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The Synthesis of Higher Aliphatic β-Keto Acids[1]

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In connection with our studies[3,4] on the relation of biotin and fatty acids we became interested in preparing a number of β-keto acids for microbiological experimentation. Although higher β-keto acids have been postulated as intermediates in the metabolism of fatty acids, little is known about the preparation and properties of these compounds. Unsaturated members of the series have not been previously prepared.

Starting materials for the present work were highly purified fatty acids[5] which were converted into the corresponding β-keto esters containing two additional carbon atoms in the chain by the excellent procedure of Ställberg-Stenhagen.[6] The properties of methyl 3-ketomyristate, palmitate, stearate and arachidate thus prepared agreed with those given in the literature.[6] Application of the same procedure to oleic acid led to the formation of methyl 3-keto-$\Delta^{11,12}$-eicosenoate (methyl oleoylacetate) which has not been previously described. This compound was related to methyl 3-ketoarachidate by catalytic hydrogenation.

The transformation of β-keto esters into the corresponding β-keto acids has been studied by several workers. Asahina and Nakayama[7] claimed the preparation of β-ketostearic acid by hydrolysis with dilute alkali of the corresponding ethyl ester. Their results are invalid since the properties of the starting ester failed to agree with those reported by other workers.[6] Breusch and Keskin[8] hydrolyzed a number of β-keto esters with potassium hydroxide and isolated extremely small quantities of the corresponding β-keto acids.

In view of the poor results obtained by the use of alkali in the saponification of β-keto esters, we turned our attention to the development of other hydrolysis procedures. As a result a method generally applicable to the preparation of higher β-keto acids was developed. This consists in cleaving the esters at room temperature with a mixture of glacial acetic and hydrochloric acids. The saturated free β-keto acids precipitate from the reaction mixture in a high state of purity in almost quantitative yields and were further purified by recrystallization from acetone. Whether this cleavage represents a straight hydrolysis or an acid-catalyzed ester interchange remains to be determined.

The pure β-keto acids are well defined crystalline substances decomposing around 100° with the evolution of carbon dioxide and the formation of the corresponding methyl ketones. The constitution of the acids followed from carbon and hydrogen analyses, neutral equivalent determinations and the fact that the correct methyl ketones were obtained on decarboxylation.

The 3-keto-$\Delta^{11,12}$-eicosenoic acid (oleoylacetic acid) was obtained in a similar manner from the corresponding methyl ester as a crystalline material which decomposed at 57–58° with the evolution of carbon dioxide and the formation of $\Delta^{10,11}$-nonadecenone-2 which was characterized as the semicarbazone.

The β-keto acids and esters were tested for their ability to replace biotin for *Lactobacillus arabinosus*. Since previous work[4] had shown that saturated fatty acids are incapable of promoting growth of this organism, it was not surprising to find the corresponding β-keto acids and esters inactive. The methyl oleoylacetate was as active as methyl oleate in contrast to the free oleoylacetic acid which was almost devoid of biological activity. The inactivity of the latter compound is probably due to its decarboxylation during the incubation period leading to $\Delta^{10,11}$-nonadecenone-2 which was also found to be inactive. The activity of the methyl oleoylacetate may be explained in two ways. Either this substance is active as such or it is converted into oleic acid by the organism.

The development of the present convenient method for the preparation of β-keto acids makes these substances readily available for further investigation.

Experimental[9,10]

Saturated β-Ketomethyl Esters.—Methyl β-ketomyristate, palmitate, stearate and arachidate were prepared according to the method of Ställberg-Stenhagen.[6] The melting points and analytical data of the compounds obtained are summarized below (Table I).

Saturated β-Keto Acids.—It is essential for the success of this procedure that β-keto esters of a high degree of purity be used.

The methyl esters (0.5 g.) were dissolved in glacial acetic acid (10 ml.) and concentrated hydrochloric acid (approximately 1 to 5 ml.) added dropwise until the solutions

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(4) Axelrod, Mitz and Hofmann, *ibid.*, **175**, 265 (1948).

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(6) Ställberg-Stenhagen, *Arkiv Kemi, Mineral, Geol.*, **20A**, No. 19 (1945).

(7) Asahina and Nakayama, *J. Pharm. Soc. Japan*, No. **526**, 3 (1925).

(8) Breusch and Keskin, *Rev. faculté sci. univ. Istambul*, **XIA**, 24 (1946).

(9) The microanalyses were performed in our microanalytical laboratory by Mr. George L. Stragand.

(10) The melting points were determined with Anschütz thermometers and are uncorrected.

TABLE I

Compound	Formula	M. p.,[a] °C.
Methyl β-ketomyristate	$C_{15}H_{28}O_3$	28.5 (28.7)
Methyl β-ketopalmitate	$C_{17}H_{32}O_3$	40 (40.1)
Methyl β-ketostearate	$C_{19}H_{36}O_3$	49 (49.2)
Methyl β-ketoarachidate	$C_{21}H_{40}O_3$	56.5 (56.4)

[a] The melting points in parentheses are those given in the literature.[6]

became slightly turbid, and the mixtures were allowed to stand at room temperature for forty-eight hours. The resulting white crystals were collected and dried *in vacuo* at room temperature over phosphorus pentoxide. Three recrystallizations from acetone gave the pure β-keto acids. The analytical data, yields and melting points of the acids prepared are summarized in Table II.

