were studied using an enzyme concentration of 3.7×10^{-6} M. For comparison purposes, the k_{OH} values for both compounds were also determined in the same solvent composition using pH 11 solutions. The data for methyl dihydrocinnamate in 20% acetonitrile were obtained using a substrate concentration range of 1–7 mM and α-chymotrypsin at 4.15×10^{-7} M. For **15** no cosolvent was required; the enzyme concentration used was 0.8×10^{-6} M with a substrate range of 1–10 mM. The kinetic data were subjected to least-squares regression analysis and the k_{cat} and K_m constants were computed using the reciprocal method of Lineweaver and Burk.[36]

α-Chymotrypsin-Catalyzed Hydrolysis of 5.—The 1β-acetoxy-6β-dihydrocinnamoyloxydecalin **5** (140 mg, 0.39 mmol) was hydrolyzed at 25° using α-chymotrypsin (10 mg) in 0.1 M aqueous potassium chloride (10 ml) containing 2% acetonitrile by the general preparative procedure; 0.5 N aqueous sodium hydroxide was used to maintain the pH at 7.8. After 24 hr, when 0.3 mmol of base had been taken up, the rate slowed dramatically and the reaction mixture was extracted with ether (3 × 50 ml). Glc analysis showed that the extract contained 0.31 mmol of the 1β-acetate product of specific 6β cleavage and 0.08 mmol of the starting diester. Tlc separation of the mixture using ethyl acetate–benzene (1:6) as developing solvent yielded the 1β-acetoxy-6β-hydroxydecalin **3** (46 mg) which was identical with an authentic sample. None of the 6β-dihydrocinnamate **10** was detected. Extraction of the acidified aqueous mother liquors of the reaction with chloroform (3 × 50 ml) give dihydrocinnamic acid (37 mg, 0.25 mmol).

α-Chymotrypsin-Catalyzed Hydrolyses of 6.—The 1β-acetate-6α-dihydrocinnamate **6** (10 mg, 0.27 mmol) in 0.1 M aqueous potassium chloride (5 ml) was treated with α-chymotrypsin (5 mg) as described above. Aqueous sodium hydroxide (0.1 N) was the base used and after 24 hr, when ~2 equiv of alkali had been consumed, the reaction was worked up as before. (The excess base uptake is due to autolysis of the enzyme.) Glc analysis showed hydrolysis to have been 96% selective for the 6α position, and the 1β-acetoxy-6α-hydroxy compound **4** (3 mg), identical with authentic material, was obtained following tlc purification.

α-Chymotrypsin-Catalyzed Hydrolysis of 15.—The hydrochloride salt of 6β-acetoxy-3α-dihydrocinnamoyloxytropane (**15** HCl, 1.55 g, 4.22 mmol) in 0.1 M aqueous potassium chloride (60 ml) was placed in the pH-Stat under the standard conditions. The pH of the reaction solution (~5.5) was adjusted to 7.8 with 1 N aqueous sodium hydroxide (2.3 mmol required), and α-chymotrypsin (60 mg) was then added. The hydrolysis stopped after 4 hr (0.2 mmol of base uptake). The reaction mixture was saturated with potassium chloride and extracted continuously with ether for 24 hr. Evaporation of the dried ($MgSO_4$) ether extract yielded the dihydrocinnamate salt of 6β-acetoxy-3α-hydroxytropane (100 mg). The aqueous solution was then saturated with potassium carbonate and again continuously extracted with ether for 24 hr. Evaporation of the dried ether solution gave 6β-acetoxy-3α-hydroxytropane (**17**, 780 mg, 3.9 mmol), identical in all respects with an authentic sample. A further 62 mg (0.21 mmol) of **17** was obtained by treatment of the dihydrocinnamate salt with aqueous potassium carbonate.

Acknowledgment.—This work was generously supported by the National Research Council of Canada.

