TABLE I

No. of fractions[a]	Collected, ml.	Weight, mg.	Melting point, °C.
I	20	9	173–175
II	20	13	174–175
III	10	15	Soft. at 174 Melted at 224
IV	15	17	224–226
V	25	28	226–227
VI	25	7	226–227
VII	25	5	226–227

[a] All fractions were eluted with ethyl acetate except for VII which was eluted with ethyl acetate and methanol.

chloric acid and kept at 38–40° for a week. The solution was evaporated off under vacuum; the residue was taken up with water, filtered and washed with water. The filtrate did not give sugar test. The residue was recrystallized from ethyl acetate, dried and weighed (98 mg., m.p. 226–227°).

(2) **With 2% Sulfuric Acid.**—One hundred mg. of corchorgenin dissolved in 5 ml. of ethanol (absolute) was refluxed on a water-bath with 2% sulfuric acid for about 4 hours. Some resinous matter separated. The filtrate did not reduce Fehling solution nor yield an osazone.

(3) **With Hydrochloric Acid and Acetone.**—One hundred mg. of corchorgenin was treated with 10 ml. of acetone containing 3–4 drops of 0.01 *N* HCl and kept at 20–21° for 6 days. The solution was distilled off under vacuum and the residue was extracted with water and filtered. The filtrate did not give a positive test for sugar.

TABLE II

Fraction	Collected, ml.	Weight, mg.	Melting point, °C.
I	10	5	240
II	15	8	240–242
III	20	26	240–242
IV	25	38	240–242
V	25	13	240–242
VI	30	7	240–242

Corchorgenin Acetate.—Two hundred mg. of corchorgenin in 2–3 ml. of pure dry pyridine was acylated with acetic anhydride (4 ml.) at room temperature. The reaction product on crystallization from ethanol yielded corchorgenin acetate, m.p. 240–242°. This acylated product (100 mg.) was chromatographed in ethyl acetate solution through a column of zinc carbonate. All the fractions were eluted with ethyl acetate, collected and analyzed (Table II).

All these fractions (97 mg.) were combined and recrystallized from ethanol.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.23; H, 7.66. Found: C, 67.08; H, 7.79.

This acetate was hydrolyzed with 2% methanolic $KHCO_3$ solution; the regenerated product on crystallization from ethyl acetate melted at 227°; the melting point was not depressed by admixture with corchorgenin.

Lactone Titration.—0.0946 g. of corchorgenin in 25 ml. of neutral ethanol was refluxed with 10 ml. of 0.1 *N* KOH on a water-bath for 30 min. The excess alkali was titrated with (1.1316) 0.1 *N* HCl with phenolphthalein as the indicator. The alkali used up in the experiment was 0.0127 g. of KOH; the theoretical amount for one lactone ring is 0.0131 g. of KOH.

Isocorchorgenin.—One hundred mg. of corchorgenin was treated with 10 ml. of 5% methanolic KOH, kept at room temperature for 3 hours, and then poured into water; the precipitate (15 mg.) was filtered off. The filtrate was acidified with dilute HCl to congo red and warmed to 40° for about 5 min. The resulting precipitate was filtered, washed free of acid, crystallized from dilute ethanol and dried over P_2O_5; m.p. 200°. It did not give a Legal test; it was soluble in alkali and could be reprecipitated with acid.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.2; H, 7.9. Found: C, 67.98; H, 8.10.

Corchorgenin yielded no oxime or phenylhydrazone.

