thyl-N'-phenylurea, m.p. 222–224°[10]; aniline hydrochloride, m.p. 197–198°; and α-naphthylamine, m.p. 48–50°.

(a) **Carbanilide.**—From 41 g. of carbanilide (prepared from phenyl isocyanate and aniline) in a run of 5.5 hours duration there was obtained 24 g. of aniline hydrochloride, a 96% yield. After aniline treatment of the toluene solution 23.6 g. of carbanilide was obtained, corresponding to a 57.6% yield of phenyl isocyanate. Also isolated from the solution was 9.5 g. of an inert liquid containing 11.05% nitrogen.

(b) ***sym*-Diethylurea.**—From 81.5 g. of Sharples *sym*-diethylurea, in a run of 4.5 hours duration, there was obtained 53.2 g. (93.0%) of ethylamine hydrochloride, m.p. 107–108°, and, after aniline treatment, 72.9 g. of N-ethyl-N'-phenylurea, corresponding to a 63.3% yield of ethyl isocyanate. A residue weighing 15.1 g. and containing 15.29% nitrogen was also obtained from the toluene solution.

(c) ***sym*-Dicyclohexylurea.**—From 58.7 g. of *sym*-dicyclohexylurea, prepared from cyclohexyl isocyanate and cyclohexylamine, m.p. 227–227.5°,[11] in a run of 5.5 hours duration there was obtained 34 g. (96.2%) of cyclohexylamine hydrochloride, m.p. 205°.[12] The yield of cyclohexyl isocyanate as determined by titration of the crude isocyanate and toluene distillate with dibutylamine was 23.4 g., or 71.5%.

(d) **N-Phenylurea.**—From 48.4 g. of N-phenylurea, prepared from phenyl isocyanate and ammonia, m.p. 145–147°, there was obtained 10.5 g. (22.8%) of aniline hydrochloride and 5.1 g. (13.5%) of carbanilide. Treatment of the toluene solution with aniline gave 27.1 g. of carbanilide, corresponding to a yield of 35.9% of phenyl isocyanate.

(e) **Urea and Aniline Hydrochloride.**—From a mixture of 7.4 g. of urea and 13.3 g. of aniline hydrochloride (1.2:1 molar ratio) in a run of one hour duration there was obtained, after treatment with aniline, 6.1 g. of carbanilide, corresponding to a 28.0% yield of phenyl isocyanate, based on the aniline hydrochloride.

(f) **Urea and α-Naphthylamine.**—From a mixture of 8.4 g. of urea and 16.5 g. of du Pont α-naphthylamine (1.2:1 molar ratio) there was obtained after aniline treatment 9.0 g. of N-α-naphthyl-N'-phenylurea.[10] The recovery of α-naphthylamine was 5.2 g., 31.5%. The yield of α-naphthyl isocyanate, based on amine used, was 43.5%.

(g) **Urea and α-Naphthylamine Hydrochloride.**—From a mixture of 10.1 g. of urea and 24.9 g. of α-naphthylamine hydrochloride (1.2:1 molar ratio) in a run of 3.5 hours duration, there was obtained a recovery of 3.6 g. of α-naphthylamine, 18.1%, and, after aniline treatment, 12.4 g. of N-α-naphthyl-N'-phenylurea.[10] The corresponding yield of α-naphthyl isocyanate was 41.5%, based on amine hydrochloride used. A residual material containing 14.04% nitrogen was also isolated from the toluene solution.

(h) **Cyanuric Acid and α-Naphthylamine.**—From 6.0 g. of cyanuric acid[13] and 17.2 g. of α-naphthylamine (1.2:1 equivalent ratio) in a run of one hour time at 415–425° there was obtained, following aniline treatment, 2.2 g. of N-α-naphthyl-N'-phenylurea.[10] The recovery of amine was 5.5 g., 31.9%. The corresponding yield of α-naphthyl isocyanate was 10.3%, based on amine. The accumulation of solids in the vaporization zone was excessive in this experiment.

(i) **Urea and Ethylamine Hydrochloride.**—From 23.8 g. of urea and 26.9 g. of ethylamine hydrochloride (1.2:1 molar ratio) in a run of one hour duration, at 370–380°, there was obtained, after aniline treatment, 4.9 g. of N-ethyl-N'-phenylurea. The corresponding yield of ethyl isocyanate was 9.1%, based on the amine hydrochloride fed. The solids found in the collector, weighing 40.9 g., were all water-soluble. The unchanged ethylamine hydrochloride was not determined, so the actual yield of isocyanate, based on amine consumed, may have been larger than 9.1%.

(j) **Urea and Cyclohexylamine Hydrochloride.**—From 25.4 g. of urea and 47.5 g. of cyclohexylamine hydrochloride, in a run of three hours duration, there was obtained, following aniline treatment, 10.7 g. of N-cyclohexyl-N'-phenylurea, m.p. 149–150°.[11] The corresponding yield of cyclohexyl isocyanate, based on amine hydrochloride fed, was 14.0%. No cyclohexylamine could be isolated from the 34.9 g. of water-soluble solids found in the collector. The toluene solution decolorized bromine in carbon tetrachloride, indicating an unsaturated compound, possibly cyclohexene.

(10) A. E. Dixon, *J. Chem. Soc.*, **79**, 102 (1901).
(11) A. Skita and H. Rolfes, *Ber.*, **53**, 1242 (1920).
(12) O. Wallach, *Ann.*, **343**, 40 (1905).
(13) R. J. Slocombe, E. E. Hardy, J. H. Saunders and R. L. Jenkins, THIS JOURNAL, **72**, 1888 (1950).

ANNISTON, ALABAMA

[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

Gliotoxin. X. Dethiogliotoxin and Related Compounds[1]

BY JOHN R. JOHNSON AND JAMES B. BUCHANAN[2]

RECEIVED AUGUST 5, 1952

Dethiogliotoxin, $C_{13}H_{16}N_2O_4$, which is formed by desulfurization of gliotoxin with amalgamated aluminum, has been converted smoothly by hot aqueous hydrochloric acid into a new crystalline compound, $C_{13}H_{14}N_2O_3$, named anhydrodethiogliotoxin. On heating with acetic anhydride, anhydrodethiogliotoxin furnishes the compound $C_{13}H_{12}N_2O_2$, which has been identified as *dl*-2,3-dimethylpyrazinoindole-1,4-dione and is identical with the product obtained by direct reduction of gliotoxin with phosphorus and hydriodic acid. Anhydrodethiogliotoxin is tentatively formulated as a hydroxyindoline derivative. The action of amalgamated zinc and dilute hydrochloric acid on gliotoxin furnishes a compound $C_{13}H_{18}N_2O_3$, which differs from dethiogliotoxin in having one less oxygen atom. With hydrogen at low pressure over Raney nickel, in the presence of aqueous ethanol and triethylamine, gliotoxin is converted to a compound $C_{13}H_{20}N_2O_4$, which corresponds formally to a tetrahydro derivative of dethiogliotoxin. New structures are proposed for dethiogliotoxin and gliotoxin.

