Synthesis and Properties of the α-Keto Acids

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I. Introduction

α-Keto acids, especially the α-keto acid analogues of the naturally occurring amino acids, are of major importance in intermediary metabolism. Thus, pyruvic acid is a metabolite involved in a number of enzyme-catalyzed intracellular phenomena, and oxaloacetic acid, α-ketoglutaric acid, and oxalosuccinic acid are intermediates in the tricarboxylic acid cycle. The α-keto acid analogues of the protein amino acids are often the penultimate products formed in the biosynthesis of amino acids, and are commonly the first product formed in the degradative metabolism of amino acids. Certain α-keto acids accumulate in the blood and tissues in pathological conditions. Recently, α-keto acids have been used in the therapy of certain conditions, e.g., uremia and nitrogen accumulation disorders. α-Keto acids are of continuing interest as intermediates in chemical syntheses, in the development of enzyme inhibitors and drugs, as model substrates of enzymes, and in other ways.

Berzelius prepared the first α-keto acid (pyruvic acid) in 1835.[1] A number of papers on the synthesis and properties of α-keto acids published during the latter part of the nineteenth century and early in the twentieth century contain information that is still of interest and thus potentially useful. With the advent of sensitive analytical techniques, especially during the last decade, interest in the biochemical importance of α-keto acids has increased substantially. Several reviews of α-keto acids of somewhat limited scope have previously appeared. In 1947, Waters published a review of the available synthetic procedures.[2] Reviews on the preparation of α-keto acids emphasizing enzymatic methods and certain chemical and enzymatic properties of α-keto acids were published by Meister in 1956, 1957, and 1965.[3-5] Cordier, in 1958 and 1964, reviewed the α-keto acids with emphasis on the synthesis of aromatic α-keto acids.[6] In the present review, we have attempted to survey all of the synthetic and enzymatic procedures now available for the preparation of α-keto acids. We include here 19 general methods for α-keto acid syn-

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thesis, and literature citations to about 200 α-keto acids. A number of the properties of the α-keto acids are given and methods for the determination of α-keto acids in biological materials are surveyed.

α-Keto acids (1) have the following structure:

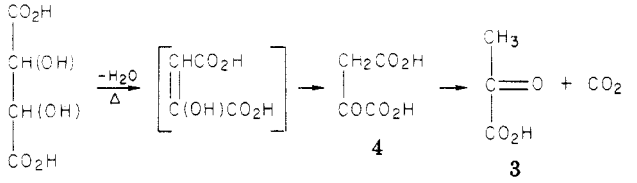
$$\underset{\mathbf{1}}{RC(O)CO_2H}$$

2, R = H
3, R = CH_3
4, R = CH_2CO_2H
5, R = $CH_2CH_2CO_2H$
6, R = $CH_2C_6H_5$

For purposes of this review, glyoxylic acid (**2**) is considered as an α-keto acid and indeed the reactions of this compound closely resemble those typical of many α-keto acids possessing larger R groups. A number of α-keto acids exist in atypical forms and thus may be less reactive than those that exist in the structure indicated above. Some of these are spontaneously or readily converted into cyclic or dimeric forms (see section VI).

II. Synthesis

The first α-keto acid to be prepared was pyruvic acid (pyroracemic acid) (**3**). This compound was prepared by Berzelius in 1835.[1] Many of the more biologically important α-keto acids were prepared over 75 years ago, e.g., glyoxylic (glyoxalic) (**2**),[7] oxaloacetic (oxalacetic) (**4**),[8,9] α-ketoglutaric (**5**),[10] and phenylpyruvic (**6**).[11] Waters lists the methods of synthesis of 36 α-keto acids, including **3**–**6**, and the date when each was first prepared.[2] Many of the α-keto acids have been prepared by unique methods (cf. Waters[2]). Thus, pyruvic acid (**3**) has been made in good yield by distilling tartaric acid.[12]



In the remainder of this section, we detail general methods that have been employed to synthesize α-keto acids. Note that some α-keto acids have a tendency to decompose as the anhydrous free acid and have only been isolated as the corresponding salt.

A. Hydrolysis of Acyl Cyanides (7)

The acyl bromide is refluxed with cuprous cyanide followed by hydrolysis in dilute acid.[13] Yields of acyl

$$RCOX + MCN \rightarrow \underset{\mathbf{7}}{RCOCN} \rightarrow \underset{\substack{\mathbf{3},\ R = CH_3\\ \mathbf{8},\ R = C_2H_5}}{RCOCO_2H}$$