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WEST BENGAL, INDIA

[CONTRIBUTION FROM THE BOTANY DEPARTMENT, CORNELL UNIVERSITY]

The Detection of the Keto Acids of Plants. A Procedure Based on their Conversion to Amino Acids

BY G. H. N. TOWERS, J. F. THOMPSON AND F. C. STEWARD

RECEIVED NOVEMBER 11, 1953

A method for the identification and quantitative determination of keto acids in plants has been described. The 2,4-dinitrophenylhydrazones of the keto acids are converted to amino acids by catalytic hydrogenolysis and determined by means of quantitative paper chromatography. This has been done for α-ketoglutaric, oxaloacetic, pyruvic and glyoxylic acids all of which occurred in the plant materials examined. It has been found, by the use of this method, that a number of hitherto unreported keto acids occur in plants and many of these have as yet to be identified. A key to the recognition of the unidentified keto acids that occur in certain plant materials is given and the amounts of the identifiable keto acids that occur in three unrelated types of plant material have been determined. Attention is drawn to the implications of these results.

Introduction

The α- and β-keto acids occupy a central position in modern views of carbohydrate and nitrogen metabolism in plants. Since the development of paper chromatography, much has been learned about the range of nitrogen compounds that occur in plants. It is now possible to apply similar techniques to the study of the keto acids. This is important because the keto acids may be expected to be indicators of metabolic pathways.

The keto acids concerned in the oxidative cycle in plants have been inferred largely from enzyme studies and it is doubtful whether a complete analysis has been made for the keto acids of any one plant.

The scant information regarding the keto acids of plants is the natural consequence of the low concentrations of these compounds in plants and the difficulties encountered in the application of current methods[1-4] for their identification and determina-

(1) S. M. Altman, E. M. Crook and S. P. Datta, *Biochem. J.*, **49**, lxiii (1951).

(2) D. Cavallini, N. Frontali and G. Toschi, *Nature*, **163**, 568 (1949).

(3) D. Seligson and B. Shapiro, *Anal. Chem.*, **24**, 754 (1952).

(4) A. I. Virtanen, J. K. Miettinen and H. Kuntu, *Acta Chem. Scand.*, **7**, 38 (1953).

tion. Most of the earlier methods were devised for animal tissues.[5-8] Some of these methods have been found unsuitable for plant materials.[9,10] Specific methods for the determination of each of the three keto acids, pyruvic, oxaloacetic and α-ketoglutaric acids, have been described.[11,12] However, these methods are unsuitable for the recognition of hitherto unidentified keto acids in plants. Since there was no generally applicable method for the recognition and estimation of these compounds in plants, the methods to be described were developed.

The Method

Paper chromatography of 4-nitrophenylhydrazones or 2,4-dinitrophenylhydrazones was tried. At the outset this technique presented problems, the chief of these being that certain hydrazones may give rise to two spots.[1,3,4,13] One-directional chromatograms of mixtures of four or five keto acids become difficult to interpret and two-directional chromatograms are even more difficult, since each of the two spots in the first direction may in turn give rise to two further spots in the second.

The hydrogenolysis of keto acids through the hydrazones to the corresponding amino acids has been known for a long time.[14] Catalytic hydrogenolysis of the oximes, phenylhydrazones, 4-nitrophenylhydrazones, 4-bromophenylhydrazones, 2,4-dinitrophenylhydrazones, nitroguanylhydrazones, 1,1-diphenylhydrazones was therefore studied in the course of this work. All of these derivatives can be reduced to give the amino acid corresponding to the keto acid, but, for one reason or another, most of them were found unsuitable with the exception of the 2,4-dinitrophenylhydrazones. For example, by the use of 1,1-diphenylhydrazones, good yields of the corresponding amino acids may be obtained in most cases (see Tables I, II and III) but oxaloacetic acid gives alanine instead of aspartic acid (Table II). Since the 1,1-diphenylhydrazone of pyruvic acid also gives alanine on hydrogenolysis, oxaloacetic could not be differentiated from pyruvic in this way. In some cases very low yields of amino acids were obtained on hydrogenolysis of the hydrazones, *e.g.*, with the nitroguanylhydrazones. The 2,4-dinitrophenylhydrazones of the keto acids were found to be most useful for the following reasons.

(1) Good yields of amino acids are obtained on hydrogenolysis of the corresponding hydrazones.

