4. A compound which is at once a salt and an acid chloride of phosphoric acid has been isolated.

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A FURTHER STUDY OF THE UTILITY OF ETHYL γ,γ-DIETHOXY-ACETO-ACETATE AS A REAGENT FOR THE SYNTHESIS OF GLYOXALINES

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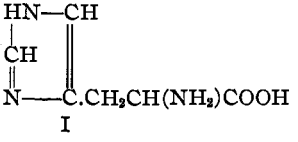
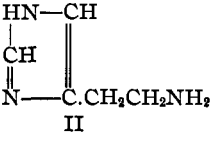
The established therapeutic value of the glyoxaline derivative histamine II, and its relationship structurally to the naturally occurring α-amino acid histidine I, have stimulated a special interest in all possible methods of preparing these two combinations or any of their derivatives synthetically. For the known methods of synthesis thus far developed for



I



II

the preparation of the amino acid (I) we are indebted to Pyman,[2] who has used citric acid (III) as his starting point and developed successfully two processes for preparing this amino acid (I), the major steps of which are expressed below.[3]

(1) $HOCH(CH_2COOH)_2$ (III) $\longrightarrow$ $OC(CH_2NH_2)_2$ (IV) $\longrightarrow$ $R.CH_2NH_2$ $\longrightarrow$ $R.CH_2Cl$ (V) $\longrightarrow$ $R.CH_2CHClCOOH$ $\longrightarrow$ $RCH_2.CH(NH_2)COOH$ (I)

(2) $R.CH_2OH$ (VI) $\longrightarrow$ $R.CHO$ (VII) $\longrightarrow$ $RCH{:}C(COOH)NHCOC_6H_5$ $\longrightarrow$ $R.CH_2.CH(NH_2)COOH$ (I)

Neither method is productive of the α-amino acid I in large yield, but of the two syntheses the second is apparently the best. Method 1 is singularly dependent for its application on the successful production of chloromethyl-glyoxaline V, while the second synthesis is wholly dependent for success on the availability of the aldehyde derivative of glyoxaline VII. Any methods for obtaining either of these two organic combinations would enhance greatly the practicability of Pyman's two syntheses.

[1] Constructed from a dissertation presented by Edward Wells Rugeley, to the Faculty of the Graduate School of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 1923.

[2] Pyman, *J. Chem. Soc.*, **99**, 1386 (1911); **109**, 186 (1916).

[3] R is the glyoxaline nucleus, HN.CH=N—CH=CH.

When one considers the preparation of the base histamine II it is apparent that the success of Windaus and Vogt's[4] original synthesis is practically dependent on the availability of β-glyoxalpropionic acid, $OCH.COCH_2CH_2COOH$, which was first synthesized by Wolff.[5] The second method of synthesis, which was patented by Pyman,[6] utilizes as a starting point chloromethylglyoxaline V, one of the intermediate products in Pyman's first method of histidine synthesis. Koessler and Hanke,[7] who have made improvements in the technique of Pyman's synthesis, report a yield of 165 g. of histamine hydrochloride from 4530 g. of citric acid. In other words, the key intermediates, which are essential for the successful application of these synthetic operations leading to histidine and histamine, are the primary alcohol and aldehyde derivatives of glyoxaline represented by Formulas VI and VII, respectively, and β-glyoxalpropionic acid first synthesized by Wolff.[5]

Johnson and Pucher[8] have previously referred to the possibility of utilizing the β-ketone ester, ethyl-γ,γ-diethoxy-aceto-acetate, as a key intermediate in the development of new methods of entering synthetically the glyoxaline series, and undertook the study of one possible method of approach, when they investigated the action of bromomethyl-phthalimide on the sodium salt of this ketone ester. Their method, however, failed to develop successfully as the phthalimido halide interacted abnormally with regeneration of phthalimide, thereby excluding this procedure as a method of approach.

In continuing our investigation of the reactions of this interesting ketone ester we have now tested experimentally several other synthetic possibilities with the hope of demonstrating the utility of this ketone ester or some derivative of it as a key for a new glyoxaline synthesis. The specific reactions with which this research has been concerned are the following.