Registry No.—**1** 1β-alcohol derivative, 878-47-7; **2**, 41163-95-5; **2** 1β-OAc derivative, 36925-35-6; **2** 1β-ODHC derivative, 41163-97-7; **2** 1β-OTHP derivative, 38538-76-0; **3**, 41163-98-8; **4**, 41163-99-9; **5**, 41164-00-5; **6**, 41164-01-6; **7**, 41164-02-7; **8**, 41164-03-8; **10**, 41164-04-9; **10** 1β-OTHP derivative, 41172-09-2; **11**, 5932-53-6; **12**, 41164-06-1; **13**, 41164-07-2; **13** HCl, 41164-08-3; **14**, 41164-09-4; **15**, 41164-10-7; **15** HCl, 41164-11-8; **16**, 41164-12-9; **17**, 41164-13-0; dihydrocinnamoyl chloride, 645-45-4.

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The Bimolecular Decarboxylative Self-Condensation of Oxaloacetic Acid to Citroylformic Acid and Its Conversion by Oxidative Decarboxylation to Citric Acid

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Received October 17, 1972

Oxaloacetic acid undergoes a bimolecular decarboxylative self-condensation to give 4-carboxy-4-hydroxy-2-ketohexane-1,6-dioic acid (**1**) (citroylformic acid). The reaction goes in high yield at pH 3–7, at 25–30°, and in aqueous media. The acid is converted to citric acid by oxidative decarboxylation and to its lactone by dehydration but is not obtainable from the previously described oxalocitrolactone ester structures.

Although the chemistry of oxaloacetic acid has been studied in considerable detail, there are unexplained observations in some of the reported data. These are reported in studies of the nmr[1] and uv[2] spectra in which anomalous behavior is attributed to unidentified dimeric structures. Since dimerization has been noted for pyruvic acid, and characterized in some detail,[3–7] it seems reasonable to postulate the dimerization[8] of oxaloacetic acid. Furthermore, because such dimeric structures are formed under physiological conditions (aqueous, pH 7) and are, therefore, possibly significant in biological systems, and since the extensive studies of the kinetics and mechanism of the decarboxylation of oxaloacetic acid[9] (based on the rate of evolution of carbon dioxide) should be interpreted for the possibility of rate-determining dimerization preceding decarboxylation,[10] additional information on this dimerization reaction is desirable. We wish to describe the results of a study of the decarboxylative self-condensation of oxaloacetic acid to citroylformic acid (**1**) (CFA) and isolation and characterization of this new acid.

Oxaloacetic acid undergoes a bimolecular aldol-type condensation in aqueous solution at room temperature (25–30°) and pH 3–7 with the loss of carbon dioxide to

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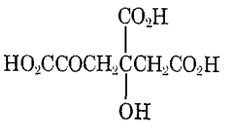
give **1**, which we have named citroylformic acid (CFA).[11] This is isolated as its water-insoluble calcium salt in



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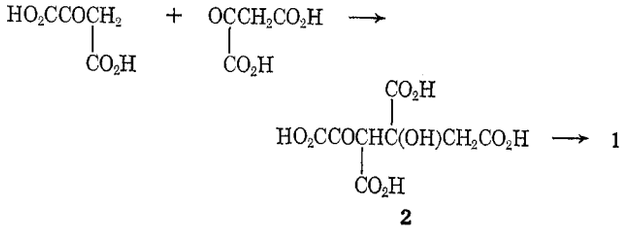
65–96% yields and converted to the free acid in 40% crude yield by treatment with sulfuric acid in dry ether. Recrystallization from ethyl acetate–carbon tetrachloride gave the analytically pure sample in 19% yield, mp 154–156° with gas evolution. Characterization data include elemental analysis, nmr, mass, and uv spectral data, titration, thin layer chromatography (tlc) R_f values (two solvent mixtures), and conversion with hydrogen peroxide or *tert*-butyl hydroperoxide to citric acid (CA). Oxaloacetic acid can be converted directly to citric acid without isolation of the intermediate **1**. Citroylformic acid sequesters calcium ions over the pH range of 2.5–10 as is shown by lowering of the pH in the presence of calcium ions. Evaporation of the decationized reaction mixture gives a solid product from which CFA is obtained as its γ-lactone by recrystallization from acetone–nitromethane. An insoluble polymeric material is also obtained. This lactone, characterized by ultimate analysis and titration, when dissolved in water gives the same tlc R_f values as does CFA.

The mechanism of the reaction is visualized as that of a base-catalyzed aldol-type condensation followed by hydroxyl-initiated decarboxylation of the β-keto acid structure of the initial product, oxalocitric acid (**2**).