(2) Although there is a slight degree of decarboxylation in the process of hydrogenolysis, oxaloacetic acid can be recognized as aspartic acid by this method.

(3) The separation and identification of the keto acids in the form of their 2,4-dinitrophenylhydrazones is facilitated by the well-defined crystalline form of these substances.

The amino acids resulting from the hydrogenolysis can be chromatographed on paper and detected with ninhydrin.[15] The extensive knowledge of the paper chromatography of amino acids should facilitate the identification of unknown keto acids.

TABLE I

YIELDS OF ALANINE FROM CATALYTIC HYDROGENOLYSIS OF SOME HYDRAZONES OF PYRUVIC ACID

Hydrazones	Catalyst	Solvent	Yield, %
Phenyl	Raney nickel	50% ethanol	None
Phenyl	Platinum oxide	50% ethanol	58.2
Phenyl	Platinum oxide	50% acetic acid	3.0
1,1-Diphenyl	Platinum oxide	95% ethanol	68.5
1,1-Diphenyl	Platinum oxide	Ether–HCl	88.1
Nitroguanyl	Platinum oxide	95% ethanol	6.0
2,4-Dinitrophenyl	Platinum oxide	Distilled water	66 0

TABLE II

YIELDS OF ASPARTIC ACID AND ALANINE FROM CATALYTIC HYDROGENOLYSIS OF SOME HYDRAZONES OF OXALOACETIC ACID

Hydrazones	Catalyst	Solvent	Yield, %
Phenyl	Palladium	50% ethanol	Alanine 5 Aspartic 0
1,1-Diphenyl	Platinum oxide	95% ethanol	Alanine 71 Aspartic 0
1,1-Diphenyl	Platinum oxide	95% ethanol–HCl	Alanine 69 Aspartic 0
2,4-Dinitrophenyl	Platinum oxide	Distilled water	Alanine 5 Aspartic 33

TABLE III

YIELDS OF GLUTAMIC ACID AND γ-AMINOBUTYRIC ACID FROM CATALYTIC HYDROGENOLYSIS OF SOME HYDRAZONES OF α-KETOGLUTARIC ACID

Hydrazones	Catalyst	Solvent	Yield, %	
1,1-Diphenyl	Platinum oxide	95% ethanol	Glutamic	86
			γ-Aminobutyric	Trace
Nitroguanyl	Platinum oxide	95% ethanol	Glutamic	5
			γ-Aminobutyric	Trace
2,4-Dinitrophenyl	Platinum oxide	Distilled water	Glutamic	36.4
			γ-Aminobutyric	1.2

Experimental

The plant material (10 g.) is killed by homogenizing in 120 ml. of ethanol containing 50 mg. of 2,4-dinitrophenylhydrazine and 0.25 ml. of concd. H_2SO_4. After standing for an hour the homogenate is centrifuged and the supernatant decanted and taken to dryness at room temperature by means of a stream of air. The tissue residue, in the centrifuge tube, is resuspended in ethyl acetate and again centrifuged and the second supernatant collected. This extraction procedure is repeated once more and the combined

(5) G. D. Lu, *Biochem. J.*, **33**, 249 (1939).

(6) T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).

(7) P. Ostern, *Z. physiol. Chem.*, **218**, 160 (1933).

(8) N. L. Edson, *Biochem. J.*, **29**, 2080 (1935).

(9) F. C. Steward and H. E. Street, *Ann. Rev. Biochem.*, **16**, 471 (1947).

(10) W. O. James, G. M. James and A. H. Bunting, *Biochem. J.*, **35**, 588 (1941).

(11) H. A. Krebs, *ibid.*, **32**, 108 (1938).

(12) A. I. Virtanen, A. A. Arhimo, J. Sundman and L. Jannes, *J. prakt. Chem.*, **162** NS, 71 (1943).

