Ethyl α-Isobutyrylcinnamate (VI).—In a 1-liter flask was placed a mixture of 159 g. (1.5 moles) of freshly distilled benzaldehyde and 237 g. (1.5 moles) of ethyl isobutyrylacetate (II). The reaction mixture was mixed thoroughly and cooled to −5° with ice–salt and 8 cc. of piperidine added. The mixture was shaken, left standing in the ice-bath for a few hours and then allowed to stand at room temperature with occasional shaking for forty-eight hours. Upon distillation under diminished pressure there was obtained 316 g. (86%) of ethyl α-isobutyrylcinnamate; b. p. 148–153 (3 mm.); n^{25}_{D} 1.5384, d^{25}_{4} 1.0543.

Anal. Calcd. for $C_{15}H_{18}O_3$: C, 73.13; H, 7.37. Found: C, 73.15; H, 7.30.

Ethyl α-Carbethoxy-α′-isobutyryl-β-phenylglutarate (III) from Benzalmalonic Ester (I) and Isobutyrylacetic Ester (II).—In a 500 cc., 3-necked flask, fitted with a separatory funnel, reflux condenser and copper blade stirrer, were placed 55.3 g. (0.35 mole) of II, 125 cc. of anhydrous ether, 7 cc. of absolute alcohol and 0.7 g. (0.035 gram atom) of sodium. When the sodium had all dissolved 87 g. (0.35 mole) of I was added to the reaction mixture and the separatory funnel washed out with an additional 75 cc. of ether. Heat was liberated upon addition of I to the ether solution. The mixture was stirred under gentle refluxing for the time indicated in Table I and then the reaction was stopped by the addition of ice water containing a slight excess of acetic acid. The ether solution was washed once with 5% sodium bicarbonate solution, once with water and finally dried over anhydrous sodium sulfate. After removal of the ether the reaction products were fractionally distilled through a 30-cm. Widmer column. The following fractions were collected and weighed: 85–90° (15 mm.) (II and V); 148–152° (3 mm.) (I and VI); 186–193° (3 mm.) (III). The determination of the amount of ethyl isobutyrylacetate (II) in the first fraction is based on the fact that this ester is saponified quantitatively by 1 *N* sodium hydroxide into sodium carbonate and the corresponding ketone whereas malonic ester is converted into sodium malonate by this reagent. Approximately 2 g. of the mixture of II and V was weighed out accurately into a Pyrex bomb tube, 50 cc. of 1 *N* sodium hydroxide added and the tube sealed and heated at 130° for eight hours to ensure complete saponification. The tube was then cooled, opened and the amount of sodium carbonate determined by acidifying with 50% sulfuric acid, bubbling carbon dioxide-free air through the mixture for one hour at room temperature. After passing through concentrated sulfuric acid and dehydrite the evolved carbon dioxide was absorbed in ascarite and weighed. Check runs on known mixtures of malonic and isobutyrylacetic esters and on pure samples of each showed this method of estimation to be accurate to within 3%.

The results obtained in these experiments are shown in Table I.

Preparation of III from Malonic Ester (V) and Ethyl α-Isobutyrylcinnamate (VI).—This reaction was carried out in the same manner as described above except that 0.35 mole each of V and VI were used instead of I and II. The results are shown in Table II.

Pure ethyl α-carbethoxy-α′-isobutyryl-β-phenylglutarate boils at 188–190° (3 mm.), n^{25}_{D} 1.4929, d^{25}_{4} 1.1198.

Anal. Calcd. for $C_{22}H_{30}O_7$: C, 64.99; H, 7.47. Found: C, 65.34; H, 7.47.

Summary

Ethyl α-carbethoxy-α′-isobutyryl-β-phenylglutarate has been prepared by the addition of ethyl isobutyrylacetate to benzalmalonic ester and also by the addition of malonic ester to ethyl α-isobutyryl cinnamate. Some data on the relative rate and retrogression of the Michael reaction are presented and discussed.