1. Production of the isonitroso derivative of ethyl-γ,γ-diethoxy-aceto-acetate and its reduction to the corresponding amino derivative (VIII).

$$(C_2H_5O)_2CH.COC:(NOH)COOC_2H_5 \longrightarrow \underset{\text{VIII}}{(C_2H_5O)_2CHCOCH(NH_2)COOC_2H_5}$$

2. Bromination of the β-ketone ester and a study of the action of the resulting halogen derivative, $(C_2H_5O)_2CH.CO.CH(Br)COOC_2H_5$, IX, on urea and ammonia.

3. The condensation of ethyl diethoxy-acetate with ethyl hydantoate

[4] Windaus and Vogt, *Ber.*, **40**, 3691 (1907). Knoop and Windaus, *Beitr. Chem. Physiol. Path.*, **7**, 144 (1906).

[5] Wolff, *Ann.*, **260**, 91 (1890).

[6] Pyman, Brit. pat. 28,538.

[7] Koessler and Hanke, THIS JOURNAL, **40**, 1718 (1918).

[8] Johnson and Pucher, *ibid.*, **44**, 817 (1922).

and ethylurethan acetate in the presence of sodium or sodium ethylate to obtain the ketone esters represented by Formulas X and XI.

$(C_2H_5O)_2CH.CO.CH(COOC_2H_5)NHCONH_2$
X

$(C_2H_5O)_2CH.CO.CH(COOC_2H_5)NHCOOC_2H_5$
XI

4. Condensation of ethyl diethoxy-acetate with diethyl succinate for formation of the ketone ester $(C_2H_5O)_2CHCOCH(COOC_2H_5)CH_2$-$COOC_2H_5$, XII, and preparation of the same ester by alkylation of ethyl γ,γ-diethoxy-aceto-acetate with ethyl chloro-acetate.

Of these four studies the only ones that have led to any results of interest from the standpoint of glyoxaline chemistry are 2 and 4. The bromo derivative IX was found to undergo hydrolysis in acid solution with formation of ethyl α-bromo-glyoxalacetate. This interesting compound which is isomeric with ethyl mucobromate, $OCH.CBr:C(OH)COOC_2H_5$,[9] was obtained in a crystalline condition and its formation is one of the most interesting features of our work. A report of its characteristic reactions is reserved for a future publication.

In Study 4 we found that Claisen's condensation could be carried out successfully with ethyl diethoxy-acetate and diethyl succinate with production of the desired ketone ester (XII). This same compound can also be obtained in good yield by direct alkylation of ethyl γ,γ-diethoxy-aceto-acetate with ethyl chloro-acetate. The most interesting property of this compound was its characteristic behavior when subjected to hydrolysis in either alkaline or acid solution. It was found to break down normally with production of the desired key intermediate already mentioned above, β-glyoxalpropionic acid. The formation of this compound as a product of hydrolysis was established by the fact that it interacted with ammonia and formaldehyde with formation of Windaus and Knoop's[10] glyoxaline-propionic acid. In other words, this is the first evidence thus far produced experimentally that ethyl γ,γ-diethoxy-aceto-acetate can serve for the preparation of a key intermediate applicable for the synthesis of glyoxaline combinations of the histidine type. While we succeeded in proving the formation of the glyoxalpropionic acid, it is formed in such small yield that the ketone ester, from which it is prepared, cannot displace levulinic acid as the best practical source of this compound.

Experimental Part

Ethyl Diethoxy-acetate, $(C_2H_5O)_2.CHCOOC_2H_5$.—The ester used in this research was prepared according to the method of Johnson and Cretcher[11] with a slight modification in procedure, however, which was found

[9] Hill and Palmer, *Am. Chem. J.*, **9**, 147 (1887).

[10] Windaus and Knoop, *Ber.*, **33**, 1166 (1900).