(13) B. Axelrod, R. S. Bandurski, C. M. Greiner and R. Jang, *J. Biol. Chem.*, **202**, 619 (1953).

(14) E. Fischer and R. Groh, *Ann.*, **383**, 363 (1911).

(15) NOTE ADDED IN PROOF.—Kulonen, *Scand. J. Clin. Lab. Invest.* **5**, 72–74, has applied a similar procedure to animal extracts.

supernatants are added to the evaporated ethanolic extract which goes into solution in the ethyl acetate. This ethyl acetate solution is filtered and washed with a small volume of water to remove excess acid and it is then extracted with 1% aqueous sodium carbonate. The alkaline extract is retained and acidified with 5 *N* sulfuric acid to a *p*H of around 2. The acidified solution containing the hydrazones is extracted with ethyl acetate and in this sequence of steps, the free amino acids, the hydrazones of neutral carbonyl compounds and a large number of other contaminating substances, including excess reagent, are removed. The final ethyl acetate solution is washed with a small volume of water and an aliquot is transferred to the vessel used in the catalytic hydrogenation. The aliquot is taken to dryness by means of a stream of air at room temperature and resuspended in distilled water. Adams catalyst[16] is added and the solution hydrogenated at room temperature and at 30 lb. per sq. inch for 6–12 hours in a Parr hydrogenator.

The products of the hydrogenation are filtered and the filtrate is evaporated to dryness at room temperature by means of a stream of air and the dried filtrate is redissolved in 80% ethanol, filtered and made to a small volume. The amino compounds in this solution can be detected by two-directional chromatography on paper and subsequent spraying with ninhydrin. A chromatogram of a comparable amount of the non-hydrogenated hydrazones is made at the same time to serve as a check in case any amino acids are present as contaminants.

Before proceeding to the catalytic reduction it is possible to learn something about the nature of the keto acids present in the extract by chromatography of the 2,4-dinitrophenylhydrazones on paper. This is done as follows.

The ethyl acetate extract containing the alkali-soluble 2,4-dinitrophenylhydrazones is passed through a column of alumina. The column is washed with ethyl acetate followed by 95% ethanol. The keto acid hydrazones are eluted with water followed by 1% aqueous sodium carbonate. The aqueous eluates are acidified and extracted with ethyl acetate. In this way, many interfering substances are removed and the technique is particularly valuable where qualitative identification is being attempted. It is not recommended for quantitative work because losses are sustained on the alumina column. At this point the solution of dinitrophenylhydrazones of the keto acids may be chromatographed on paper.