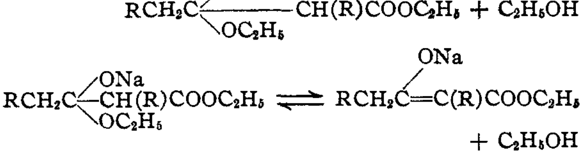
MADISON, WISCONSIN RECEIVED JANUARY 25, 1934

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Acetoacetic Ester Condensation.
VI. A Study of the Mechanism of the Reaction

BY RICHARD F. B. COX, EDWIN H. KROEKER AND S. M. MCELVAIN

In a previous paper[1] it was pointed out that Dieckmann's interpretation of the acetoacetic ester condensation as a two-phase reversible reaction



$$RCH_2C(ONa)(OC_2H_5)(OC_2H_5) + HCH(R)COOC_2H_5 \rightleftharpoons RCH_2C(ONa)(OC_2H_5)\text{—}CH(R)COOC_2H_5 + C_2H_5OH$$

$$RCH_2C(ONa)(OC_2H_5)\text{—}CH(R)COOC_2H_5 \rightleftharpoons RCH_2C(ONa){=}C(R)COOC_2H_5 + C_2H_5OH$$

failed to explain the significant fact that an ester, such as ethyl isobutyrate, having only one hydrogen on the α-carbon atom, does not undergo the first phase of the reaction even when conditions are such as to allow for the removal of the alcohol as it is formed in the reaction mixture.

Nevertheless, certain types of esters have been reported to undergo an internal acetoacetic ester condensation in which an α-carbon atom possessing only one hydrogen atom is involved. Perkin and Thorpe[2] have reported the condensation of 1,1 - dimethyl - 2,3 - dicarbethoxy - 3 - diethyl-

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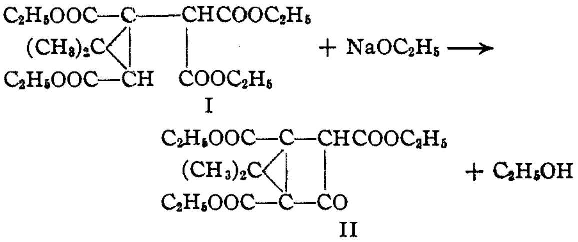
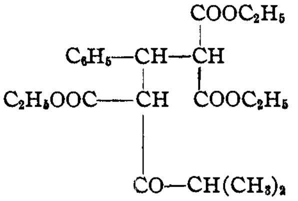
[1] McElvain, THIS JOURNAL, **51**, 3124 (1929).

[2] Perkin and Thorpe, *J. Chem. Soc.*, **79**, 736 (1901); *cf.* also Ingold and Thorpe, *ibid.*, **115**, 330 (1919); Farmer and Ingold, *ibid.*, **117**, 1362 (1920).

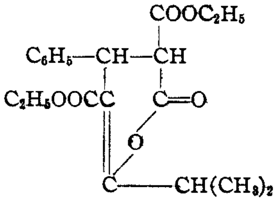
malonate-cyclopropane (I) to 2,3,4-tricarbethoxy-3,4-endoisopropylidene cyclobutanone-1 (II) by means of sodium ethoxide.



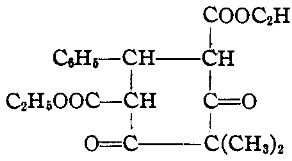
Also Dieckmann and Kron[3] found that benzalmalonic ester and isobutyrylacetic ester condensed in the presence of sodium ethoxide to form 2,2 - dimethyl - 5 - phenyl - 4,6 - dicarbethoxycyclohexanedione-1,3 (V). This latter product apparently was produced by an internal acetoacetic ester condensation of ethyl α-carbethoxy - α′ - isobutyryl - β - phenylglutarate (III) which resulted from a Michael condensation of the initial reactants.[4] Dieckmann postulated that the condensation of such esters was due to the presence of a high enolic acidity in the condensation product, a condition which rendered it sufficiently stable to resist reversal of the acetoacetic ester condensation.