The action of amalgamated aluminum on gliotoxin under mild conditions brings about elimination of the sulfur atoms as hydrogen sulfide and furnishes a colorless, crystalline sulfur-free product of the molecular formula $C_{13}H_{16}N_2O_4$, which is designated as dethiogliotoxin.[3] This compound appears to be formed by simple reductive desulfurization in which the two sulfur atoms of gliotoxin are replaced by two hydrogen atoms

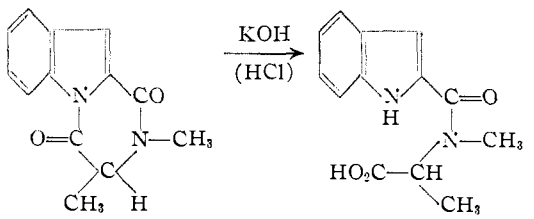
$$C_{13}H_{14}N_2O_4S_2 + 6H(Al—Hg) \longrightarrow C_{13}H_{16}N_2O_4 + 2H_2S$$

Dethiogliotoxin, like gliotoxin itself, is strongly levorotatory and its ultraviolet absorption spectrum is similar to that of gliotoxin. Since it seems likely that the desulfurization is effected without any profound structural alteration, the study of dethiogliotoxin is expected to contribute significantly to establishing the structure of gliotoxin.

Dethiogliotoxin reacts rapidly with hot methan-

(1) Preceding paper, THIS JOURNAL, **73**, 3749 (1951).
(2) The Wm. S. Merrell Company Fellow, 1948–1949.
(3) J. D. Dutcher, J. R. Johnson and W. F. Bruce, THIS JOURNAL, **67**, 1736 (1945). In the present paper the name *dethiogliotoxin* is used instead of *desthiogliotoxin*, in conformity with recent recommendations for nomenclature of such compounds.

olic potassium hydroxide solution and gives rise to two compounds. The main product, amounting to about three-fourths of the total, is the 2-indolecarbonyl derivative of *dl*-N-methylalanine (II), which undoubtedly is formed by way of 2,3-dimethylpyrazinoindole-1,4-dione (I). The latter has been obtained also by direct reduction of glio-

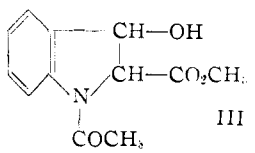


I ($C_{13}H_{12}N_2O_2$, m.p. 122°) II ($C_{13}H_{14}N_2O_3$, m.p. 187°)

toxin with hydriodic acid and phosphorus, and the constitution of these compounds has been established by synthesis.[4] The composition of the dimethylpyrazinoindoledione (I) corresponds to the elimination of *two* molecules of water from dethiogliotoxin.

The accessory product of the action of methanolic alkali on dethiogliotoxin is a very sparingly soluble, crystalline substance of high melting point (m.p., with dec., *ca.* 365°), having the empirical formula $C_{13}H_{12}N_2O_2$. The constitution of this compound has not been established and its relationship to dethiogliotoxin is obscure.

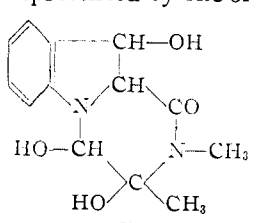
A significant *intermediate* transformation product has now been obtained by the action of mineral acids on dethiogliotoxin. Strong aqueous hydrochloric or sulfuric acid at moderate temperatures brings about elimination of *one* molecule of water and gives a colorless optically active compound having the molecular formula $C_{13}H_{14}N_2O_3$, named anhydrodethiogliotoxin. The ultraviolet absorption spectrum of this compound differs from that of dethiogliotoxin, or any other degradation product that we have encountered hitherto, but is strikingly similar to that of 3-hydroxyindoline-2-carboxylic acid derivatives (III) recently synthesized as model compounds[5] (*cf.* Fig. 1). The behavior of



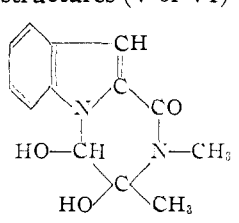
III

anhydrodethiogliotoxin toward hot methanolic potassium hydroxide is similar to that of dethiogliotoxin itself. The principal product (57% by weight) is *dl*-N-2-indolecarbonyl-N-methylalanine (II) and a smaller amount (36% by weight) of a sparingly soluble, crystalline compound, m.p. *ca.* 365°, is formed. The latter has the empirical formula $C_{13}H_{12}N_2O_2$ and appears to be identical with the high-melting compound obtained from dethiogliotoxin and methanolic alkali.

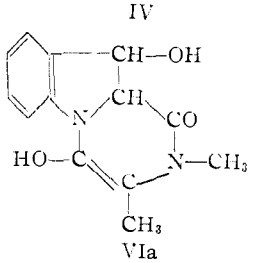
On heating with acetic anhydride at 235° in a sealed tube, anhydrodethiogliotoxin loses the elements of one molecule of water and is converted almost quantitatively into 2,3-dimethylpyrazinoindole-1,4-dione (I), m.p. 121–122°. The product was identified by its ultraviolet absorption spectrum and by direct comparison with an authentic specimen. On the basis of our tentative structure for dethiogliotoxin (IV), anhydrodethiogliotoxin may be represented by one of the structures (V or VI)[6]



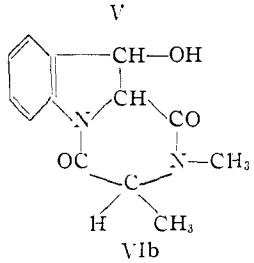
IV



V



VIa



VIb

The striking similarity of the ultraviolet absorption of anhydrodethiogliotoxin to that of model hydroxyindolines and the absence of the character-