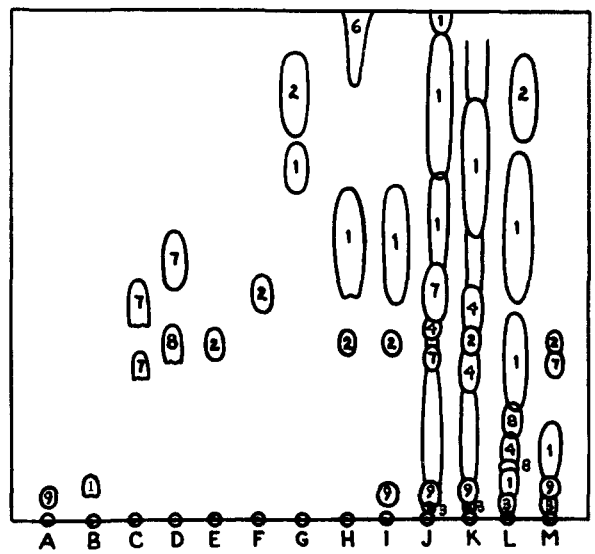

Fig. 1.—Diagram of one-directional chromatograms of 2,4-dinitrophenylhydrazones of acidic carbonyl compounds obtained from plants and of synthetic keto acids: A, α-ketoglutaric; B, oxaloacetic; C, glyoxylic; D, pyruvic; E, unknown from *Mentha* leaves; F, unknown from *Tulipa* leaves; G, α-ketoisocaproic; H, *Chlorella;* I, *Mentha* leaves; J, potato tuber; K, *Mentha* roots; L, *Tulipa* leaves; M, *Glycine* root nodules. Solvent: isoamyl alcohol:ethanol: water, 5:1:4. NH_3 vapors in chromatography chamber. Key to colors with alkali: 1, brown; 2, yellow; 3, blue; 4, pink; 5, brown on heating; 6, grey; 7, salmon; 8, chocolate; 9, olive green.

(16) Platinum oxide obtained from J. Bishop & Co., Malvern, Penna.

For quick identification, 5 × 5 inch papers of Whatman No. 1 are used. The ascending method is adopted using standard American museum jars (6″ × 6″ × 3½″) as the chromatographic cabinet. The solvent[2] used is a 5:1 mixture of isoamyl alcohol and ethanol equilibrated with water and it is advisable to place a small vial of ammonium hydroxide in the cabinet. In 2 to 3 hours sufficient separation is obtained to give some idea of the substances present. A diagram showing representative chromatograms at this stage is shown in Fig. 1. The chromatograms are finally sprayed with alkali to intensify the color and to make them more representative of the specific dinitrophenylhydrazones.

Experimental Results

By the use of these procedures it is readily apparent that pyruvic, oxaloacetic and α-ketoglutaric acids are not, by any means the only keto acids present in plant materials. In Table IV are listed the keto acids which have been found in plants by this method and which are recognizable because they yield known amino acids. The great range in the amounts of these substances in different plants and organs is noteworthy.

TABLE IV

QUANTITATIVE DETERMINATIONS OF KETO ACIDS IN PLANT TISSUES[a]

Keto acid	Amino acid by hydrogenolysis	Amount of keto acid (μmole × 10^{-4}/g.f.w.) Mint[b] leaves	Carrot[c] roots	Potato[d] tuber
α-Ketoglutaric	Glutamic	1279	279	25.0
	γ-Aminobutyric acid	20	16	2.0
Oxaloacetic	Aspartic acid	Trace	15	Trace
Pyruvic	Alanine	123	21	4.4
Glyoxylic	Glycine	Trace	4	19.0

[a] The unidentified keto acids are shown as their amino compounds in Fig. 2. [b] *Mentha piperita*. These plants were grown under artificial conditions at 60°. Plants under other conditions have been found to contain even greater amounts of keto acids in their leaves. For example mint plants with α-ketoglutarate as high as 7 μmols per g. f. w. have been encountered. [c] Carrot roots have been analyzed in which the amounts of α-keto glutarate, pyruvate, and glyoxylic acid were almost equal. [d] Potato tuber tissue has been analyzed in which the main keto acid was glyoxylic reaching values of the order of 0.07 μmol per g. f. wt.

In Fig. 2 are shown the locations of a number of amino acids whose identity is still unknown and which correspond to keto acids whose identity is therefore also unknown at present. None of these compounds could have been derived from ascorbic acid by these procedures. The possibility that osazones (formed by 2,4-D.N.P. and which are sufficiently soluble in 1% sodium carbonate, *e.g.*, of uronic acids) may contribute ninhydrin reactive compounds after hydrogenolysis has not been eliminated.

From these results two outstanding conclusions may be drawn. First, it is now apparent that more keto acids occur in plants than had been recognized previously (Fig. 1). Secondly, it is evident that it is necessary to identify the several unknown keto compounds as they may be expected to be important intermediates in metabolism. The metabolic implications of these results have yet to be worked out.