III



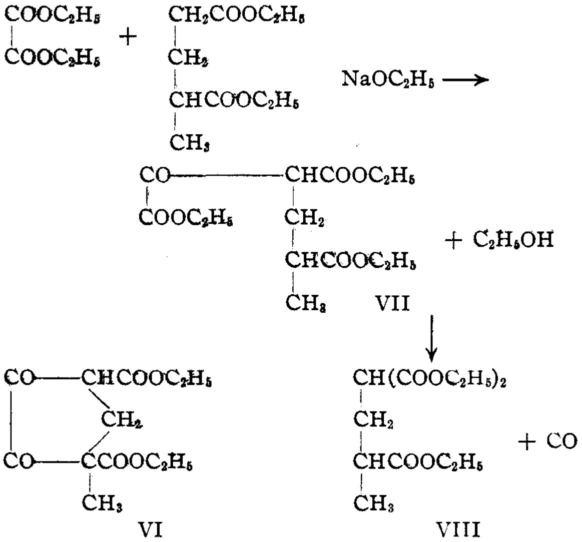
IV



V

Before concurring in the supposition that V was formed from III through an acetoacetic ester condensation it seemed necessary to ascertain whether or not V might have been produced by the rearrangement of IV, a lactone which could conceivably be formed from an enolic modification of III. However, the fact that III could not be caused to lose alcohol when heated alone to quite high temperatures (250°) for twenty-four hours, but did lose alcohol readily to give as high as 42% yields of V when heated to 120° in the presence of sodium ethoxide for one hour, showed beyond a doubt that an internal acetoacetic ester condensation was responsible for the formation of V.

The question then arises as to what type of structure will allow an α-carbon atom carrying only one hydrogen atom to function in an acetoacetic ester condensation. Certainly, it does not seem that a high enolic acidity in the condensation product is the prime requisite, for Dieckmann himself found that the expected cyclic condensation product (VI) could not be obtained when ethyl oxalate and diethyl α-methylglutarate were allowed to react in the presence of sodium ethoxide.[5] It would seem that VI should have as high enolic acidity as II, and have the further advantage of possessing the more stable cyclopentanone structure. When the condensation of ethyl oxalate and diethyl α-methylglutarate in the presence of sodium ethoxide was carried out in this Laboratory, it was found that slightly more than the theoretical amount of alcohol for the formation of VII distilled from the reaction mixture. During the reaction as well as while the reaction products were being distilled, there was a vigorous evolution of carbon monoxide, indicating that VII was decomposing into diethyl α-methyl-α′-carbethoxyglutarate (VIII). This latter product was isolated in 37% yields. The remainder of the reaction products consisted of small amounts of higher boiling material and an undistillable tar. There was no indication that VI (which would be expected to be a solid[6] and boil



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[3] Dieckmann and Kron, *Ber.*, **41**, 1260 (1908).

[4] Kroeker and McElvain, THIS JOURNAL, **56**, 1171 (1934).

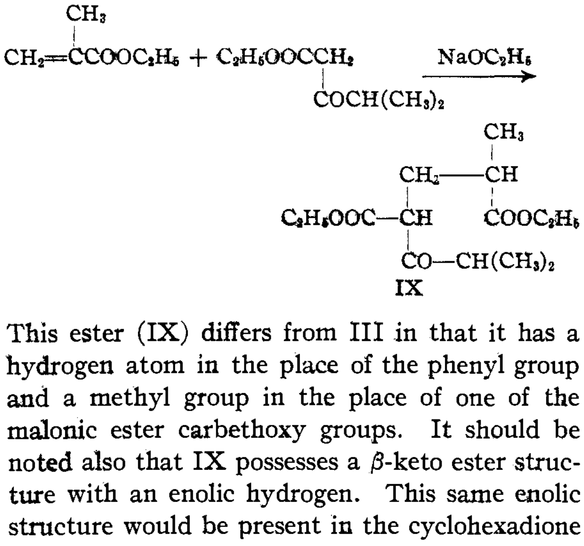
[5] Dieckmann, *Ber.*, **32**, 1930 (1899).

[6] Ref. 5, p. 1931.

in the neighborhood of VIII) was present in the reaction products.

An inspection of the structures of the esters I and III shows that the carbethoxy group which functions in the condensation is attached to a carbon atom which carries another carbethoxy group, *i. e.*, it is a part of a malonic ester structure. This particular structure is absent in the ester VII which would be the expected precursor of VI. The failure of VII to undergo further condensation to form the cyclic structure VI must be due, therefore, to either the absence of an essential structure or to its proneness to decompose into VIII. In order to ascertain whether or not a particular structure was necessary for this type of acetoacetic ester condensation it seemed advisable to prepare an ester which contained the $(CH_3)_2CHCO\underset{|}{C}HCOOC_2H_5$ structure of III and the $-CH(CH_3)COOC_2H_5$ structure of VII.