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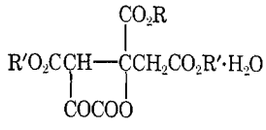
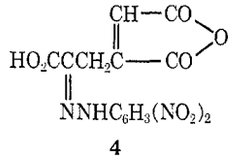
Since there is no indication that these reactions are mechanistically unique, kinetic and mechanistic studies of the decarboxylation of oxaloacetic acid,[9] particularly in the presence of ions which catalyze dimerization,[10] need to be reevaluated to determine whether or not dimerization precedes decarboxylation. There is no evidence for the independent existence of the initial condensation product, oxalocitric acid (**2**), under the conditions used in our studies.

The structure **1**, assigned to the product of the decarboxylative condensation, is consistent with all of the available data. The key points in these data, in addition to all of the confirming analytical data, are the nearly quantitative oxidative decarboxylation to citric acid and the absence of both aldehydic reaction (Schiff's fuchsin aldehyde test) and aldehydic proton in the nmr spectrum. Alternative structures involving the loss of any one of the other three carboxyl groups in the intermediate **2** are inconsistent with these key points. Thus, loss of one of either of two of the other carboxyl groups would give a structure incapable of conversion to citric acid. Loss of the third would give a β-aldehydo acid for which there is no confirming support, *i.e.*, no aldehydic proton, no enol test, no aldehyde test, and comparative stability toward decarboxylation. In the course of our studies we have repeated and confirmed earlier reports[12–15] of the base-catalyzed formation of trimethyl and triethyl oxalocitrolactone esters **3a**. We have observed for the first time that partial hydrolysis of their esters gives the corresponding monoester or its hydrate, which, short of degradation, is resistant to further hydrolysis. We take this to indicate that the unhydrolyzed ester is the most hindered one and assign the formula **3b** in which the molecule of



3a, R, R′ = CH_3 or C_2H_5
b, R = CH_3 or C_2H_5; R′ = H

water is present as a hydrated carboxyl. The neutralization equivalent data and the ease of removal of the molecule of water indicate that the lactone ring has not been opened by addition of the molecule of water. Such hydrates are formed from α-keto acids.[10,16] The dinitrophenylhydrazine (DNPH) derivatives of CFA have been obtained and structures have been assigned on the basis of their analytical data. Mass spectral data confirm the structures **4**–**7**. The ethyl esters are formed from ethanolic DNPH solutions.



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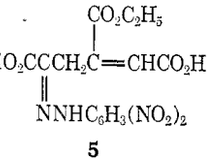

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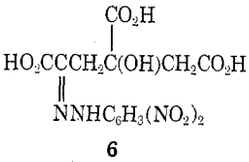

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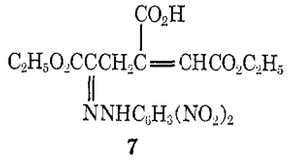

7

Experimental Section

Equipment.—The mass spectra were obtained with a Varian M66 and are accurate to well within ±0.2 amu, except as noted for the precision analysis of the oxalocitrolactone ester. The ultraviolet spectra were determined with a Cary Model 14 and the nmr with a Varian A-60A.

Chromatographic Techniques.—Thin layer chromatograms were on 3 × 3 in. sheets of Eastman chromagram silica gel sheets, no. 6061. They were developed with BFW (1-butanol–90% formic acid–water 8:3:2 by volume; the upper layer which separates on standing is used)[17] and with EAW (ethanol–water–concentrated ammonium hydroxide, 78:9.5:12.5 by volume).[18]

(11) The systematic name for this compound is 4-carboxy-4-hydroxy-2-ketohexane-1,6-dioic (or -adipic) acid. The name citroylformic acid is used here to show the relation to citric acid. Similar names have been used for benzoylformic and acetylformic (pyruvic) acids. The name is admittedly ambiguous in not specifying which of the citric acid carboxyls (1- or 2-) is involved in the citroyl radical. A prefix 1- or 2- may be used if specification on this point is desired.

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R_f values for citric and aconitic acid are 60 ± 3 and 85 ± 5 (BFW) and 20 ± 2 and 34 ± 3 (EAW).