Some Quantitative Data on the Content of Keto Acids in Plants.—A quantitative method for the accurate determination of the amino acids which is based on two-directional paper chromatography, has been described.[17] Whatman No. 1 (18 × 23 inches) paper, washed with 0.3 *N* HCl followed by 0.5 *N* NaOH and then distilled water to remove salt and excess alkali, is used. The papers are dried and 0.1% sodium phosphate buffer, *p*H 7.0 sprayed on. The solvent systems are water-saturated phenol (*p*H 5.5) for the first direction and 3:1 lutidine:collidine saturated with water and at *p*H 8.5 for the second direction. After chromatography, the paper is dried at room temperature and treated with 1% ninhydrin in 95% ethanol. The color, resulting from reaction with amino acids, is developed in a chamber containing CO_2 in equilibrium with liquid ethanol at 60° for 30 minutes. The colored spots, representing the amino acids, are cut out, weighed and extracted with 50% ethanol. The density of coloration is determined spectrophotometrically at 570 mμ for blue and purple spots and 330 mμ for brown spots, *e.g.*, proline. Blank determinations on weighed pieces of paper unoccupied by ninhydrin spots are determined for every paper sheet. The amounts of amino acids as so determined, are then calculated from standard curves.

Since the keto acid 2,4-dinitrophenylhydrazones are converted to amino acids by catalytic hydrogenolysis, quantitative determinations of the keto acids may also be made in this way. However, it is necessary to ask how quantitative is the catalytic hydrogenolysis. The yields of amino acids from hydrogenolysis of the corresponding hydrazones are shown in Tables I, II, III and V. The determinations of the total amino acids were originally made by the method of Moore and Stein.[18] By this means, the values were somewhat high because of the color of the hydrogenated solution, but a more accurate determination can be made by paper chromatography of the hydrogenated solution and analysis of the individual amino acid spots by the method of Thompson and Steward.[17] It is possible, therefore, to estimate the keto acid content of plant materials in this manner. Data for three different types of plant tissue are given in Table IV.

TABLE V

YIELDS OF AMINO ACIDS FROM CATALYTIC HYDROGENOLYSIS OF KETO ACID 2,4-DINITROPHENYLHYDRAZONES[a]

Keto acid 2,4-dinitrophenylhydrazone	Amino acid by hydrogenolysis	Yield, %
α-Ketoglutaric	Glutamic	36.4
	γ-Aminobutyric	1.2
Oxaloacetic	Aspartic	33.0
	Alanine	4.9
Pyruvic	Alanine	65.3
Glyoxylic	Glycine	97.8

[a] Average of three determinations except in the case of glyoxylic acid where only one determination was made.

γ-Aminobutyric acid can arise in two ways, (a) by decarboxylation of α-ketoglutaric acid 2,4-dinitrophenylhydrazone during hydrogenolysis or (b) by hydrogenolysis of the 2,4-dinitrophenylhydrazone of succinic semialdehyde. At present, the only way in which one could distinguish between the γ-aminobutyric acid arising in these two ways would be in those analyses where the quantity of γ-aminobutyric acid exceeded the amount that could be accounted for by characteristic decarboxylation of α-ketoglutaric 2,4-dinitrophenylhydrazone, *i.e.*, about 3%. Where γ-aminobutyric appears (after hydrogenolysis of the hydrazones) in amounts greater than this, it is open to the interpretation that it arises from succinic semialdehyde.

(17) J. F. Thompson and F. C. Steward, *Plant Physiol.*, **26**, 375 (1951).

(18) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1949).