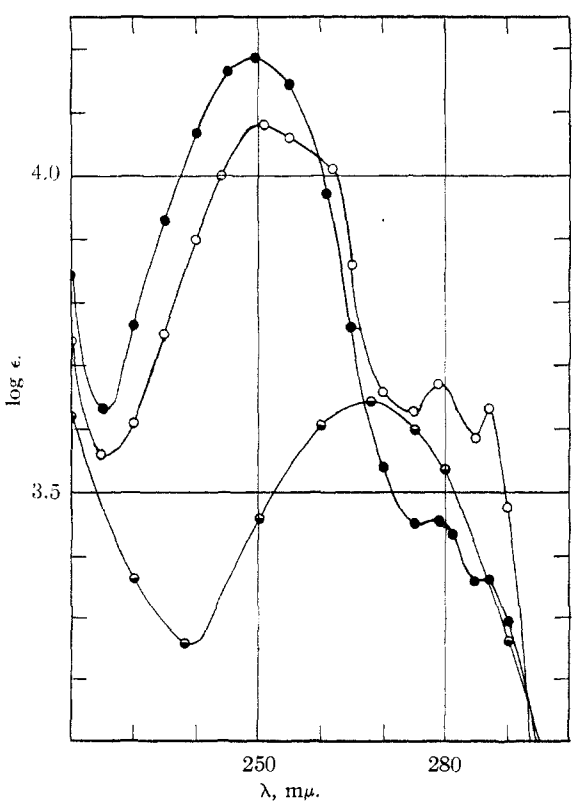


Fig. 1.—Ultraviolet absorption spectra: O, anhydrodethiogliotoxin; ◒, dethiogliotoxin; ●, methyl 1-acetyl-3-hydroxyindoline-2-carboxylate.

(4) J. D. Dutcher, J. R. Johnson and W. F. Bruce, THIS JOURNAL, **66**, 617 (1944).

(5) J. R. Johnson and J. H. Andreen, *ibid.*, **72**, 2862 (1950).

(6) In these formulas the location of the hydroxyl group shown at position 10 of the pyrazinoindole system is not definitely established, since the experimental evidence indicates merely the presence of hydroxyl at either position 10 or 11. We have chosen position 10 as being much more probable and have shown only this formulation, as the present work does not concern that aspect of the structural problem.

istic absorption exhibited by 2-indolecarboxylic acid derivatives, furnish good evidence in favor of formula VI.

During the course of the present work Elvidge and Spring[7] reported an investigation of the action of dry hydrogen chloride on dethiogliotoxin in the presence of anhydrous organic solvents (dioxane, methanol and ethanol). They obtained eight transformation products, of which six were definitely characterized: *compounds B, B'* and *G*, of the molecular formula $C_{13}H_{18}N_2O_5$, corresponding to addition of the elements of water to dethiogliotoxin; *compound E*, m.p. 168°, of the molecular formula $C_{13}H_{16}N_2O_4$, which is therefore an isomer of dethiogliotoxin; *compounds A and F*, of the formula $C_{13}H_{14}N_2O_3$, corresponding to removal of the elements of one molecule of water from dethiogliotoxin.

One of their transformation products, compound F, resembles anhydrodethiogliotoxin very closely, as shown in the following comparison: *Anhydrodethiogliotoxin:* m.p. 157–158°, $[\alpha]_D$ −91 ± 3°; absorption maxima, 251 mμ (ε 12,700), 279 mμ (ε 4,690), 287 mμ (ε 4,310); prisms from methanol, crystal bundles from dioxane–petroleum ether; active hydrogen, in anisole, 1.4 at 25°, 2.1 at 95°. *Compound F:* m.p. 156° $[\alpha]_D$ −100 ± 5°; absorption maxima, 253 mμ (ε 10,370), 275 mμ (ε 5,980), 282 mμ (ε 3,800); prismatic needles or sheaves of laths from dioxane–petroleum ether; active hydrogen, 0.73 (conditions not specified).

In spite of the discrepancy in the values of active hydrogen for anhydrodethiogliotoxin and compound F, the two substances are actually the same compound. A sample of compound F prepared by their method, from dethiogliotoxin and methanolic hydrogen chloride, melted at 156–158° and showed no depression when mixed with our anhydrodethiogliotoxin.

Anhydrodethiogliotoxin crystallizes from water as a hydrate which seems to be identical with the compound G of Elvidge and Spring.[7] It was reported and we have confirmed that this hydrate ($C_{13}H_{14}N_2O_3 + 2H_2O$) regenerates the anhydro compound on heating in a vacuum. They have assigned to compound F the structure VIb, in agreement with our formulation of anhydrodethiogliotoxin, basing their structural argument mainly on the supposition that a compound of structure VIb would show only one active hydrogen by the Zerewitinow method, whereas one of structure V would show two active hydrogens. Our recent experiments on the Zerewitinow method (Table I) show that the active hydrogen values for various compounds in this series are subject to significant variations, depending on the solvent and the temperature. Since compound I shows *one* active hydrogen in anisole at 95°, it follows that the hydroxyindoline compound VIb should show *two* active hydrogens under the same conditions. This is in satisfactory agreement with our observation that anhydrodethiogliotoxin shows two active hydrogens in anisole at 95°.

In the course of the present work active hydrogen determinations have been carried out by the Zerewitinow method, on gliotoxin and a group of twelve related compounds. The apparatus and procedures employed were based on those of Roth[8] and of Pregl–Grant.[9] Most of the determinations were made in anisole at 25° and at 95°; gliotoxin, gliotoxin dibenzoate and dethiogliotoxin were run in pyridine at 25 and at 70°. The results are collected in Table I.