Materials.—The oxaloacetic acid used in these experiments was obtained commercially (Calbiochem Co., no. 5000) or prepared from diethyl oxaloacetate by hydrolysis with concentrated hydrochloric acid.[19] It was stored at 0° and used as a white powder with a neutralization equivalent (neut equiv) of 67–69 (theory 66) and ϵ (265 nm) 7200 for a 0.01% solution in *tert*-butyl alcohol–chloroform (5:95, v/v) acidified with 6 *N* sulfuric acid. Some commercially available materials do not meet these specifications. The preferred procedure for this hydrolysis has not been given in detail before, and, since it has certain advantages over other procedures,[20] it is given below. R_f data from thin layer chromatography were obtained for oxaloacetic acid by conducting the operations rapidly and spraying with ferric chloride solution and by low-temperature techniques, but these are neither highly reliable nor sensitive.

Preparation of Oxaloacetic Acid.—The following procedure is much simpler and gives purer product than preparations described previously. A sample of 20 g of commercial (EK Co., no. P4830) sodium diethyl oxaloacetate was partially dissolved in 200 ml of water at 30° and filtered to remove insoluble material. The solution was acidified with sulfuric acid and extracted with ether to give 12 g (80% recovery) of diethyl oxaloacetate. This ester (5 ml) was added to 10 ml of concentrated hydrochloric acid in an ice bath. The mixture was stirred for 2 hr and the solution was stored in the cold (0°) for 9 days, during which time crystals of oxaloacetic acid separated. The supernatant liquid was decanted and the crystals were dried for 10 hr at 50°. The residue was washed with cold carbon tetrachloride–ethyl acetate (4:1) and again dried to give 35% yield. This material, with ϵ (265 nm) 7200 and neut equiv 67–69, was comparable in purity to the best commercial materials that we have received.

The mass spectral data for the DNPH derivative of oxaloacetic acid, but not that of the free acid itself, is of assistance in detecting small quantities of oxaloacetic acid. The spectrum of oxaloacetic acid showed a prominent peak at 113 amu for the molecular ion less 19 amu but no peak for the molecular ion itself, which is presumably lost in the source heater. The mass spectrum of the DNPH of oxaloacetic acid recrystallized from ethyl alcohol showed peaks at 268 and 222 amu for the dinitrophenylhydrazones of pyruvic acid and acetaldehyde. There was also an intense peak at 368 amu corresponding to the carbethoxydinitrophenylpyrazolone. This peak was also prominent in the spectrum of the dinitrophenylhydrazine derivative obtained from diethyl oxaloacetate itself.

Citroylformic Acid (1).—A solution of 1.5 g of oxaloacetic acid in 10 ml of water was adjusted to a pH of 7.5 with 20% sodium hydroxide and held at 25–30° for 48 hr. To the reaction mixture was added 3 ml of 60% calcium chloride solution and the precipitate was collected and dried to give 1.62 g (95%) of the calcium salt of citroylformic acid. On warming to 60° for 10 min the calcium salt of oxaloacetic acid (0.14 g, dried) precipitated from the filtrate. Two grams of the calcium citroylformate and 0.6 g of anhydrous magnesium sulfate in 30 ml of dry ether were treated with 0.6 ml of concentrated sulfuric acid in 20 ml of dry ether.[21] The addition was dropwise with cooling and stirring. The mixture was filtered and the filtrate was evaporated to give 0.6 g (40%) of a crystalline residue of 1. Recrystallization from ethyl acetate–carbon tetrachloride gave 0.26 g (19%) of pure 1: mp (with gas evolution and decomposition) 154–156° (slow heating), 164–165° (rapid heating); ϵ (240 nm) 7300. The acid turned brown at 145°. The Schiff fuchsin aldehyde test was negative. The analysis was for samples vacuum dried at room temperature.

Anal. Calcd for $C_7H_8O_8$: C, 38.19; H, 3.66; neut equiv, 73.3, 110, 220. Found: C, 38.12; H, 3.47; neut equiv, 73.9, 108.6, 217.2.

The mass spectrum of citroylformic acid ($C_7H_8O_8$, mol wt 220) showed, as the most intense peaks above mass 100, peaks at the following mass values: 112 ($C_5H_4O_3$, theory 112), 139 ($C_6H_3O_4$, theory 139), and 184 ($C_7H_4O_6$, anhydrocitroylformic acid anhydride or lactone, theory 184). These fragments were derivable by thermal or electron impact degradation reactions from citroylformic acid in processes observed with oxaloacetic acid and citric acid in their mass spectra. The molecular ion of citroylformic acid was not observed.