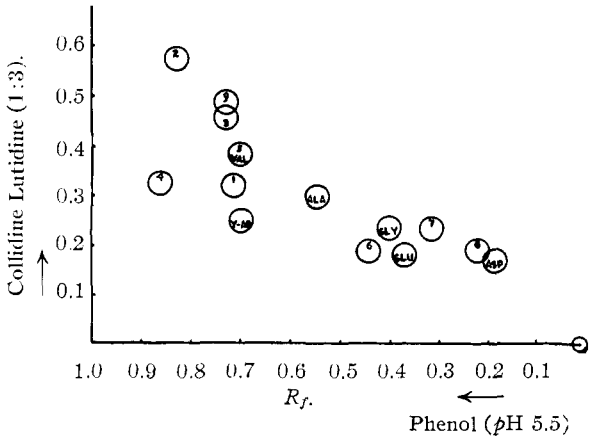

Fig. 2.—Location of ninhydrin reacting compounds on paper chromatograms resulting from hydrogenolysis of 2,4-dinitrophenylhydrazones of plant keto acids. Where the identity of the amino compound is known it is designated by name; where it is unknown it is designated by number. 1, occurs in all plant tissues examined. Not an α-amino acid. Stable to acid hydrolysis; m.p. of 2,4-dinitrophenylhydrazone 214–215° dec.; 2, occurs in root nodules of *Glycine max*, leaves of *Tulipa* and *Mentha piperita;* 3, occurs in leaves of nearly all plants which have been examined—is distinguishable from the leucines in other solvents; 4, occurs in potato tuber; 5, occurs in leaves and bulbs of *Tulipa*. Co-chromatographs with valine in three solvents; 6, occurs in leaves and bulbs of *Tulipa*. Co-chromatographs with γ-methylglutamic acid in three solvents; m.p. of derivative with 2,4-dinitrophenylhydrazine 244°; 7, occurs in leaves of *Tulipa* and *Mentha piperita;* 8, occurs in leaves of *Tulipa;* 9, occurs in leaves of *Mentha piperita* and in carrot tissue; co-chromatographs with the leucines in phenol and in colidine:lutidine but is separable from them in butanol:acetic acid. There is some indication that this substance becomes more conspicuous if the tissue is allowed to stand at room temperature in the acid–alcohol–hydrazine solution for a few days or longer.

The general implications of this work are obvious. Further investigation is necessary to determine the full range of keto compounds that occur in plants and their distribution in the plant kingdom and also to determine the reasons for the large differences met with in one organism. The extension to the list of familiar keto acids which it has been possible to make by this first application of the method suggests that more knowledge of these highly labile compounds can be obtained by this means. This may have important implications in the field of intermediary metabolism of carbohydrates and, through transamination, in the field of nitrogen metabolism. The prominent occurrence of several as yet unidentified keto compounds in green leaves also raises the question of the extent to which their role is determined by light and their possible relation, if any, to photosynthesis.

Acknowledgments.—This work commenced in the Botany Department, University of Rochester,

at which time one of us (J. F. T.) worked as a Post Doctoral Fellow of the National Institute of Health. The association of G. H. N. T. with the work was made possible by a Lalor Foundation Fellowship tenable at Cornell University. The assistance of Mr. R. Rabson with the quantitative work is acknowledged. The completion of the work at Cornell University was assisted by the Grasselli Grant to Cornell University for work being directed by one of us (F. C. S.). Grateful acknowledgment of all this help is now made.

ITHACA, NEW YORK

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MERCK & CO., INC., RAHWAY]

Isomerization and Displacement Reactions in the Cyanopregnene Series. Synthesis of Cortisone 21-Methyl Ether

BY HUANG-MINLON,[1a] ROGER TULL AND JOHN BABCOCK[1b]

RECEIVED NOVEMBER 10, 1953

20-Cyano-17-pregnene-3(α),21-diol-11-one has been found to undergo geometrical inversion under alkaline conditions accompanied by ether formation at position 21. This discovery has enabled the synthesis of 17-hydroxydehydrocorticosterone-21-methyl ether.

