether added to near turbidity. More ether was added from time to time as the crystallization proceeded; yield 0.85 g., m. p. 137–139°. Further addition of ether to the mother liquor resulted in the precipitation of an oil which could not be induced to crystallize. The material in this mother liquor was used to prepare the methiodide and picrate of the lactone VII (see below). The crystalline material was purified by several crystallizations from absolute ethanol–ether; yield 0.55 g., m. p. 147–148° (cor.), unchanged on further crystallization from methanol–ether. The compound crystallized in colorless cubes which are very soluble in water, methanol and ethanol; insoluble in ether, dioxane and benzene. In aqueous solution the substance gave upon standing a good yield of iodoform on addition of an aqueous solution of sodium hypoiodite.

Anal. Calcd. for $C_8H_{16}O_3NCl$: C, 45.82; H, 7.69; N, 6.68. Found: C, 45.86; H, 7.94; N, 6.49.

Rotation. 0.1381 g. made up to 5 cc. with absolute ethanol at 32° gave $\alpha^{32}D$ −1.50; *l*, 1; $[\alpha]^{32}D$ −54.31°.

2-(1-Hydroxyethyl)-1-pyrrolidineacetic Acid (VI).—A solution of 1.04 g. of the hydrochloride of 2-(1-hydroxyethyl)-1-pyrrolidineacetic acid in 20 cc. of distilled water was treated with a slight excess of freshly precipitated silver carbonate. After shaking for thirty minutes, carbon dioxide was no longer liberated and the silver chloride was removed by filtration. The slightly turbid filtrate was saturated with hydrogen sulfide, boiled with 0.3 g. of a mixture of equal parts of Darco and Norite, and filtered. The colorless, crystalline residue remaining after removal of solvent *in vacuo* at 50°, was purified by crystallization from absolute ethanol–ether; yield 0.6 g. (70%) of colorless prisms; m. p. 186.5–187.5° (cor.), unchanged on further crystallization from absolute ethanol.

Anal. Calcd. for $C_8H_{15}O_3N$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.26; H, 8.75; N, 7.93.

Rotation. 0.2127 g. made up to 5 cc. with distilled water at 28° gave $\alpha^{28}D$ −2.70; *l*, 1; $[\alpha]^{28}D$ −63.47.

Betaine Hydrochloride of VI.—To 30 cc. of moist ether containing a considerable excess of diazomethane was added 0.39 g. of 2-(1-hydroxyethyl)-1-pyrrolidineacetic acid. An immediate slow evolution of nitrogen took place and the solid material gradually changed to an oil. After standing overnight at room temperature, the ether which still contained diazomethane was decanted from the residual oil. This was taken up in 10 cc. of water and the solvent removed *in vacuo* at 45°. The colorless oil which remained crystallized on standing for six hours in an evacuated desiccator. The free betaine was very hygroscopic

(9) If the temperature of the bath was higher than 30° serious decomposition occurred. Not only was the yield materially decreased but the product was much more difficult to obtain in a pure state.

(10) Shriner and Fuson, "Identification of Organic Compounds," John Wiley and Sons, New York, N. Y., 2nd ed., 1940, p. 53.

and, therefore, it was converted to the hydrochloride by treating with one equivalent of *N* hydrochloric acid. Removal of solvent *in vacuo* left a crystalline solid which was purified by several crystallizations from absolute ethanol–ether; yield 0.2 g., m. p. 176–177° (cor.), softens at 170°. The substance formed colorless elongated prisms which are slightly hygroscopic.

Anal. Calcd. for $C_9H_{18}O_3NCl$: C, 48.32; H, 8.11; N, 6.26. Found: C, 48.25; H, 8.29; N, 6.25.

Methiodide of VII.—The mother liquor from the crystallization of 2-(1-hydroxyethyl)-1-pyrrolidineacetic acid hydrochloride (see above) was taken to dryness *in vacuo*. The residual oil (1.2 g.) was taken up in 40 cc. of distilled water and shaken with a slight excess of freshly precipitated silver carbonate for twenty-five minutes. After filtering off the silver chloride formed, the filtrate was extracted with several 30-cc. portions of ether. The combined ethereal extracts were dried over anhydrous potassium carbonate and the ether removed by distillation. The basic oil which remained (0.5 g.) was divided into two equal portions and used for the preparation of a methiodide and picrate. The methiodide was prepared by dissolving the first portion in 5 cc. of dry acetone and refluxing the solution with 1 cc. of methyl iodide for thirty minutes. On cooling crystallization occurred; yield 0.25 g. The substance was purified by several crystallizations from absolute ethanol–ether; long fine prisms, m. p. 242–243° (cor.).