The compound chosen for this purpose was diethyl α-methyl-α'-isobutyrylglutarate (IX), which was prepared by the addition of isobutyryl acetic ester to α-methylacrylic ester

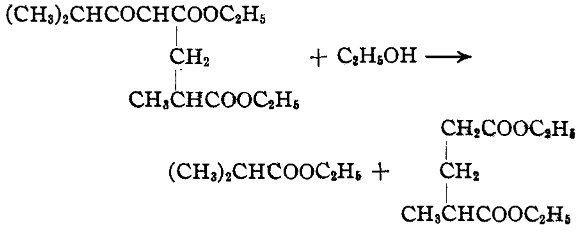


$$CH_2{=}\overset{CH_3}{\overset{|}{C}}COOC_2H_5 + C_2H_5OOC\underset{COCH(CH_3)_2}{\underset{|}{C}}H_2 \xrightarrow{NaOC_2H_5} \begin{array}{l} CH_2\text{——}\overset{CH_3}{\overset{|}{C}}H \\ C_2H_5OOC\text{—}\underset{CO\text{—}CH(CH_3)_2}{\underset{|}{C}}H \quad COOC_2H_5 \end{array}$$

IX

This ester (IX) differs from III in that it has a hydrogen atom in the place of the phenyl group and a methyl group in the place of one of the malonic ester carbethoxy groups. It should be noted also that IX possesses a β-keto ester structure with an enolic hydrogen. This same enolic structure would be present in the cyclohexadione which would be obtained if IX were to undergo the acetoacetic ester condensation in the manner of III to V.

When one mole of the ester IX was heated with a molecular equivalent of sodium ethoxide at 115–125° for two and one-half hours, a liquid, consisting of approximately 0.85 mole of alcohol and 0.63 mole of ethyl isobutyrate, distilled from the reaction mixture. The presence of this latter product in the distillate indicated that the ester IX had been cleaved to the extent of 63% by alcohol which was formed in the reaction mixture. This cleavage would produce an equivalent amount of diethyl α-methylglutarate, thus



$$\begin{array}{l} (CH_3)_2CHCOCHCOOC_2H_5 \\ \qquad\quad | \\ \qquad\quad CH_2 \qquad + C_2H_5OH \longrightarrow \\ \qquad\quad | \\ \quad CH_3CHCOOC_2H_5 \end{array} \qquad (CH_3)_2CHCOOC_2H_5 + \begin{array}{l} CH_2COOC_2H_5 \\ | \\ CH_2 \\ | \\ CH_3CHCOOC_2H_5 \end{array}$$

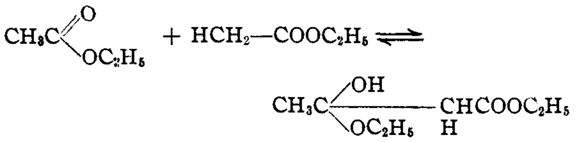
Since an amount of alcohol equivalent to the ethyl isobutyrate is consumed in this reaction, it appears that 1.48 (0.63 + 0.85) moles of alcohol was produced by the original reactants. The formation of such an amount of alcohol indicates that some acetoacetic ester condensation had taken place. When the reaction mixture was worked up and the products fractionated, a relatively small yield of diethyl α-methylglutarate was obtained. Practically all of the original ester IX which was not accounted for as ethyl isobutyrate was recovered unchanged when the reaction products were fractionated. The remainder of the reaction products were materials boiling higher than the ester IX. There was no evidence of a cyclohexanedione having formed from IX, for such a product would boil lower than IX and would in all probability be a solid.

The difference between the yield of ethyl isobutyrate and diethyl α-methylglutarate gave a clew as to the nature of the higher boiling products. Theoretically the yields of these two substances should be the same. The relatively low yield of diethyl α-methylglutarate indicated that this reaction product had been used up through a further reaction with sodium ethoxide. Indeed when diethyl α-methylglutarate was heated with sodium ethoxide an amount of alcohol corresponding to about 75% of the theoretical (based on one acetoacetic ester condensation per two moles of ester) distilled from the reaction mixture. Fractionation of the products of this reaction gave fractions boiling over the same range as those higher boiling materials obtained from the reaction of sodium ethoxide with IX.

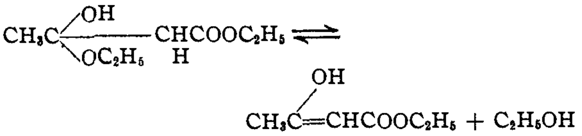
It would seem safe to conclude from these results that the ester IX will not undergo an internal acetoacetic ester condensation. While it is true that IX suffers cleavage under the conditions of the experiment, nevertheless this cleavage is not sufficiently extensive to prevent a considerable quantity of the unchanged ester from being isolated from the reaction products. Consequently, the failure of IX to undergo the condensation cannot be ascribed to the greater speed of a competitive reaction as might be done in the case of VII. It appears, therefore, that the ester IX does not have the requisite structure to allow the condensation to occur.