TABLE I

DETERMINATIONS OF ACTIVE HYDROGEN[a]

Compound[d]	Atoms active H per mole At 25°	At 95°
Gliotoxin	2.03, 2.02 (2.6)[a]	2.5 +[c] (2.90, 2.88)[b]
Gliotoxin dibenzoate	.....	0.5–1.0[c] (0.95, 1.19)[b]
Dethiogliotoxin	(2.5, 2.4)[a]	(2.84, 2.86)[b]
Anhydrodethiogliotoxin	1.61, 1.32	2.02, 1.96
Methyl 1-acetyl-3-hydroxyindoline-2-carboxylate (III)	1.29, 1.13	1.99, 1.83
2-Methylpyrazino[1,2-a]-indole-1,4(2,3)dione	0.64, 0.69	0.90, 0.85
2,3-Dimethylpyrazino[1,2a]-indole-1,4(2,3)dione (I)	0.16, 0.10	1.33, 1.11
2,3,10-Trimethylpyrazino-[1,2-a]-indole-1,4(2,3)dione	0.04, 0.03	0.12, 0.13
2-Methylpyrazino[1,2-a]-indole-1,3,4(2)trione	0.04, 0.04	0.15, 0.08
Raney nickel desulfurized product ($C_{13}H_{20}N_2O_4$), m.p. 194–195°	2.03, 2.11	3.06, 2.99
Gliotoxin mono-acetate, from *Penicillium terlikowski* ($C_{15}H_{16}N_2O_5S_2$)	1.26, 1.42	1.88, 211
Deoxydethiogliotoxin	1.04, 0.99	1.64+, 1.59+[c]
Anhydro derivative of deoxydethiogliotoxin	0.18, 0.21	1.06, 1.02

[a] Determinations were made in anisole, except for values given in parentheses, which were run in pyridine. [b] In pyridine, at 70°. [c] In these experiments methane continued to be evolved slowly, at a gradually diminishing rate. [d] Elvidge and Spring[7] reported the following values for active hydrogen, determined under conditions not specified: gliotoxin, 3.06; gliotoxin dibenzoate, 1.26; dethiogliotoxin, 2.03, 2.45; dethiogliotoxin diacetate, 1.35; compound F (anhydrodethiogliotoxin), 0.73.

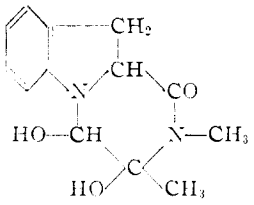
Several new transformation products have been obtained as a result of further studies of the action of desulfurizing agents on gliotoxin. Amalgamated zinc in the presence of dilute aqueous hydrochloric acid converts gliotoxin into a mixture of colorless, crystalline compounds, m.p. 160–190° (70% by weight). By means of a seven-stage countercurrent distribution between water and chloroform the mixture was separated into two principal fractions. The smaller fraction, which was more soluble in water and amounted to about 10% of the total, melted at 235–240° and proved to be dethiogliotoxin; it showed no depression of the melting point when mixed with authentic dethiogliotoxin, m.p. 246–247°, $[\alpha]_D$ −265°. The larger fraction, which was more soluble in chloroform and amounted to almost 90% of the reduction products, melted at 160–175° and was optically

(7) J. A. Elvidge and F. S. Spring, *J. Chem. Soc.*, 2935 (1949).

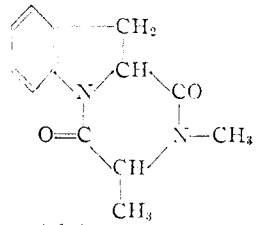
(8) H. Roth, *Mikrochem.*, **11**, 140 (1932).

(9) Grant, "Quantitative Organic Microanalysis (of Fritz Pregl)," The Blakiston Company, Philadelphia, Penna., 1946, p. 134.

active, $[\alpha]_D$ −215°. This fraction consists mainly of a compound which has the same ultraviolet absorption as dethiogliotoxin (maximum at 268 mμ), together with a small amount of an impurity (absorption maximum at 262 mμ) that could not be eliminated. After five recrystallizations from ethanol this zinc amalgam reduction product melted at 177–183° but was still not quite pure. Analytical data give the formula $C_{13}H_{16}N_2O_3$, which corresponds to a deoxydethiogliotoxin and



VII



VIII

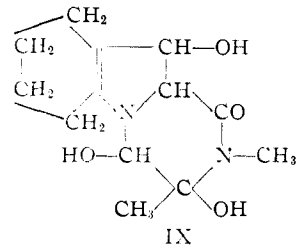
suggests the structure VII, in which the indole hydroxyl of dethiogliotoxin has been eliminated.

When the zinc amalgam reduction product was warmed with concentrated hydrochloric acid, under conditions like those used for the conversion of dethiogliotoxin to anhydrodethiogliotoxin, there was formed a crystalline product, m.p. 90–100°, which appeared to be analogous to anhydrodethiogliotoxin. The new substance exhibits an ultraviolet absorption very similar to that of anhydrodethiogliotoxin. It has the formula $C_{13}H_{14}N_2O_2$, corresponding to the elimination of water from VII, and presumably can be represented by structure VIII.

Desulfurization of gliotoxin by means of Raney nickel and hydrogen at low pressures and room temperature, in the presence of ethanol and triethylamine, furnished a colorless crystalline compound, m.p. 194–195°. The new substance is optically active, $[\alpha]^{25}D$ +73°, and the analytical data indicate a formula $C_{13}H_{20}N_2O_4$, which corresponds to a *tetrahydro* derivative of dethiogliotoxin.

By refluxing an ethanolic solution of gliotoxin with Raney nickel, Dutcher[10] had obtained earlier a desulfurized product which had the same molecular formula as ours but different physical properties: m.p. 155–156°, $[\alpha]D$ +50°. We have not encountered Dutcher's isomer in our work but in his later experiments Dutcher obtained a small amount of a desulfurized product, m.p. 191°, $[\alpha]D$ +75°, which is evidently identical with our tetrahydrodethiogliotoxin.

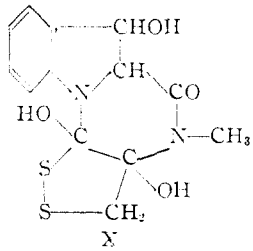
In terms of our previous formulation of gliotoxin the action of Raney nickel must be considered to effect the addition of four atoms of hydrogen to the carbocyclic ring of the indoline system and give rise to the structure IX. This interpretation is at variance with our observation that model compounds of the hydroxyindoline series, such as III, do not undergo hydrogenation of the carbocyclic ring with Raney nickel under mild conditions. On the other hand, it has been reported[11]



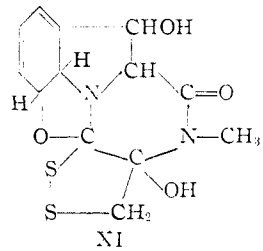
IX

that 2- and 3-methylindoles take up four atoms of hydrogen in the presence of nickel to furnish the corresponding 4,5,6,7-tetrahydroindoles.