[11] Johnson and Cretcher, THIS JOURNAL, **37**, 2144 (1915); *J. Biol. Chem.*, **26**, 106 (1916).

to be advantageous for large scale work. Instead of alkylating the dry silver salt of diethoxy-acetic acid, suspended in dry ether, with ethyl iodide by heating under pressure, it was found more practical to accomplish this reaction by simply agitating the moist silver salt in moist ether with the alkyl halide at ordinary temperature for a period of 10–12 hours. After the separation of inorganic salts by filtration the ether solution was separated and dried over sodium sulfate and the ethyl ester finally recovered and purified by distillation in a vacuum. Substitution of ethyl iodide by ethyl bromide or diethyl sulfate did not enable us to improve the method of synthesis. For the practical success of this method of operating, it is necessary to have available pure dichloro-acetic acid. A halogenated acid synthesized from chloral by the action of potassium ferrocyanide[11,12] always led to a good yield of the ester of diethoxy-acetic acid. On the other hand, dichloro-acetic acid furnished by the Dow Chemical Company and the Eastman Kodak Company always gave yields of the ester averaging 15–20% less than that obtainable from pure synthetic dichloro-acetic acid. The results obtained by us would indicate that these commercial acids are mixtures of mono-, di- and trichloro-acetic acids.

Ethyl γ,γ-Diethoxy-aceto-acetate, $(C_2H_5O)_2CH.CO.CH_2COOC_2H_5$.—The best method for synthesizing this ester is that employed by Dakin and Dudley,[13] and modified later by Johnson and Cretcher.[11] An attempt to prepare this ketone ester according to the procedure described by Hamel[14] was unsuccessful. This involves a condensation of ethyl chloro-acetate with ethyl diethoxy-acetate in the presence of magnesium, but no ketone ester was obtained and the two original esters were recovered unaltered.

Ethyl α-Isonitroso-γ,γ-diethoxy-aceto-acetate, $(C_2H_5O)_2CH.CO.C:(NOH)-COOC_2H_5$.—This nitroso compound has never been obtained in a pure state. Following Jovitschitsch's[15] method of synthesizing esters of this type, 22 g. of ethyl γ,γ-diethoxy-aceto-acetate was mixed with 14 g. of sodium nitrite dissolved in 10 cc. of water, and to the mixture was added slowly 10 g. of concd. sulfuric acid diluted with 40 cc. of water. After one and a half hours at 0°, sufficient water was added to dissolve inorganic salts and the oil was extracted with ether and dried over sodium sulfate. In no case were we able to purify this ester by distillation under diminished pressure due to intensive decomposition with the formation of a charred residue when the substance was heated. Our best success at purification was attained by heating the ester at 100° under a high vacuum. Under these conditions the purest product obtainable represented approximately 63–65% of the nitroso compound; yield, 20 g.

Behavior on Reduction.—Several attempts were made to prepare the unknown amino derivative, VIII, by reduction of the above-mentioned impure isonitroso compound, as follows: (1) by the catalytic method with platinum as catalyst; (2) by stannous chloride and hydrochloric acid in both aqueous and alcoholic solution;[16] and (3) by means of aluminum amalgam, but in no case were we successful in obtaining the desired compound.

Ethyl α-Bromo-γ,γ-diethoxy-aceto-acetate, IX.—While ethyl γ,γ-diethoxy-aceto-

[12] Wallach, *Ber.*, **10**, 1525 (1877).

[13] Dakin and Dudley, *J. Chem. Soc.*, **105**, 2453 (1924).

[14] Hamel, *Bull. soc. chim.*, [4] **29**, 390 (1921).

[15] Jovitschitsch, *Ber.*, **23**, 2683 (1890).

[16] See Kalischer, *Ber.*, **28**, 1519 (1895).

acetate apparently interacts normally with bromine in carbon disulfide solution to form this substituted ester, it has never been possible to purify the compound by distillation in a vacuum, due to intense decomposition. The crude ester was obtained in the form of a light red oil which was purified by heating in a high vacuum at 60°. From 10 g. of the ketone ester we obtained 10.6 g. of the crude bromo derivative. The literature reveals no mention of the action of urea on an α-bromoketo structure of the type represented by ethyl α-bromo-aceto-acetate. Kunckell[17] has shown, however, that amidines interact normally to form glyoxalines. We now find that our new bromoketone ester also shows no tendency to interact with urea and form a glyoxaline derivative. Also the halogenated ester failed to interact smoothly with ammonia. Evidence of substitution of bromine by the amino group was obtained, but no product could be isolated in a state of purity. The reaction product always contained nitrogen and bromine.