The nmr spectrum in perdeuteriodimethyl sulfoxide showed a singlet at 2.95 ppm [(measured with reference to the residual DMSO-d_5 peak as corrected to TMS) for CH_2], 3.16 ppm (CH_2 adjacent to CO), broad absorption at *ca.* 10 ppm, and no low-field absorption as required for HCO. These data were in accord with the citroylformic acid structure. The sample turned brown at once on dissolution in DMSO, presumably owing to dehydration as is evidenced by a peak at 6.35 ppm whose intensity as integrated indicates that the ratio of unsaturated product to citroylformic acid is 4:1.[22]

On chromatographic analysis by the method of Kesner[23] the acid appeared before malic and after aconitic acids.[24] The ferric chloride enol test with citroylformic acid showed a slight change in color about like that observed with pyruvic acid.

Citric Acid from Citroylformic Acid and Oxaloacetic Acid.—Citroylformic acid was converted to citric acid, with or without isolation as such from the oxaloacetic acid condensation, by oxidative decarboxylation with aqueous acidic potassium permanganate, hydrogen peroxide, or *tert*-butyl hydroperoxide. Similar reagents have been used previously for the decarboxylation of other α-keto acids.[25,26] Details of the reactions with hydrogen peroxide and *tert*-butyl hydroperoxide are given.

A solution of 100 mg of 1 in 1 ml of water and 0.5 ml of 30% hydrogen peroxide was allowed to stand at room temperature for 23 hr. The presence of citric acid was confirmed by tlc: R_f (BFW) 61 (CA), 73 (CFA); R_f (EAW) 21 (CA), 28 (CFA).

A solution of 5 mg of 1 in 0.25 ml of water was heated to 70° for 4 hr with 0.1 ml of *tert*-butyl hydroperoxide. The presence of citric acid was confirmed by tlc: R_f (BFW) 59 (CA), 71 (weak CFA); R_f (EAW) 19 (CA), 28 (CFA).

The bimolecular decarboxylative self-condensation of the oxaloacetic acid product can be converted directly to citric acid without isolation of 1. The procedures with hydrogen peroxide and *tert*-butyl hydroperoxide are given.

A solution of 3 g of oxaloacetic acid in 20 ml of water was adjusted to a pH of 7.5 with 20% sodium hydroxide and allowed to stand at room temperature for 3 days. To this was added 2.5 ml of *tert*-butyl hydroperoxide and the resulting mixture was stirred for 4 hr at 50°. To this solution was added 6 ml of 60% calcium chloride to precipitate 3 g (93%) of calcium citrate hydrate (vacuum dried at room temperature). This calcium salt was suspended in 100 ml of dry ether with 1 g of anhydrous magnesium sulfate and treated with 0.8 ml of concentrated sulfuric acid in 50 ml of dry ether with stirring and cooling. The mixture was stirred for 4 hr and filtered. Evaporation of the filtrate gave 0.2 g (9.3%) of crystalline citric acid hydrate identified by tlc: R_f (BFW) 58, R_f (EAW) 23.

A solution of 10 g of oxaloacetic acid in 40 ml of water was adjusted to pH 7 with 20% sodium hydroxide. After 3 days at room temperature the pH was 8.5. To this solution was added 10 ml of 30% hydrogen peroxide and the mixture was heated to 45–50° for 1 hr. Calcium chloride (60%, 30 ml) was added; the mixture was boiled for 15 min and filtered. The filtrate was washed with water and methanol and dried to give 9.5 g (87%) of hydrated calcium citrate. This was suspended in 350 ml of dry ether with 3 g of anhydrous magnesium sulfate. To this was added a solution of 2.7 ml of concentrated sulfuric acid in 50 ml of dry ether. After stirring for 6 hr the mixture was filtered and the filtrate was evaporated to give 3.1 g (41.6%) of citric acid hydrate identified by tlc: R_f (BFW) 57, R_f (EAW) 21.