Any mechanism, then, that attempts to explain the acetoacetic ester condensation must recognize the following facts: (1) the condensation takes place between a carbethoxy group of a simple ester and a carbon atom which carries at least two hydrogen atoms and a negative group such as carbethoxy, acyl, cyano, etc. (2) If the reacting carbethoxy group is attached to a carbon atom which carries another carbethoxy group, then the condensation can take place with a carbon atom which carries a suitable negative group and only one hydrogen atom. Up to the present this latter type of condensation has been studied only with compounds that contain both of the reacting groups in a single molecule.

On the basis of these facts it would seem to the authors that the acetoacetic ester condensation could be best interpreted by an extension of the aldol-like mechanism, first suggested by Michael,[7] in which the primary condensation product, through the loss of a molecule of alcohol, passes into the enolic form of a β-keto ester (or cyanide, etc.). To illustrate with ethyl acetate



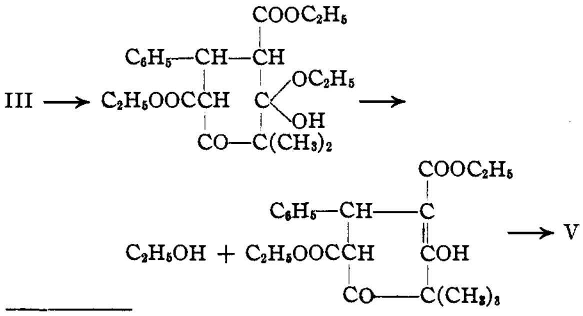
$$CH_3C\genfrac{}{}{0pt}{}{\diagup O}{\diagdown OC_2H_5} + HCH_2{-}COOC_2H_5 \rightleftharpoons CH_3C\genfrac{}{}{0pt}{}{\diagup OH}{\diagdown OC_2H_5}{-}\underset{H}{C}HCOOC_2H_5$$



$$CH_3C\genfrac{}{}{0pt}{}{\diagup OH}{\diagdown OC_2H_5}{-}\underset{H}{C}HCOOC_2H_5 \rightleftharpoons CH_3\overset{OH}{C}{=}CHCOOC_2H_5 + C_2H_5OH$$

This interpretation would presume that the formation of the hemi-acetal structure on the reacting carbethoxy group would destroy the activating effect of the latter group on the hydrogen atoms on the γ-carbon atom of the primary condensation product. The only hydrogens remaining sufficiently reactive to split out with the ethoxy group as alcohol would be those on the α-carbon atom. The elimination of alcohol from such a hemi-acetal structure would seem likely in view of the tendency of the acetals of β-keto esters to decompose with the loss of alcohol into β-alkoxycrotonic esters.[8] Sodium ethoxide may function catalytically in bringing about both phases of this reaction, but its most obvious purpose is to form a salt with the enolic form of the condensation product.

From such a picture of the mechanism of the reaction it is apparent why such an ester as ethyl isobutyrate does not condense with itself while esters of the type of I and III condense through a carbon atom containing only one hydrogen. In the former case the intermediate hemi-acetal product would have no available hydrogen on the α-carbon atom to be eliminated with the ethoxy group. The same situation would exist, so far as this particular carbon atom is concerned, in the intermediate product derived from I or III, but the hemi-acetal group in this intermediate is adjacent to another carbon atom carrying a hydrogen which is activated by a carbethoxy group. The complete condensation, therefore, can proceed because there are available two active hydrogens although they are attached to different carbon atoms. The mechanism of the condensation, in the case of such an ester as III, may, therefore, be illustrated as follows