Ethyl α-Bromo-glyoxalacetate, $OCH.CO.CH(Br)COOC_2H_5$.—This interesting and very reactive compound is obtained as a product of hydrolysis when the above-mentioned diethoxy compound is saturated with hydrogen bromide and the mixture allowed to stand at ordinary temperature. Under these conditions the acetal grouping is slowly destroyed and this glyoxal derivative separates in a crystalline condition. The compound is readily soluble in acetone, ethyl acetate, ether and alcohol, slightly soluble in chloroform and practically insoluble in carbon tetrachloride and water. The compound separates in the form of colorless, elongated crystals which melt at 119° to a clear oil. It is our intention to investigate very thoroughly the conditions influencing the formation of this interesting compound and to study some of its characteristic reactions.

Anal. Calcd. for $C_6H_7O_4Br$: C, 32.2; H, 3.17; Br, 35.8. Found: C, 32.24, 32.01; H, 3.4, 3.23; Br, 36.2, 36.3.

Attempts to Apply Claisen's Reaction with Ethyl Hydantoate and Ethyl Urethan-acetate.—Neither ethyl diethoxy-acetate nor ethyl formate condenses with ethyl hydantoate,[18] $NH_2CONH.CH_2COOC_2H_5$, in the presence of sodium ethylate to form β-ketone esters. The condensation reaction with ethyl diethoxy-acetate was applied in absolute alcohol at a temperature of 60–80° for eight hours. We recovered the hydantoate in the form of its corresponding sodium salt, $NH_2CONH.CH_2COONa$, and over 50% of the ethyl diethoxy-acetate was recovered in pure condition, distilling at the correct temperature.

With ethyl formate and ethyl hydantoate we operated according to the procedure of Erlenmeyer, Jr.,[19] and the condensation was allowed to proceed for 12 days at ordinary temperature. Here again, when the reaction product was examined practically all of the hydantoate was recovered in the form of its sodium salt, there being no evidence of the formation of a condensed product.

Failure to obtain condensation products was also experienced when attempts were made to bring about reactions between ethyl urethan-acetate,[20] $C_2H_5OOC.NH.CH_2COOC_2H_5$, and ethyl diethoxy-acetate and ethyl formate under the catalytic influence of sodium ethylate.

[17] Kunckell, *Ber.*, **34**, 637 (1901).

[18] Harries and Weiss, *Ber.*, **33**, 3418 (1900).

[19] Erlenmeyer, *Ann.*, **337**, 251 (1904).

[20] Fischer and Otto, *Ber.*, **36**, 2107 (1903).

Ethyl α-Methoxymethyl-γ,γ-diethoxy-aceto-acetate, $(C_2H_5O)_2CH.CO.CH.(CH_2OCH_3)COOC_2H_5$. The chlorodimethyl ether employed in these experiments was prepared according to the directions of Favre.[21] Alkylation of the ketone ester was conducted in ether as follows.

The required quantity of sodium in wire form was added slowly to an ether solution of ethyl γ,γ-diethoxy-aceto-acetate to avoid too energetic reaction. The change was decidedly exothermic with vigorous evolution of hydrogen and after two hours the solution of sodium was complete. The required quantity of chlorodimethyl ether was then added, when alkylation took place at once with the separation of sodium chloride. After it had been allowed to interact for 12 hours and then digest for about five to eight hours the solution was neutral to litmus, indicating complete reaction and, after removal of the ether and distillation of the resulting oil we obtained the substituted ester in a pure condition; b. p., 130° (4 mm.); yield, 44%; n_{23}, 1.4387.

Anal. Calcd. for $C_{12}H_{22}O_6$: C, 54.96; H, 8.46. Found: C, 54.41, 54.59; H, 8.19, 8.23.

Diethyl γ,γ-Diethoxy-acetosuccinate, $(C_2H_5O)_2CH.COCH(CH_2COOC_2H_5)COOC_2H_5$.—This new ketone ester can be prepared by either of the following two methods: (1) alkylation of ethyl γ,γ-diethoxy-aceto-acetate with ethyl chloro-acetate or (2) by application of Claisen's condensation reaction with diethyl succinate and ethyl diethoxy-acetate.