Dinitrophenylhydrazone Derivatives of Citroylformic Acid.—A solution of 50 mg of 1 in 1 ml of 95% ethanol was combined with 2 ml of DNPH solution prepared by adding 0.4 g of DNPH to 2 ml of concentrated sulfuric acid and adding 3 ml of water and 10 ml of ethanol. The crystalline precipitate which formed was collected, washed with cold ethanol, and dried under vacuum, mp 119–127° dec. The mass spectrum of this material showed, as the most intense peaks above 190 amu, two peaks at 364–365 amu and above that a set of peaks at 392–394 amu. The 364 peak corresponded to the DNPH of citroylformic acid less two molecules of water and can be formulated as the unsaturated anhy-

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(21) G. Payne and P. H. Williams, *J. Org. Chem.*, **24**, 54 (1959), and references cited therein.
(22) The authors wish to thank R. Lichter for assistance with these measurements.
(23) L. Kesner and E. Muntwyler, *Anal. Chem.*, **38**, 1164 (1966).
(24) The authors wish to thank L. Kesner for this determination.
(25) L. Claisen, *Ber.*, **12**, 632 (1879).
(26) A. F. Holleman, *Recl. Trav. Chim. Pays-Bas*, **23**, 169 (1904).

dride 4 or possibly as a lactam. The 393 peak corresponded to the anhydride of the monoethyl ester formulated as 5. Elemental analysis gave values in accord with those for an equimolar mixture of the DNPH of CFA 6 and its monoethyl ester.

Anal. Calcd for $(C_{13}H_{12}N_4O_{11}$–$C_{15}H_{16}N_4O_{11})0.5$; C, 40.5; H, 3.4; N, 13.5. Found: C, 40.2; H, 3.8; N, 13.35.

Slow recrystallization from ethanol containing sulfuric acid gave crystals, mp 150–155°, with analysis corresponding to the diethyl ester 7.

Anal. Calcd for $C_{17}H_{18}N_4O_{10}$: C, 46.57; H, 4.11; N, 12.78. Found: C, 46.12; H, 4.09; N, 12.08.

Citroylformic Acid Lactone.—A solution of 20 g of oxaloacetic acid in 60 ml of water was adjusted to pH 3 with 20% sodium hydroxide and allowed to stand for 19–20 hr at 25–30°. Carbon dioxide was evolved during this period. The solution was decationized by passing through an acidified ion exchange resin bed (22 × 500 mm column of Dowex 50W-X8) and evaporated to dryness under a vacuum evaporator at 35°. The final drying was completed in a vacuum desiccator over anhydrous calcium sulfate to give 12–15 g. The dried residue was partially dissolved in a minimum amount (30 ml) of warm, dry acetone. Nitromethane (dry, about 4 volumes) was added to incipient cloudiness. The mixture was filtered and the solution was allowed to stand at room temperature to deposit crystals. Recrystallization gives the pure lactone of citroylformic acid, mp 160–164° dec, turns brown at 150°. Toluene or xylene can be used in place of the nitromethane. The acetone-insoluble residue is apparently polymeric. The lactone is assigned the butyro-γ-lactone structure arbitrarily. The ir data show absorption (in mineral oil) at 1770, 1740, 1700, and 1660 cm^{-1}.

Anal. Calcd for $C_7H_6O_7$: C, 41.6; H, 2.99; neut equiv, 67.3. Found: C, 41.51; H, 2.98; neut equiv, 67.9.

Oxalocitrolactone Monomethyl Ester Hydrates.—The oxalocitrolactone used in this preparation was prepared from dimethyl oxaloacetate and trimethylamine as previously described[13,14] and was obtained analytically pure (neut equiv calcd, 288; found, 285) on recrystallization from water. The molecular weght (mass spectrometer) was found to be 288.048 (theory 288.050) with reference to the $C_6{}^{35}Cl_3{}^{37}Cl_3$ peak at 287.8043 amu. We confirmed the mp 104–106° reported by Dieckmann.[15]

A mixture of 1.0 g of citrolactone trimethyl ester prepared as above and 20 ml of concentrated hydrochloric acid was heated on a steam bath at 50° for 20 min. The residual oil on cooling deposited crystals in 55% yield. The solution remaining was evaporated at room temperature to 10 ml to deposit additional crystals. The yield is 0.3 g, mp 150–154° dec, R_f 24 (EAW).