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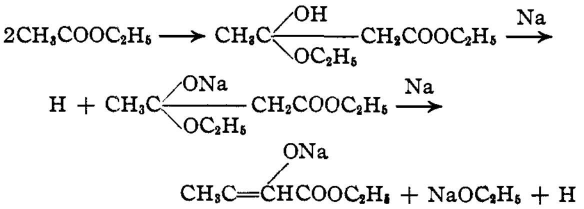
(7) Michael's suggestion, *J. prakt. Chem.*, [2] **37**, 483 (1888), was concerned with the condensation of ethyl acetate by sodium metal and may be illustrated thus



$$2CH_3COOC_2H_5 \longrightarrow CH_3C\genfrac{}{}{0pt}{}{\diagup OH}{\diagdown OC_2H_5}{-}CH_2COOC_2H_5 \xrightarrow{Na} H + CH_3C\genfrac{}{}{0pt}{}{\diagup ONa}{\diagdown OC_2H_5}{-}CH_2COOC_2H_5 \xrightarrow{Na} CH_3\overset{ONa}{C}{=}CHCOOC_2H_5 + NaOC_2H_5 + H$$

However, recent work [Snell and McElvain, THIS JOURNAL, **53**, 2310 (1931)] has indicated that sodium metal is probably not the active condensing agent in the acetoacetic ester condensation and that when sodium is used to bring about the condensation the relative amounts of the various reaction products are totally different than those indicated by Michael's interpretation of the reaction.

(8) Moureu, *Bull. soc. chim.*, [3] **31**, 509 (1904).

Experimental

Attempt to Form the Lactone IV.—Sixty grams of ethyl α-carbethoxy-α′-isobutyryl-β-phenylglutarate[4] (III) was placed in a flask fitted with a condenser for distillation and heated in an oil-bath at 250° for twenty-four hours. No material distilled from the reaction flask during this period of heating. Fractionation of the heated ester indicated that the ester had been pyrolyzed into lower boiling (retrogression) products and a considerable amount of an undistillable tar.

Reaction of III with Sodium Ethoxide.—To 10.2 g. (0.15 mole) of sodium ethoxide contained in a flask fitted with a vacuum sealed stirrer and a condenser for distillation was added 60.9 g. (0.15 mole) of III and the mixture heated at 120° for one hour. The distillate receiver was cooled by immersion in a solid carbon dioxide-acetone mixture. The reaction was run under diminished pressure in order that the alcohol might be removed rapidly. During the course of the reaction 11.4 g. (0.25 mole) of alcohol was collected. Of this alcohol 0.15 mole would obviously result from the reaction of the enol form of III with sodium ethoxide, and approximately this amount distilled over quite rapidly. The remainder of the alcohol (0.10 mole) came over more slowly during the course of the reaction and represented the amount of condensation which was taking place.

The reaction mixture soon became gummy and increasingly difficult to stir as the reaction progressed. When cool it set to a brittle, amber-clear solid. This material was broken up, ground to a powder and decomposed with ice water containing a slight excess of acetic acid. The insoluble layer was taken up in ether, washed and dried, and, after removal of the ether, recrystallized from 95% alcohol. A yield of 21.7 g. (42%) of 2,2-dimethyl-5-phenyl-4,6-dicarbethoxycyclohexanedione-1,3 (V), m. p. 144–145°, was obtained. The alcohol was removed from the mother liquor and the residue fractionated. The following fractions were obtained: (1) 9.4 g. lower boiling (under 184° (3 mm.)) products; this material was found to be a mixture of the retrogression products[4] of III; (2) 12.5 g. (21%) of unchanged III, b. p. 184–187 (3 mm.); (3) 8.9 g. of an undistillable residue.

Diethyl α-Methyl-α′-isobutyrylglutarate (IX).—To 300 cc. of absolute alcohol containing 2 g. of sodium ethoxide, was added 57.0 g. (0.5 mole) of ethyl α-methylacrylate[9] and 85 g. (0.537 mole) of ethyl isobutyrylacetate. This mixture was heated for ten hours on a steam-bath. At the end of this time, the alcohol and low boiling material were removed under reduced pressure. The residue was poured onto 100 g. of ice and neutralized with 4 cc. of acetic acid in 25 cc. of water. The ester layer was separated and the water layer was extracted once with ether. After drying the combined extracts and fractionally distilling, 40 g. of the ester IX boiling at 144–147° (10 mm.) was obtained; n_D^{25} 1.4384, d_4^{25} 1.015, mol. wt. (ethylene bromide) calcd. for $C_{14}H_{24}O_5$, 272, found 278.

Anal. Calcd. for $C_{14}H_{24}O_5$: C, 61.76; H, 8.83. Found: C, 61.87; H, 8.87.