A new contribution to the structure of gliotoxin has been made by Professor R. B. Woodward[12] as a result of infrared studies of gliotoxin and certain related compounds. He obtained evidence for the absence of an aromatic system in gliotoxin and the presence, instead, of a 1,3-diene structure. On this basis he has suggested that our preferred formula for gliotoxin (X) should be modified by a ring closure involving the hydroxyl of position 4 and the carbon atom of position 5, leading to the pentacyclic structure XI. This formula would account for the ease of hydrogenation with Raney

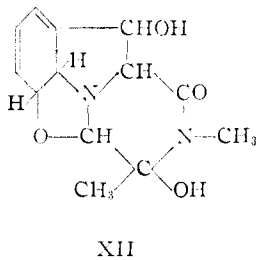


X

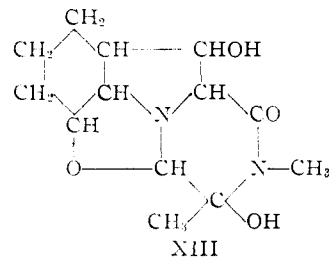


XI

nickel and for the uptake of precisely four atoms of hydrogen by the carbocyclic system. A corresponding modification of the structure of dethiogliotoxin and of the Raney nickel desulfurization product would give formulas XII and XIII, respectively.



XII



XIII

There is another structural feature of the formulas for gliotoxin and dethiogliotoxin, XI and XII, which also appears to require revision. The view that dethiogliotoxin contains a carbon–methyl group is not supported by recent new evidence. We had reported[3] that gliotoxin and dethiogliotoxin gave 0.12 and 0.93 mole of acetic acid, respectively, in the Kuhn–Roth determination of carbon-methyl groups and concluded that dethiogliotoxin contains one C–CH_3 which is not present in gliotoxin but is formed in the course of the desulfurization by aluminum amalgam. This con-

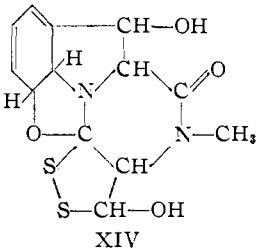
(10) Private communication from Dr. James D. Dutcher, Squibb Institute for Medical Research, New Brunswick, N. J.: (a) February 15, 1946; (b) March 30, 1949. We are indebted to Dr. Dutcher for permission to include his experimental work on tetrahydrodethiogliotoxin in this paper.

(11) J. V. Braun, O. Bayer and G. Blessing, *Ber.*, **57**, 392 (1924).

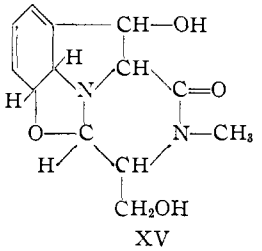
(12) The modified formula for gliotoxin was kindly communicated to me by Professor R. B. Woodward in the course of an informal exchange of views on gliotoxin chemistry at Harvard in November 1950. (J. R. J.)

clusion was supported by the formation of iodoform and later by independent carbon-methyl determinations of Elvidge and Spring,[13] who reported 0.2 carbon–methyl for gliotoxin and 0.76 for dethiogliotoxin. Subsequent determinations of carbon-methyl by the modified procedure of Barthel and LaForge[14] have given values of 0.20, 0.00 and 0.19 for dethiogliotoxin, which indicate that the earlier results[15] and conclusions are incorrect.

The absence of C–CH_3 in dethiogliotoxin implies that the hydroxyl group assigned to position 3 in formula IV or XII should be shifted to the side chain, position 3a, so that this becomes –CH_2OH instead of –CH_3. A corresponding change must also be made in the formulas for gliotoxin and deoxydethiogliotoxin (VII). The structures XIV and XV represent what we believe to be the best expression of our present knowledge of the chemistry of gliotoxin and dethiogliotoxin. The lack of C–CH_3 in dethiogliotoxin has been confirmed by



XIV

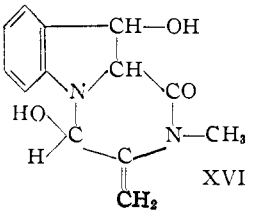


XV

studies of infrared spectra now in progress, which indicate absence of C–CH_3 in gliotoxin and dethiogliotoxin but show C–CH_3 to be present in anhydrodethiogliotoxin.

A plausible explanation of the contradictory results of the Kuhn–Roth and Barthel–LaForge methods for carbon-methyl determinations may be based upon differences in the concentration of acid to which the dethiogliotoxin is subjected. In the Kuhn–Roth method the sample is mixed with concentrated sulfuric acid and then treated with aqueous chromic acid solution, while in the Barthel–LaForge modification the sample is treated with previously prepared chromic acid solution in 20% sulfuric acid. Spectrophotometric studies have shown that dethiogliotoxin is converted to anhydrodethiogliotoxin (which does contain C–CH_3) by only a few minutes contact with cold concentrated sulfuric acid but this transformation is very slow with cold 20% sulfuric acid. Consequently, in the Kuhn–Roth procedure the formation of acetic acid can be attributed to conversion of dethiogliotoxin to the anhydro compound (VI) before oxidation occurred. In the Barthel–LaForge procedure the oxidation of dethiogliotoxin proceeds rapidly at room temperature and occurs before the anhydro compound is formed. These considerations and the data from infrared studies indicate that the Barthel–LaForge method has given the correct result for this compound. The observed values of 0.20 and 0.19 are within a range often found for compounds that do not contain C–CH_3. In a control experiment, serine, which has an α-amino-β-hydroxy system similar to the relevant portion of the revised dethiogliotoxin structure (XV), gave 0.25 mole of acetic acid by the Barthel–LaForge method.[15]

On the basis of the new formula for dethiogliotoxin (XV) the transformation to anhydrodethiogliotoxin (VI) is not so simple and straightforward as from the older formula (IV). A possible sequence of steps would involve elimination of the elements of water and opening of the oxygen bridge to form an intermediate such as XVI, which would furnish the anhydro compound (VIa) by isomerization. It is possible that the insoluble high-melting



XVI

compound of the formula $C_{13}H_{12}N_2O_2$, formed by the action of hot alkali on dethiogliotoxin, would be capable of furnishing valuable structural evidence but we have not been able to establish its identity. Studies of tetrahydrodethiogliotoxin and other new transformation products are being pursued actively.

Experimental

Determination of Active Hydrogen.—The apparatus and procedure used were based on the method described by Pregl–Grant,[9] adapted to a semi-micro scale. The Grignard reagent was prepared in di-*n*-butyl ether instead of isoamyl ether. The results of the determinations are collected in Table I.