Anal. Calcd for $C_9H_8O_9 \cdot H_2O$: C, 38.84; H, 3.59; neut equiv, 92.6. Found: C, 38.93; H, 3.53; neut equiv, 91.37. On prolonged drying under vacuum the material lost water.

Anal. Calcd for $C_9H_8O_9$: neut equiv, 86.6. Found: neut equiv, 85.0. The nmr spectrum of the methyl ester in perdeuteriodimethyl sulfoxide showed maxima at about 3.5 (5 H, two carboxylic, one enolic, two water), 3.8 (3 H, one methyl), and 3.3–3.4 ppm (doublet, two methylene H on asymmetric carbon).

The monomethyl ester hydrate was converted to citric acid in low yield by refluxing with lithium hydroxide, R_f (BFW) 63, but is otherwise stable to alkaline hydrolysis short of further degradation.

Oxalocitrolactone Monoethyl Ester Hydrates.—This compound was prepared following the above procedure for the methyl ester, mp 72–75°, R_f (BFW) 65.

Anal. Calcd for $C_{10}H_{10}O_9 \cdot H_2O$: C, 41.1; H, 4.14; neut equiv, 97.3. Found: C, 41.1; H, 4.13; neut equiv, 94.19.

Acknowledgment.—The authors are indebted to the Miles Laboratories Inc. for a grant in support of this research.

Registry No.—**1**, 39118-31-5; **3** (R = R′ = H), 41118-38-1; **3a** (R = R′ = CH_3), 41118-39-2; **3b** (R = CH_3; R′ = H), 41118-40-5; **3b** (R = C_2H_5; R′ = H), 41118-41-6; **4**, 41118-42-7; **5**, 41118-43-8; **6**, 41118-44-9; **6** monoethyl ester, 41118-45-0; **7**, 41118-46-1; diethyl oxaloacetate, 108-56-5; oxaloacetic acid, 328-42-7; citric acid, 77-92-9; DNPH, 119-26-6; citroylformic acid γ-lactone, 41118-47-2.

The Beckmann Fragmentation Reaction of Some α-Hydroxy Ketoximes

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Received March 13, 1973

The Beckmann fragmentation reaction of 3β,17β-dihydroxy-16-oximino-5-androstene (**2**) and 2-*exo*-hydroxy-3-oximinobornane (**8**) was readily achieved with *p*-toluenesulfonyl chloride in pyridine at 0° or by short boiling with 20% (v/v) H_2SO_4. The structures of the primary fragmentation products **3** and **9** were deduced on the basis of spectral and analytical properties of the corresponding 2,4-dinitrophenylhydrazones **4** and **10**; in addition, **3** was converted into the known lactone **6**. Anti configurations were assumed for the hydroxy oximes **2** and **8** obtained by $NaBH_4$ reduction of **1** and **7**, based on studies of the $NaBH_4$ reduction of *syn*- and *anti*-benzil monoximes.

The fragmentation reaction of some α-oxo and α-hydroxy oximes, under the conditions of Beckmann rearrangement was discovered by Werner and Piguet in 1904.[1] The mechanism and synthetic utility of the reaction has recently been discussed[2] and the geometry (syn or anti) of the oximes has been shown to influence the nature of the fission products.[1,3] Thus, the *anti*-benzoin oxime (anti to the α-hydroxyl group), on treatment with benzenesulfonyl chloride and pyridine, gives equimolar amounts of benzaldehyde and benzonitrile, whereas the syn isomer, under the same experimental conditions, forms benzaldehyde and phenyl isocyanide.[1] Generally, the anti isomers of α-hydroxy oximes, when treated with variety of reagents which induce Beckmann rearrangement, yield aldehydes or ketones, and nitriles, whereas the syn isomers occasionally yield isocyanides[1,3] The Beckmann fission of α-hydroxy oximes has not been widely studies but a recent report described the fragmentation reaction of 5-hydroxy-5α-cholestan-4- and -6-one oximes with thionyl chloride at −20°. A nearly quantitative yield of 5-oxo-4,5-*seco*-cholestano-4-nitrile and 5-oxo-5,6-*seco*-cholestano-6-nitrile was obtained. It should be noted that no direct evidence was given for the geometries of the starting oximes; owing to exclusive formation of oxo nitriles the authors assumed them to have anti configurations.[4]

We wish to report the results of our studies which were undertaken in order to explore the possibility of

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