In applying the alkylation experiment we operated with both ether and absolute alcohol solutions and in each trial the yield was practically the same, namely, 43–45%. In both solvents the reaction is hastened by introduction of a small quantity of potassium iodide as catalyst. Twenty g. of the original ketone ester yields about 23 g. of the alkylation product which on redistillation gives about 15–17 g. of ester; b. p., 150–170° (3 mm.). The purified ester boils at 156° (3 mm.).

In applying the condensation reaction we found that good results can be obtained by using either sodium or sodium ethylate as the condensing agent. The yield of condensation product is, however, somewhat higher when sodium is used. Using the proportions of 7 g. of ethyl diethoxy-acetate and 7 g. of diethyl succinate per gram of sodium the reaction was allowed to proceed in alcohol at ordinary temperature for one hour and finally at 80° for seven to eight hours, when the change was considered complete. On working up the reaction product we obtained a 40% yield of pure ester. When we applied the condensation by introducing finely divided sodium into a mixture of diethyl succinate and ethyl diethoxy-acetate heated to 80° and continued the time of heating for six hours we obtained a reaction product that yielded the desired β-ketone ester in a 48% yield. Diethyl γ,γ-diethoxy-acetosuccinate is a yellow oil; b. p., 156° (3 mm.); n_{21}, 1.4370.

Anal. Calcd. for $C_{14}H_{24}O_7$: C, 55.26; H, 7.95. Found: C, 55.00; H, 7.67.

The Behavior of Diethyl γ,γ-Diethoxy-acetosuccinate on Hydrolysis The Formation of Glyoxalpropionic Acid, $OCH.CO.CH_2.CH_2.COOH$.

Hydrolysis with Barium Hydroxide.—The diethyl ester was digested with barium hydroxide (1 molecular equivalent) in aqueous solution for eight hours and the solution then cooled when a mixture of barium carbonate and barium succinate separated weighing 3.3 g. When these salts were decomposed with sulfuric acid the free succinic acid was precipitated in the form of its characteristic benzylpseudothio-urea salt, $(C_7H_7SC-$

[21] Favre, *Bull. soc. chim.*, [3] **11**, 881, 1096 (1894).

$(NH_2):NH)_2.(CH_2COOH)_2.2H_2O$. This crystallized from 95% alcohol in the form of prisms; m. p., 153°. This has proved to be a very valuable reagent for the identification[22] of many fatty acids and in this case served to show that practically one-half of the ketone ester used has undergone a ketone hydrolysis by the action of barium hydroxide.

Anal. Calcd. for $C_{20}H_{30}O_6N_4S_2$: N, 11.53. Found: 11.67, 11.69.

Identification of Glyoxalpropionic Acid, $OCH.CO.CH_2CH_2COOH$.—The filtrate left after removing the barium carbonate and barium succinate as described above, was first acidified with sulfuric acid and the solution finally freed from barium and sulfate ions. The clear filtered solution was then concentrated to a small volume and an application made at once of Windaus and Knoop's[10] method for converting glyoxalpropionic acid into glyoxaline-propionic acid. To the solution was added, therefore, 3 g. of 40% formaldehyde solution and 7 g. of concd. aqueous ammonia. The mixture was then preserved for five days and finally heated on a water-bath to remove the excess of ammonia and formaldehyde and reduced to a small volume before acidification with acetic acid. This acidification with acetic acid, followed by evaporation at 100°, was repeated thrice and the resulting residue finally triturated with 20 times its volume of methyl alcohol to precipitate any inorganic material. Evaporation of the methyl alcoholic filtrate yielded 1.9 g. of a crystalline residue which dissolved immediately in water. When this aqueous solution was diluted with acetone beautiful, elongated rods separated which melted sharply at 212°, corresponding exactly to the melting point assigned to glyoxaline-propionic acid by Windaus and Knoop,[10] and the substance agreed in its chemical behavior with their description of this compound. That the filtrate still contained more of the glyoxaline compound was shown by the fact that the acetone solution gave a strong positive glyoxaline color test when treated with Pauly's reagent, *p*-diazobenzene-sulfonic acid. It was impossible to regulate the course of the reaction during alkaline hydrolysis of the ketone ester with barium hydroxide, so as to reduce the quantity of succinic acid formed and increase correspondingly that of glyoxalpropionic acid.