Dethiogliotoxin.—Dethiogliotoxin was prepared as previously described,[3] except that it was found advantageous to use smaller quantities of aluminum amalgam, initially 4 g. for 2 g. of gliotoxin, with the addition of further smaller quantities as the reaction proceeded, and to dissolve the gliotoxin in a larger volume of absolute ethanol (2 g. in 500 ml.). Although these variations facilitated recovery of the product, the yield was only about 40%. After repeated recrystallization from absolute ethanol, a final crystallization was effected by dissolving the material in hot acetonitrile, adding hot methyl *n*-butyl ether to incipient turbidity, and chilling overnight. The purified dethiogliotoxin formed colorless prisms, m.p. 246–247° (cor.),[16] $[\alpha]^{25}D$ −265° (*c* 0.1 in ethanol); previously reported, m.p. 243–244°,[3] and 248–249°.[7] We believe that the optical rotation reported in our earlier paper,[3] $[\alpha]D$ −130° (*c* 0.2 in ethanol), is incorrect owing to an arithmetical error; the value reported in the present work has been checked several times.

Dethiogliotoxin showed three active hydrogens (2.84, 2.86) in pyridine at 70° by the Zerewitinow method, which is the same number observed for gliotoxin under similar conditions (2.90, 2.88).

The earlier observation[3] that dethiogliotoxin furnishes iodoform on treatment with hypoiodite solution was confirmed. A solution of 20 mg. of dethiogliotoxin in 2 ml. of water was treated with 0.4 ml. of 20% aqueous potassium hydroxide and a 10% solution of iodine in potassium iodide was added dropwise until a large excess had been introduced. Crystals began to form slowly; after an hour they were collected and washed. The crude iodoform weighed 5 mg. (18% of the theoretical) and melted at about 119°. It was contaminated with a substance that turned black and decomposed at higher temperatures.

In an exploratory experiment it was found that dethiogliotoxin can be hydrogenated over platinum with an uptake of four atoms of hydrogen per mole. The relationship

(13) J. A. Elvidge and F. S. Spring, *J. Chem. Soc.*, S136 (1949).

(14) W. F. Barthel and F. B. LaForge, *Ind. Eng. Chem., Anal. Ed.*, **16**, 434 (1944).

(15) We are indebted to Mr. J. F. Alicino of the Squibb Institute for Medical Research, for the recent determinations of carbon–methyl as well as those reported earlier.

(16) All melting points are corrected.

of this product to the tetrahydro compound obtained by reduction of gliotoxin with Raney nickel, as described below, is under investigation.

Action of Acids on Dethiogliotoxin: Anhydrodethiogliotoxin. (a) **Hydrochloric Acid.**—A solution of 200 mg. of dethiogliotoxin in 10 ml. of concentrated hydrochloric acid in an open flask was heated at 70° for 40 minutes. The reaction mixture was poured into 40 ml. of water, 10 g. of sodium bicarbonate was added to neutralize the acid, and the neutral solution was extracted repeatedly with chloroform. After the chloroform had been dried with magnesium sulfate and the solvent removed, there remained a crystalline residue of 147 mg. (80% yield), melting at 154–155°. Repeated recrystallization from methanol yielded prisms, m.p. 157–158°, $[\alpha]^{21}D$ −91° (*c* 0.5 in ethanol).

Anal. Calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.47, 63.29; H, 6.25, 6.14; N, 11.53, 11.54.

Anhydrodethiogliotoxin is slightly soluble in cold water, ethanol and methanol, but dissolves readily in all three on warming. It is soluble in chloroform and pyridine in the cold, in benzene and carbon tetrachloride only at elevated temperatures, and is insoluble in petroleum ether. Its partition coefficient between water and carbon tetrachloride is 12/1. When a chloroform solution of anhydrodethiogliotoxin is shaken with aqueous acid or alkali there is no significant decrease in concentration of the compound in the chloroform layer, indicating that it has no basic or acidic properties. In alcoholic solution it shows no color with ferric chloride, and it does not react with 2,4-dinitrophenylhydrazine.

(b) **Methanolic Hydrogen Chloride.**—In an attempt to repeat the procedure of Elvidge and Spring,[7] 300 mg. of dethiogliotoxin was dissolved at 0° in 40 ml. of anhydrous methanol saturated with dry gaseous hydrogen chloride. The solution was allowed to warm to 20° and left 24 hours, after which time the solution was distilled to dryness at 10 mm. pressure. The residue was taken up in 1 ml. of methanol and 6 ml. of ethyl ether added dropwise. After two days, when some of the solvent had been allowed to evaporate, 13 mg. of dense prisms, m.p. 168–170°, had separated. This substance, which appears to be different from any of the products described by Elvidge and Spring, was found to contain 9.54 and 9.35% nitrogen and the material exhibited a low order of ultraviolet absorption, at 250 mμ, $E^{1\%}_{1\ cm}$ 50. After the filtrate had been reduced to 1 ml. it yielded another 4 mg. of crystals, m.p. 164–170°, which were filtered off. No further attempt was made to characterize this substance.

The filtrate on evaporation to dryness left a semi-crystalline oil. Admixture of 3 ml. of water with the residue produced immediately a voluminous mass of fine needles, 103 mg., m.p. 156–158° with softening *ca.* 60–70°. After this material, presumably compound G of Elvidge and Spring,[7] had been heated at 100° and 5 × 10^{-3} mm. for two hours, the product melted at 156–158°. This substance, which is presumed to be their compound F, showed no depression of melting point when mixed with anhydrodethiogliotoxin. The ultraviolet absorption spectra of compound G and compound F appeared to be identical with the spectrum observed for anhydrodethiogliotoxin.

When anhydrodethiogliotoxin was recrystallized from water the crystal habit of the product was similar to that exhibited by compound G, indicating that compound G is a hydrated form of anhydrodethiogliotoxin (compound F).

Action of Alkali on Anhydrodethiogliotoxin.—Anhydrodethiogliotoxin (100 mg.) was refluxed with 3 ml. of 2 *N* methanolic potassium hydroxide for 20 minutes. After less than one minute of heating a copious crystalline precipitate had appeared in the refluxing solution. After the conclusion of the reaction the insoluble product was removed by centrifuging and reserved. The filtrate was acidified with 3 *N* hydrochloric acid and extracted repeatedly with chloroform. Removal of the chloroform yielded 57 mg. of crystalline material, m.p. 170–175°. This material melted at 184–185° after one recrystallization from chloroform and was identical with N-(2-indolecarbonyl)-N-methylalanine, m.p. 187–188°. A mixture of the two materials melted at 185–186°.