Anal. Calcd. for $C_9H_{18}O_2NI$: C, 36.38; H, 5.42. Found: C, 36.53; H, 5.55.

Rotation. 0.1232 g. made up to 5 cc. with methanol at 29° gave $\alpha^{29}D$ −0.37; *l*, 1; $[\alpha]^{29}D$ −15.02.

Picrate of VII.—The second portion of the basic oil was dissolved in 5 cc. of absolute ethanol and a saturated solution of picric acid in ethanol added. The picrate that separated was purified by crystallization from 95% ethanol; yellow prisms, m. p. 169–170° (cor.).

Anal. Calcd. for $C_{14}H_{18}N_4O_9$: C, 43.75; H, 4.20; N, 14.58. Found: C, 44.06; H, 4.40; N, 14.77.

Conversion of 2-(1-Hydroxyethyl)-1-pyrrolidineacetic Acid to the Lactone (VII).—A mixture of 0.20 g. of the hydrochloride of 2-(1-hydroxyethyl)-1-pyrrolidineacetic acid and 8 cc. of acetic anhydride was heated on the steam cone for one and one-half hours. All of the hydrochloride was in solution after fifteen to twenty minutes heating. The acetic anhydride was removed *in vacuo* at 60° and the dark brown product which remained was taken up in 5 cc. of water. This solution was treated with freshly precipitated silver carbonate, filtered, and extracted with several portions of ether. After drying, the ethereal extract was concentrated to 6–8 cc. and 5 cc. of a saturated solution of picric acid in ethanol added. The yield of purified picrate was 0.07 g.; m. p. 169–170°, mixed melting point with the picrate described above produced no depression.

Summary

1. The unsaturated base, isoheliotridene (IV), has been prepared from desoxyretronecine (II) in two steps.

2. Ozonolysis of the hydrochloride of isoheliotridene leads to the formation of 2-acetyl-1-pyrrolidineacetic acid (V). This substance was characterized by preparation of a 2,4-dinitrophenylhydrazone and by reduction to the carbinol (VI). The carbinol lost water readily to yield a lactone and formed a betaine upon treatment with diazomethane.

3. The isolation of this amino acid on ozonolysis definitely establishes the position of the double bond in isoheliotridene and retronecine as being between carbon atoms 1 and 2.

URBANA, ILLINOIS RECEIVED JULY 2, 1943

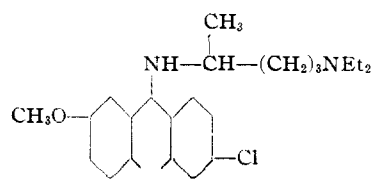
[CONTRIBUTION FROM THE PARKE, DAVIS & COMPANY RESEARCH LABORATORIES]

N-Substituted 2-Methoxy-6-chloro-9-aminoacridines

BY J. H. BURCKHALTER, ELDON M. JONES, W. F. HOLCOMB AND L. A. SWEET

Since the discovery of atabrine (I) by Mietzsch and Mauss,[1] a great number of acridine compounds have been synthesized by chemists throughout the world. The most promising of these from the standpoint of antimalarial activity have been derivatives of 9-aminoacridine, many of which follow a rather uniform pattern (II).

In this Laboratory, several compounds of Type II in which there are wide variations of the R group, as well as a few simple aromatic and heterocyclic derivatives of 9-aminoacridine, were



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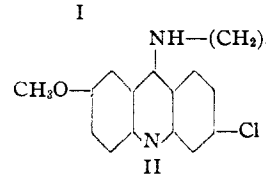

II

n = 1, 2, 3, etc.
R = NEt_2, NMe_2

prepared. The variations include aliphatic, aro-

(1) Mietzsch and Mauss, *Angew. Chem.*, **47**, 633 (1934) [*C. A.*, **28**, 7360 (1934)].