Condensation of Ethyl Oxalate with Diethyl α-Methylglutarate. Diethyl α-Methyl-α′-carboxyglutarate (VIII).—A mixture of 29.2 g. (0.2 mole) of diethyl oxalate[10] and 20.2 g. (0.1 mole) of diethyl α-methylglutarate[11] was added to 6.8 g. (0.1 mole) of sodium ethoxide in a 200-cc. flask fitted with a vacuum-sealed stirrer and a 15-cm. upright side arm. The side arm, which served as a fractionating column, was connected to a receiver cooled with a mixture of solid carbon dioxide and acetone. In a typical run the reaction flask was heated in a Woods metal bath at 125–130° for four hours, during which time gentle, intermittent suction was applied to the receiver. After the reaction was over the distillate which collected in the receiver was redistilled and weighed. It was pure alcohol, b. p. 78–79°, and amounted to 9.45 g. (0.205 mole).

The solid residue in the reaction flask was pulverized and dissolved in 300 cc. of ice water containing 8 cc. of glacial acetic acid. The oily layer which separated was extracted with four 50-cc. portions of ether and, after drying, fractionally distilled. No oxalyl methyl glutaric ester (VII) could be isolated as it decomposed both during the reaction and on subsequent distillation with the loss of carbon monoxide into VIII. The distillate was separated into the following fractions: ethyl oxalate, b. p. 183–184°, 15 g. (0.103 mole); diethyl α-methylglutarate, b. p. 122–124° (19 mm.), 3.g. (0.015 mole); diethyl-α-methyl-α′-carbethoxyglutarate (VIII), b. p. 165–166° (22 mm.), 10 g. (0.037 mole); higher boiling (180–210° (5 mm.)) material 2 g.; undistillable residue, 2 g. These fractions together with small amounts of intermediate fractions accounted for practically all of the starting materials.

Increasing the amount of sodium ethoxide used in the reaction served merely to increase the yield of higher boiling material at the expense of compound VIII.

Reaction of IX with Sodium Ethoxide.—To 6.8 g. (0.1 mole) of sodium ethoxide was added 27.2 g. (0.1 mole) of diethyl α-methyl-α′-isobutyrylglutarate (IX) and the condensation carried out as described above for two and one-half hours at 115–125°. The distillate consisted of a mixture of alcohol and ethyl isobutyrate. The amount of the latter compound, estimated by saponification with standard alkali, was 7.3 g. (0.063 mole) while the yield of alcohol, estimated by difference, was 3.9 g. (0.085 mole). The residue in the reaction flask was taken up in ice water, decomposed with a slight excess of glacial acetic acid and extracted with ether. The combined ether extracts, after drying, were then fractionated. The following fractions were obtained: (1) diethyl α-methylglutarate, b. p .108–112° (11 mm.), 2 g.; (2) a mixture of unchanged diethyl α-methylglutarate and IX, b. p. 130–143° (11 mm.), 4 g.; (3) unreacted IX, b. p. 143–148° (11 mm.), 6 g.; (4) b. p. 149–154° (11 mm.), 3 g.; (5) b. p. 154–184° (11 mm.), 2 g.; undistillable residue, 3 g. These fractions accounted for practically all of the starting materials.

The yield of 0.063 mole of ethyl isobutyrate from the reaction indicates that 63% of the original ester IX had been cleaved. The remainder (37%) of the ester IX which was put into the reaction amounted to 10.1 g. of which 6 g. was recovered in pure state as fraction 3.

Fraction 2 was mainly low boiling unchanged ester IX and did not contain any of the cyclic condensation product

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[9] Ruzicka, *Helv. Chim. Acta*, **2**, 152 (1919).

[10] "Organic Syntheses," 1925, Vol. V, p. 5.

[11] Ingold, *J. Chem. Soc.*, **119**, 339 (1921).

as shown by hydrolysis with 85% acetic acid.[12] This hydrolysis gave two fractions, one acidic and one neutral. The acidic fraction formed a semicarbazone, m. p. 161–162°, corresponding to the semicarbazone of α-methyl-δ-keto-iso-octanic acid.[13] The neutral fraction proved to be the ethyl ester of this acid. It is obvious that both of these compounds result from the ketonic hydrolysis of the ester IX. No trace of a cyclic ketone could be detected.

It seems reasonably certain that fraction 4 also contains some unchanged ester IX.