The insoluble product from the alkaline hydrolysis was collected and after washing with methanol weighed 36 mg.; it melted with darkening at 370°. The crude insoluble material obtained in a similar manner through the action of alkali on dethiogliotoxin[3] melted at 365°, and the melting point of a mixture of the two materials showed no depression. Their properties were similar in all respects. This high-melting substance was insoluble in aqueous acid and alkali, ethanol, chloroform and petroleum ether (60–70°), but sparingly soluble in acetic acid.

Anal. Calcd. for $C_{13}H_{12}N_2O_2$: C, 68.40; H, 5.30; N, 12.28. Found: C, 68.50, 68.30; H, 5.59, 5.46; N, 12.56, 12.72.

Recrystallization from acetic acid radically altered the behavior of the insoluble product at higher temperatures. The purified crystals browned slightly at 390°, and at this temperature a dark oil began to condense in the capillary above the melting-point block; at about 450° the crystals sintered and blackened, and liquid began to distil more rapidly from the capillary.

The ultraviolet absorption spectrum of the insoluble product shows three maxima, at 259, 277 and 285 mμ, with $E^{1\%}_{1\ cm}$ values of 325, 201 and 173, respectively (in glacial acetic acid). An attempt was made to determine optical rotation: 0.86 mg. in 1 ml. of glacial acetic acid (a supersaturated solution) in a 1-dm. tube at 25° showed a negligible rotation (+0.006°). Thus, the insoluble product is probably optically inactive.

Action of Acetic Anhydride on Anhydrodethiogliotoxin.—Anhydrodethiogliotoxin (100 mg.) and 1 ml. of acetic anhydride were heated in a sealed, evacuated Pyrex tube for one hour in an oil-bath at 235–240°. The reaction mixture was dissolved in 25 ml. of ethyl ether and the solution was washed four times with 5% sodium bicarbonate solution and once with water. After drying over magnesium sulfate and removal of the ether, there was obtained 87 mg. (94% yield) of a brownish oil, which crystallized after persistent rubbing under petroleum ether; m.p. 110–116°. This material was recrystallized once from methanol, with addition of Norit, to yield 36 mg. of dense, rhombic crystals, m.p. 121–122°. The melting point of a mixture of this material with authentic 2,3-dimethylpyrazinoindole-1,4-dione showed no depression. The ultraviolet absorption spectra[4] of the two substances were identical.

Reduction of Gliotoxin by Amalgamated Zinc: Deoxydethiogliotoxin.—A solution of 1.0 g. of gliotoxin in 200 ml. of ethanol (99.5%) was stirred rapidly at 25° with 30 g. of amalgamated zinc (granular, 10 mesh). A stream of nitrogen was bubbled slowly through the system and passed into a solution of lead plumbite to absorb hydrogen sulfide. When 2 ml. of concd. hydrochloric acid was added, evolution of hydrogen sulfide commenced immediately. More acid was added gradually as the stream of hydrogen slackened, until a total of 3.5 ml. had been introduced. After five hours there was no further evolution of hydrogen sulfide. The ethanolic solution was decanted from the unreacted zinc amalgam and the ethanol was distilled off under reduced pressure. The residue was taken up in 100 ml. of water and shaken with two 50-ml. portions of chloroform. The combined chloroform extracts were shaken three times with 100-ml. portions of water to remove dethiogliotoxin. After the chloroform solution had been dried with magnesium sulfate and solvent removed, 0.74 g. of crystalline material was obtained, m.p. 150–165°. In order to remove a persistent impurity, 0.37 g. of this material dissolved in 70 ml. of carbon tetrachloride was chromatographed on silica gel (50 g. of Davison Co., comm. grade, 200 mesh, in a column 22 × 170 mm.). The column, wet with carbon tetrachloride, initially was transparent except for the uppermost 2 cm., upon which the mixture was adsorbed. When a large volume of chloroform (U.S.P.) was passed through the column, a diffuse band gradually moved downward and away from the opaque upper band; the latter descended only slightly. The position of the upper band was marked, the corresponding portion of adsorbent removed from the column, and the adsorbate eluted with hot methanol, from which was obtained 0.27 g. of crystalline material, m.p. 160–170°. Upon repeated recrystallization from ethanol-petroleum ether (60–70°), prismatic needles were obtained, m.p. 183–185°, $[\alpha]^{25}D$ −238 ± 10° (*c* 0.9 in ethanol). Qualitative tests showed the presence of nitrogen and absence of sulfur.

Anal. Calcd. for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.29. Found: C, 63.01, 63.14; H, 6.53, 6.50; N, 11.40, 11.29.

The ultraviolet absorption of deoxydethiogliotoxin is al-

most identical with that of dethiogliotoxin[3] in the region 220–300 mμ, showing a maximum at 268 mμ, log ε 3.615. The infrared spectrum shows absorption in the C=C region, similar to that of gliotoxin and dethiogliotoxin. It is soluble in cold chloroform and dioxane, soluble in warm ethanol, methanol and benzene, insoluble in water and petroleum ether.

In an earlier experiment some dethiogliotoxin was isolated from the action of amalgamated zinc on gliotoxin in an aqueous medium. A slurry of 1.0 g. of gliotoxin in 150 ml. of water was stirred with 30 g. of zinc amalgam. When 1 ml. of concentrated hydrochloric acid was added, evolution of hydrogen sulfide commenced immediately. More acid, in all 3.5 ml., was added gradually until the reaction was complete. After removal of the zinc as sulfide, the filtered solution was evaporated to dryness in a vacuum. The dried product was powdered and extracted with hot chloroform in a soxhlet extractor. This gave 560 mg. of crystalline material m.p. 160–190°.

A sample of 260 mg. of this material was subjected to a seven-plate countercurrent distribution between 25-ml. portions of chloroform and water, and thereby separated into two distinct substances, one with maximum concentration at tube 1, and the second with maximum concentration at tube 7. Removal of solvent from tubes 1 and 2 yielded 30 mg. of material melting at 235–240°, which showed no depression of melting point when mixed with authentic dethiogliotoxin. The partition coefficient between water and chloroform for authentic dethiogliotoxin was found to be 3.1/1, which is in agreement with the position of the compound in the distribution scheme. The material from tubes 6 and 7 consisted of impure deoxydethiogliotoxin, m.p. 160–175°.