Hydrolysis with Sulfuric Acid.—Five g. of the above β-ketone ester was heated at 100° for seven hours with 25 cc. of 10% sulfuric acid. The ester dissolved completely within four hours, giving a deep red solution. A portion of this, when neutralized with sodium hydroxide and tested with benzylpseudothio-urea, gave no precipitate, indicating the absence of free succinic acid. The sulfuric acid was then removed by exact precipitation as barium sulfate, yielding a highly colored filtrate. This required three successive treatments with active Norite before removal of color was complete. The solution was then concentrated to a small volume and the usual treatment with formaldehyde and ammonia applied for conversion of the dissolved glyoxal derivative into glyoxaline-propionic acid. By working up the reaction product it was possible to isolate a small quantity of the glyoxaline derivative, giving a strong color reaction when treated with *p*-diazobenzene-sulfonic acid. The acid was very highly colored and was obtained in a pure condition with great difficulty. The low yield may partially be accounted for by the fact that very probably a large proportion of the glyoxalpropionic acid was removed by adsorption during the intensive treatment to remove color by digestion with Norite.

Hydrolysis with Sodium Hydroxide.—In this experiment we subjected the ketone ester to hydrolysis by digestion with 2 *N* sodium hydroxide solution for four hours at 100°. The alkali was then neutralized with nitric acid when there was a vigorous evolution of carbon dioxide and on the addition of silver nitrate a heavy precipitate of a

[22] Unpublished results.

colorless silver salt was obtained. This quickly turned dark colored when exposed to the light. After washing this salt thoroughly with cold water, it was triturated with 7 cc. of concd. hydrochloric acid to remove silver when we obtained practically 5 g. of silver chloride, indicating that our hydrolysis had led to a smooth production of glyoxalpropionic acid. The filtrate from the silver chloride precipitation gave no precipitate when treated with benzylpseudothio-urea, showing the absence of succinic acid from this fraction. Glyoxalpropionic acid was identified in the usual manner by conversion into glyoxaline-propionic acid, which was obtained in a crystalline condition, m. p. 212°, and gave a strong color reaction when tested with *p*-diazobenzene-sulfonic acid. To summarize, therefore, the β-ketone ester breaks down during both alkaline and acid hydrolysis to give glyoxalpropionic acid, but the yield is very small due to secondary reactions leading to destruction of a large proportion of the β-ketone ester. This decomposition during hydrolysis is evidenced by the intense color developed in the solution during hydrolysis.

Attempts were made to improve the synthesis of the glyoxalpropionic acid by first alkylating ethyl γ,γ-diethoxy-aceto-acetate with chloro-aceto-amide and iodo-acetonitrile and then subjecting the alkylation products to hydrolysis in both alkaline and acid solution. In neither case were we successful in obtaining the corresponding alkylation product in a pure condition but, on the other hand, hydrolysis of the crude reaction products led to the formation of glyoxalpropionic acid in both cases. This was identified in the usual manner by conversion into glyoxaline-propionic acid.

Summary

1. Improvements have been made in the method of preparing ethyl diethoxy-acetate from dichloro-acetic acid.

2. For the success of this preparation it is necessary that pure dichloro-acetic acid be employed.

3. Ethyl γ,γ-diethoxy-aceto-acetate interacts with nitrous acid and bromine to form the corresponding isonitroso and bromo derivatives. The latter compound undergoes hydrolysis in presence of acids, giving the glyoxal compound, $OHC.CO.CHBr.COOC_2H_5$.

4. Ethyl diethoxy-acetate has been condensed with ethyl succinate successfully with formation of ethyl γ,γ-diethoxy-acetosuccinate. This compound is also formed by direct alkylation of ethyl γ,γ-diethoxy-aceto-acetate with ethyl chloro-acetate. This new ketone ester undergoes hydrolysis in acid and alkaline solution with formation of glyoxalpropionic acid.

5. Glyoxalpropionic acid obtained from the new ketone ester has been shown to interact normally with ammonia and formaldehyde with formation of glyoxaline-propionic acid.

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