20-Cyano-17-pregnene-3(α),21-diol-11-one 3,21-diacetate (Ia) is deacetylated readily by brief treatment with methanolic alkali to give the corresponding diol (I).[2] It has been found that prolonged treatment of Ia with methanolic alkali leads to the formation of two new substances: A, m.p. 234–235° and B, m.p. 221–222°. Although the ultraviolet and infrared spectra of these substances clearly indicated the retention of the cyanoethylene system, their non-identity with 20-cyano-17-pregnene-3(α),21-diol-11-one (I) was demonstrated by mixed melting point comparison. The same two substances, A and B, were formed by reaction of the pure diol I with methanolic alkali. The proportion of the two products formed was found to depend on the length of time the reaction mixture was allowed to stand. Thus, when a solution of the diol I in 3% methanolic alkali was allowed to stand for 24 hours or longer, substance B, m.p 221–222°, could be isolated in over 80% yield in addition to a small amount of substance A, m.p. 234–235°. When the reaction time was shortened to three or four hours, however, a mixture of substances A and B together with some unchanged starting material was obtained in which substance A, m.p. 234–235°, predominated.

It was suggested from the analytical data alone that substance B might be a configurational or positional isomer of the starting diol I having structure II or XI, respectively. It was found, however, that substance B formed only a monoacetate, m.p. 158–159° even after prolonged refluxing with acetic anhydride. This monoacetate derivative, moreover, exhibited no free hydroxyl band in the infrared spectrum thereby excluding structures II or XI for this substance. The possibility remained that the 21-hydroxyl group of the starting diol I had been replaced by a methoxyl group to give the methyl ether (III). Consistent with this consideration III should form a monoacetate possessing no free hydroxyl function. Structure III for substance B was confirmed by active hydrogen and methoxyl group determinations performed on III and its monoacetate, respectively. Ozonolysis of III, furthermore, yielded the known 17-ketone (V),[3] whereas hydroxylation with osmium tetroxide afforded pregnane-3(α),17(α),21-triol-11,20-dione 3-monoacetate 21-methyl ether (VII) (see below) in analogy with the conversion of I to dihydrocortisone (VI).[2]

In order to demonstrate that the 21-hydroxyl function of I had become displaced by a methoxyl group from the solvent, methanol, I was in turn treated with ethanolic alkali. In this case a new substance, m.p. 144–145°, was obtained together with the above-mentioned substance A, m.p. 234–235°. The analysis, ultraviolet and infrared spectra of the new product, m.p. 144–145°, agreed with the 21-ethoxy structure IV. The latter also formed a monoacetate, m.p. 139.5–140.3°, and the presence of an ethoxyl group was confirmed by Zeisel determination.

Substance A, m.p. 234–235°, was found to be the $\Delta^{17,20}$-geometrical isomer of the starting diol I and is therefore to be formulated as II.[4] Thus when the diol I was treated with alkali in aqueous dioxane, only II along with some unchanged I could be isolated. II formed a diacetate, m.p. 135°, still in possession of the cyanoethylene system and exhibiting no hydroxyl band in the infrared spectrum. The structure of II was confirmed by conversion of its 21-monoacetate through osmylation and oxidation to VI, identical with that obtained from the 21-monoacetate of I by the same sequence of reactions.[5]

It is interesting to note that II may be the intermediate product in the formation of III from I. As mentioned above, I is largely converted to II on short standing with methanolic alkali but gives III

(1) (a) Huang-Minlon, 178 Sin Lo Road, Shanghai, China; (b) John Babcock, Upjohn Company, Kalamazoo, Mich.

(2) L. H. Sarett, THIS JOURNAL, **70**, 1454 (1948).

(3) L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

(4) Inspection of the models of I and II indicates that in I there is a definite crowding of groups between C_{12} and C_{21} as well as a distinct restriction to free rotation of the C_{21}-hydroxymethylene group about the axis of the C_{20}-C_{21} bond. In contrast thereto no apparent steric interference is offered to the linear —C≡N group in II. It may therefore be concluded tentatively that II represents the more stable geometrical species and consequently is the configuration to be assigned to the predominant isomer arising from the base-catalyzed inversion.

(5) Private communication from Dr. R. E. Jones and Mr. S. A. Robinson of these laboratories.