Action of Acids on Deoxydethiogliotoxin.—A solution of 50 mg. of deoxydethiogliotoxin dissolved in 2 ml. of concentrated hydrochloric acid was heated to 70° for 30 minutes. After 5 ml. of water and 2 g. of sodium bicarbonate had been added to the reaction mixture, it was extracted with chloroform. From the chloroform extract there was obtained 43 mg. of an oil, which yielded crystals, m.p. 91–100°, after persistent rubbing under petroleum ether. The material was recrystallized from petroleum ether (60–70°), from which it was obtained in characteristic colonies of needles, m.p. 95–98°, $[\alpha]^{25}D$ −68 ± 3° (*c* 1.9 in ethanol).

Anal. Calcd. for $C_{13}H_{14}N_2O_2$: C, 67.87; H, 6.13; N, 12.17. Found: C, 67.65, 67.59; H, 6.02, 6.07; N, 12.22, 12.25.

This compound, designated as the anhydro derivative of deoxydethiogliotoxin, exhibits an ultraviolet absorption spectrum very similar to that of anhydrodethiogliotoxin (Fig. 1). It has three maxima, at 251, 279 and 287 mμ, log ε 4.080, 3.611 and 3.548, respectively. It is soluble in most organic solvents with the exception of petroleum ether.

Desulfurization of Gliotoxin by Raney Nickel. (a) Tetrahydrodethiogliotoxin, M.p. 194–195°.—A solution of 0.25 g. of gliotoxin, 5 ml. of triethylamine, and 3 ml. of water in 150 ml. of ethanol was shaken with 5 g. of Raney nickel[17] at 25° under 45 p.s.i. of hydrogen for 40 minutes. The catalyst was removed by centrifuging and eluted twice with 50 ml. of ethanol. Three further 0.25-g. samples of gliotoxin were treated similarly, the four solutions and washings were combined, and the solvent was distilled off at 7 cm. pressure under nitrogen, yielding 0.67 g. of a clear oil. When 25 ml. of chloroform was mixed with the oil, most of it dissolved and the solution was decanted from a small amount of a crystalline, chloroform-insoluble residue.

When the chloroform solution was decolorized with Norit, filtered and the solvent removed, 0.65 g. of slightly yellowish oil was obtained, which crystallized in rosettes, m.p. 185–190°, after standing overnight at 5°. When the material had been recrystallized three times from chloroform–petroleum ether, it formed elongated plates, m.p. 194–195°, $[\alpha]^{25}D$ +73° (*c* 0.5 in chloroform). Qualitative tests showed the presence of nitrogen and the absence of sulfur.

Anal. Calcd. for $C_{13}H_{20}N_2O_4$: C, 58.19; H, 7.51; N, 10.44. Found: C, 58.48, 58.24; H, 7.51, 7.04; N, 10.56, 10.27.

The tetrahydrodethiogliotoxin derived from Raney nickel is soluble in water, ethanol, chloroform and acetone, but is insoluble in ethyl ether and petroleum ether. It possesses a partition coefficient water/chloroform of 6.3/1. The ultraviolet absorption spectrum shows only low general absorption (log ε 2.56 at 240 mμ, 2.25 at 260 mμ, 2.03 at 300 mμ).

The chloroform-insoluble material separated from the crude reduction product (18 mg.), darkened at 200° and decomposed at 310°. It was soluble in ethanol. The ultraviolet absorption spectrum exhibited only low general absorption.

Countercurrent distribution studies using water and chloroform as the two immiscible phases were made of the mixtures of products obtained by the action of Raney nickel under various conditions. When Raney nickel desulfurization was carried out at 25° in the presence of triethylamine as described above, the tetrahydrodethiogliotoxin, m.p. 194–195°, was the principal component of the mixture. When the triethylamine was omitted but conditions were otherwise similar, the above compound comprised only about 40% of the mixture, and the main component had a partition coefficient water/chloroform of 0.6/1. The compound formed in this manner was not characterized further. Refluxing an ethanolic solution of gliotoxin with Raney nickel yielded a mixture of which all the components were far more soluble in chloroform than in water and the mixture could not be resolved by this scheme.

(b) Tetrahydrodethiogliotoxin, M.p. 155–156°.—The following procedure was used by Dr. Dutcher for desulfurization with Raney nickel[10]: A sample of gliotoxin was refluxed in ethanol with a large excess of Raney nickel until no more hydrogen sulfide was evolved and a test portion of the solution gave no coloration when treated with alkali. The catalyst was filtered off and the filtrate evaporated to dryness. The weight of the residue was about 50% of the weight of gliotoxin used. The residue was dissolved in a small amount of chloroform, and the solution was diluted with benzene and chromatographed over alumina. More chloroform was added to develop the column.

Fractional elutions were made arbitrarily and the solvent evaporated off. From the early fractions some gliotoxin crystals were obtained as well as a small amount of a nitrogen-free compound which melted at 59° (*Anal.* C, 76.2; H, 11.8). This substance is soluble in hexane and in chloroform, insoluble in ethanol or water.

The early fractions furnished also a small amount of crystalline material which, when purified, melted at 122° and proved to be identical with the hydriodic acid reduction product of gliotoxin (I).

The next fractions gave the principal product, m.p. 155–156°, $[\alpha]D$ +50°. This substance is readily soluble in water, ethanol, acetone and chloroform. Its composition agrees with that of a tetrahydrodethiogliotoxin.

Anal. Calcd. for $C_{13}H_{20}N_2O_4$: C, 58.19; H, 7.51; N, 10.44. Found: C, 58.31; H, 7.58; N, 10.45; carbon-methyl,[14] 0.0.

The reduction product does not give an iodoform test, does not give the Ehrlich color test, and does not give a precipitate with 2,4-dinitrophenylhydrazine. It shows only end absorption in the ultraviolet.

The final eluates, which were nearly pure chloroform, furnished a small amount of crystals by addition of ether; m.p. 191°, $[\alpha]D$ +75° (in water). This material is believed to be identical with the compound described in the preceding preparation, m.p. 194–195°, $[\alpha]^{25}D$ +75° (in chloroform).

(17) R. Mozingo, *Org. Syntheses*, **21**, 15 (1941).

ITHACA, N. Y.