TABLE II

Compound	Formula	Yield, %	M. p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Neut. equiv. Calcd.	Neut. equiv. Found
β-Ketomyristic acid	$C_{14}H_{26}O_3$	90	93–94	69.38	69.14	10.82	10.64	242	240
β-Ketopalmitic acid	$C_{16}H_{30}O_3$	93	98–98.5	71.07	70.86	11.18	10.82	270	271
β-Ketostearic acid	$C_{18}H_{34}O_3$	90	102–103	72.43	72.65	11.48	11.68	299	297
β-Ketoarachidic acid	$C_{20}H_{38}O_3$	86	104–105	73.57	73.83	11.73	11.66	327	327

In order to further characterize these acids, small samples of them were heated at 10° above the decomposition point for one hour and the melting points of the resulting methyl ketones determined. The agreement between the melting points observed and those given in the literature is shown in Table III.

TABLE III

Compound	M. p., °C.
Tridecanone-2	28–29(29)[11]
Pentadecanone-2	38–39(39)[12]
Heptadecanone-2	48–49(49)[13]
Nonadecanone-2	55–56(56)[14]

Methyl 3-Keto-$\Delta^{11,12}$-eicosenoate (Methyl Oleoylacetate).—An ethyl sodioacetoacetate suspension was prepared in the usual manner from 13.6 g. of the ester and 2 g. of powdered sodium in 100 ml. of dry benzene. This suspension was cooled in an ice-bath and oleoyl chloride (prepared from 20 g. of oleic acid[15]) added dropwise with shaking over a period of fifteen minutes. The mixture was refluxed for fifteen minutes, cooled, poured on crushed ice and acidified to congo red with 5% sulfuric acid. Ethanol (70 ml.) was added and the two layers separated. The benzene layer was washed three times with 10% ethanol, dried over sodium sulfate and the solvent removed *in vacuo*. A sodium methoxide solution prepared by dissolving sodium (2.1 g.) in absolute methanol (63 ml.) was added to the residue and the solution kept at room temperature for ninety minutes. The mixture was then decomposed by pouring on crushed ice and acidified to congo red with 10% sulfuric acid. The organic material was extracted with ether, the ether solution washed with water, dried over sodium sulfate and the ether removed under reduced pressure. The resulting oily material on fractionation in a miniature molecular still[16] gave a main fraction (9.9 g., 41% yield) which boiled at a bath temperature of 140–150° at 0.001 mm. and melted at 5–6° ($n^{25}D$ 1.4566).

Anal. Calcd. for $C_{21}H_{38}O_3$: C, 74.51; H, 11.32. Found: C, 74.50; H, 11.41.

Hydrogenation.—The above methyl ester (678 mg.) dissolved in ethanol (5 ml.) was hydrogenated in the presence of a 5% palladium-on-charcoal catalyst (50 mg.) until one mole equivalent of hydrogen was absorbed. Purification of the resulting hydrogenation product through the copper chelate gave 491 mg. (73% yield) of methyl β-ketoarachidate melting at 55–56°. No depression of the melting point was observed when this sample was mixed with a sample of the same ester prepared from stearic acid.[6]

3-Keto-$\Delta^{11,12}$-eicosenoic Acid (Oleoylacetic Acid).—To a solution of the above methyl oleoylacetate (1 g.) in glacial acetic acid (20 ml.) concentrated hydrochloric acid (about 1 ml.) was added dropwise until a slight turbidity appeared, and the mixture was allowed to stand at room temperature for forty-eight hours. The solvents were evaporated *in vacuo* at room temperature leaving an almost dry solid from which the last traces of solvents were removed by further drying over potassium hydroxide pellets *in vacuo* at a temperature of 7–10°. The resulting white crystalline material (m. p. 55–56°) was purified by recrystallization from petroleum ether (b. p. 30–32°) at −20° when 400 mg. (42% yield) of oleoylacetic acid was obtained as white crystals which melted at 57–58° with the evolution of carbon dioxide. The compound is unstable and should be stored in the refrigerator.

Anal. Calcd. for $C_{20}H_{36}O_3$: C, 74.02; H, 11.18; neut. equiv., 325. Found: C, 73.98; H, 10.91; neut. equiv., 326.

Decarboxylation.—A sample of the above acid was decarboxylated by heating at 70° for one hour and the resulting methyl ketone distilled *in vacuo*.

Anal. Calcd. for $C_{19}H_{36}O$: C, 81.36; H, 12.94. Found: C, 81.19; H, 13.09.

The semicarbazone after recrystallization from ethanol melted at 101–102°.

Anal. Calcd. for $C_{20}H_{39}ON_3$: N, 12.45. Found: N, 12.33.

Microbiological Procedures.—Microbiological activity, *i. e.*, the ability to replace biotin in the metabolism of *L. arabinosus* was determined by the method of Wright and Skeggs[17] with the addition of 0.01% of L-asparagine to the basal medium. For testing purposes all compounds were dissolved in redistilled 95% ethanol. In the case of oleoylacetic acid and its methyl ester, the alcoholic solutions were added aseptically to the medium thereby avoiding exposure of these compounds to the autoclaving temperature. The amount of ethanol added to each culture tube was always below the tolerance limit of these organisms to this compound.

Summary

1. A practical procedure for the synthesis of higher β-keto acids was developed and applied to the preparation of a number of these compounds.

2. Methyl 3-keto-$\Delta^{11,12}$-eicosenoate was found to be as active as methyl oleate in replacing biotin in the nutrition of *Lactobacillus arabinosus*.

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