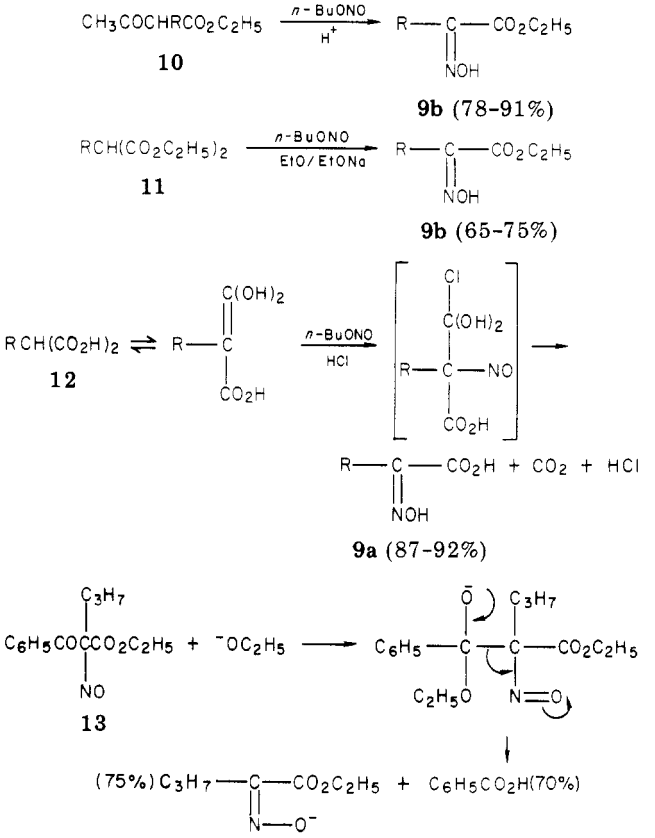
cyanide varied from 65 to 70%. In the case of the acyl cyanides of pyruvic and α-keto-*n*-butyric acid, hydrolysis with cold concentrated HCl gave yields of 75% α-keto acid. Hydrolysis of isovaleryl cyanide to α-ketoisovaleric acid could not be demonstrated, and it was suggested that the method is only applicable to the synthesis of straight-chain α-keto acids with no more than five carbon atoms.[13]

B. Hydrolysis of α-Keto Acid Oximes or α-Keto Acid Oxime Esters (9)

This method was originally proposed by Bouveault and Locquin in 1902.[14] The corresponding oxime (**9a**) or oxime ester (**9b**) is dissolved in water in the presence

$$\underset{\substack{\mathbf{9a},\ R' = H\\ \mathbf{9b},\ R' = C_2H_5}}{RC(=NOH)CO_2R'} \rightarrow RCOCO_2R' \rightarrow \underset{\mathbf{1}}{RCOCO_2H}$$

of formic acid at 0 °C and nitrosylsulfuric acid is added slowly.[14] Although the method has been criticized for often giving poor yields,[15] it is generally useful since the oximes or oxime esters can be made in excellent yield from substituted acetoacetic acids (**10**),[16,17] diethyl alkylmalonates (**11**),[17] alkylmalonic acids (**12**),[17] or nitrosated β-keto ester (**13**) (prepared from alkylated benzoylacetic ester in the presence of ethyl nitrite and sodium ethoxide).[18] Waters lists 10 α-keto acids that



have been made by this procedure.[2] The six α-keto acids originally prepared by Bouveault and Locquin are listed in Table I. The method has not always been successful. Attempts to make phenylpyruvate from the corresponding oxime resulted in formation of α-hydroxy-β-phenylcrotonolactone.[19]

C. Hydrolysis of Ethyl Esters of Oxalo Acids (14)

This method was first described by Adickes and Andresen in 1943.[15] Eleven α-keto acids were prepared this way as the barium salts in yields of 8–94%[15] (see

Arthur J. L. Cooper was born in 1946 in London, England. He obtained his B.Sc. and M.Sc. degrees from the University of London. In 1974 he obtained his Ph.D. degree in Biochemistry from Cornell University Medical College. After 2 years as a postdoctoral fellow at Brandeis University working with Professor Redfield in the area of biological applications of NMR, he returned to Cornell University Medical College, where he is currently Associate Professor of Biochemistry in Neurology. Dr. Cooper's interests include α-keto acid, amino acid, and ammonia metabolism, pyridoxal 5′-phosphate enzymes, and the use of metabolic inhibitors. Recently Dr. Cooper has become involved in the synthesis of compounds labeled with short-lived positron emitters for the study of in vivo metabolism in the human brain.

Alton Meister was born in New York in 1922. In 1942 he graduated (cum laude) from Harvard and obtained his M.D. from Cornell Medical College in 1945. Following internship and residency at New York Hospital (1945–1946) he became a Research Investigator (Commissioned Officer, USPHS) at the National Institutes of Health, Bethesda. In 1951 he was appointed head of the Clinical Biochemical Research Section, National Cancer Institute, NIH. In 1955 he became Professor and Chairman of the Department of Biochemistry, Tufts University School of Medicine. In 1967 he returned to New York to become Professor and Chairman of the Department of Biochemistry, Cornell University Medical College. Since 1971 he has also held the position as Biochemist-in-Chief at the New York Hospital–Cornell Medical Center. Dr. Meister's research interests include mechanism and scope of transamination reactions; properties of the α-keto acids; the role of pyridoxal 5′-phosphate in amino acid metabolism; specificity of lactate dehydrogenase; tumor enzymology; phenylketonuria; mechanism by which ATP is used in biosynthetic reactions; mapping of the active site of glutamate- and glutamine-utilizing enzymes; enzymology and metabolism of glutamate, glutamine, and asparagine; enzymology and metabolism of glutathione and γ-glutamyl amino acids; the γ-glutamyl cycle; inhibition of enzymes involved in the synthesis and utilization of glutathione and glutamine by active-site directed reagents; 5-oxoproline metabolism; and physiological functions of glutathione. In 1954, Dr. Meister received the Paul–Lewis Award in Enzyme Chemistry of the American Chemical Society. He has served as Chairman of the Division of Biological Chemistry of the American Chemical Society, and is a member of the National Academy of Sciences. Dr. Meister is the author of "Biochemistry of the Amino Acids" (1957; Second Edition (volumes I and II), 1965).