The products boiling higher than fraction 4 appear to have been formed by the condensation of diethyl α-methylglutarate with itself. This conclusion seems likely in view of the low yield of this ester (relative to ethyl isobutyrate) from the reaction. In fact when diethyl α-methylglutarate was heated alone with sodium ethoxide under the conditions described above for the reaction of IX, alcohol distilled from the reaction mixture and when the reaction product was worked up and fractionated, the same series of higher boiling fractions as were obtained in the case of IX resulted.

Summary

A study of the acetoacetic ester condensation between a carbethoxy group and an alpha carbon atom carrying a single hydrogen atom has been made. It has been found that such a condensation takes place only when the condensing carbethoxy group is a part of a monosubstituted malonic ester structure. A mechanism, which explains the facts now known, is suggested for the acetoacetic ester condensation.

MADISON, WISCONSIN RECEIVED JANUARY 25, 1934

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[12] This reagent has been shown by Dieckmann and Kron (Ref. 3, p. 1266) to decarboxylate the cyclic β-ketonic esters to yield the cyclic ketones.

[13] Wallach, *Ann.*, **327**, 138 (1903).

[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

The Biuret Reaction. IV. (a) A Biuret Salt of the Tetrapeptide Triglycylglycine. (b) A Biuret Salt of Glycine Anhydride. (c) The Barium Biuret Salt of Succinimide[1,2]

BY MARY M. RISING, FRANCIS M. PARKER AND DOROTHY R. GASTON

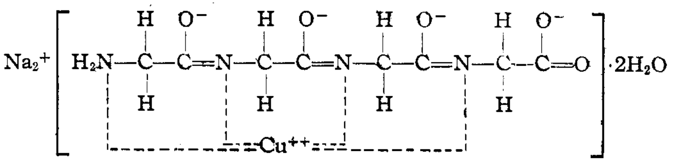
Following the investigation of the biuret reaction of amino acid amides,[3] and in preparation for a study of the biuret reaction of the proteins, the biuret salt of the tetrapeptide triglycylglycine, $NH_3^+ (CH_2CONH)_3CH_2CO_2^-$ has been isolated and examined.

The synthesis of triglycylglycine was accomplished in a series of reactions described by Fischer.[4] Glycine was converted into its ester hydrochloride, from which there was obtained glycine anhydride. The anhydride yielded upon hydrolysis and treatment with chloroacetyl chloride in one operation chloroacetylglycylglycine, which was converted by the use of ammonia into diglycylglycine. By treatment of the tripeptide successively with chloroacetyl chloride and ammonia, triglycylglycine was obtained. It was found that liquid ammonia acts more efficiently in the ammonolysis of certain of the chloroacetyl peptides than does the aqueous ammonia recommended by Fischer.

The biuret salt sodium copper triglycylglycine

$Na_2^+[Cu^{++}(NH_2(CH_2CO^- = N)_3CH_2CO_2^-)]\cdot 2H_2O$

was prepared by treatment of the tetrapeptide with copper acetate and sodium hydroxide. The analytical data for the salt point to the empirical formula just given. It is of peculiar interest to note that the molecule of the salt contains only one peptide molecule, which furnishes the four basic nitrogen atoms and the four acid hydrogen atoms which have already been found requisite for the occurrence of a number of typical biuret reactions. The molecular weight of the salt has not been ascertained. If its molecular and empirical formulas are identical the structure of the salt may be



$$\mathrm{Na_2^+}\left[\mathrm{H_2N{-}\overset{H}{\underset{H}{C}}{-}\overset{O^-}{C}{=}N{-}\overset{H}{\underset{H}{C}}{-}\overset{O^-}{C}{=}N{-}\overset{H}{\underset{H}{C}}{-}\overset{O^-}{C}{=}N{-}\overset{H}{\underset{H}{C}}{-}\overset{O^-}{C}{=}O}\;\;\mathrm{Cu^{++}}\right]\cdot 2\mathrm{H_2O}$$

Sodium copper triglycylglycine

It will be noted that formation of a salt molecule of this structure involves (1) neutraliza-

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[1] The contents of this paper were reported at the National Meeting of the American Chemical Society held at Chicago, September, 1933.

[2] The work here described constitutes part of the dissertations of Francis M. Parker and Dorothy R. Gaston, presented in partial fulfilment of the requirements for the doctorate degree at the University of Chicago.

[3] Rising and Yang, *J. Biol. Chem.*, **99**, 755 (1933).

[4] E. Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," Vols. I and II, Verlag von Julius Springer, Berlin, 1906.