James Z. Ginos was born in 1923 in Hillsboro, IL. He received his B.A. degree in chemistry from Columbia College and a M.S. degree of Chemical Engineering and a Ph.D. degree in organic chemistry in 1964 from the Stevens Institute of Technology. In 1964, he was appointed Assistant Scientist in the Medical Department of Brookhaven National Laboratories, where he collaborated under Dr. Katsoyannis in the synthesis of human insulin and analogues thereof. Following Dr. Katsoyannis's appointment to the Chairmanship of the Biochemistry Department at the Mt. Sinai School of Medicine, New York City, Dr. Ginos joined his staff in 1968 as a Research Assistant Professor, where he continued his work and taught until 1970. In 1970, he returned to Brookhaven National Laboratories to join Dr. George Cotzias's staff as an Associate Scientist in the Medical Department, where he worked on the synthesis and study of pharmacological and behavioral effects of dopamine and apomorphine analogues in the laboratory animal models. He followed Dr. Cotzias in 1975 to the Cornell University Medical College, where he continued his work as a Research Associate Professor of Biochemistry in the Department of Neurology. Presently he holds a joint appointment at Cornell and Memorial Sloan–Kettering Cancer Center, where he is currently engaged in the synthesis of short-lived radiopharmaceuticals to be used in human studies with positron emission tomography.

Table I). For lower molecular weight α-keto acids (<

$$H_5C_2O_2CCO_2C_2H_5 + RCH_2CO_2C_2H_5 \rightarrow$$
$$\underset{\mathbf{14}}{H_5C_2O_2CCOCHRCO_2C_2H_5} \xrightarrow[20\%\,HCl]{\Delta} RCH_2COCO_2H$$

C_{11}), the oxalo acid ethyl ester (**14**) was generated in ether/sodium ethoxide with an excess (20%) of ethyl oxalate; for higher molecular weight α-keto acids (≥ C_{11}) the reaction was carried out in pyridine/potassium ethoxide according to the method of Grundmann.[20] Schreiber describes a modified and improved oxalo acid synthesis; seven aliphatic α-keto acids were synthesized by this procedure (Table I).[21] Vogel and Schinz have modified the method for the synthesis of α-keto acid esters and α-keto acid ester ketals.[22]

D. Hydrolysis of Addition Product (15) of Grignard Reagents with Diethyloxamates

This method was originally described by Barré.[23] The proportion of **15** to **16** can be varied according to the conditions employed. Under appropriate conditions

$$H_5C_2O_2CCO_2C_2H_5 + HN(C_2H_5)_2 \rightarrow$$
$$(H_5C_2)_2NCOCO_2C_2H_5 \xrightarrow{RMgX} \underset{\mathbf{16}}{(HO)CR_2CON(C_2H_5)_2}$$
$$+ \underset{\mathbf{15}}{RCOCON(C_2H_5)_2} \rightarrow \underset{\mathbf{1}}{RCOCO_2H}$$

1, R =
CH_3(**3**), $C_6H_5CH_2$(**6**), C_2H_5(**8**), C_6H_5(**17**), *n*-C_3H_7(**18**)

15 (R = C_2H_5) was obtained in 75–80% yield, whereas in another experiment 16 (R = C_2H_5) was obtained in 85–90% yield; 15 but not 16 readily hydrolyzes in strong base to the corresponding α-keto acid.[23] The method has been used to synthesize pyruvic (3), phenylpyruvic (6), phenylglyoxylic (17), α-ketobutyric (8), and α-ketovaleric acids (18) in yields of 60–70%.[23]

E. Hydrolysis of Dehydropeptides (19)

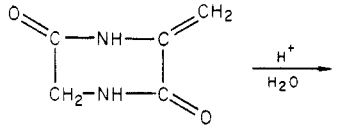
Dehydropeptides (19), in which R^2 = H, are readily converted to the corresponding α-keto acid by heating with dilute HCl or by enzymatic ("dehydropeptidase") methods.[24,25] Although the method is often stated as

$$\underset{\mathbf{19}}{RCH_2CONHC(=CHR^1)CO_2R^2} \rightleftarrows RCH_2CON=C(CH_2R^1)CO_2R^2 \rightarrow RCH_2CO_2H + NH_3 + R^1CH_2COCO_2R^2$$
$$\mathbf{20,21}$$

20, R^1 = H; R^2 = $NHCH_2CO_2H$

21, R^1 = Ph; R^2 = $NHCCH_2CO_2H$

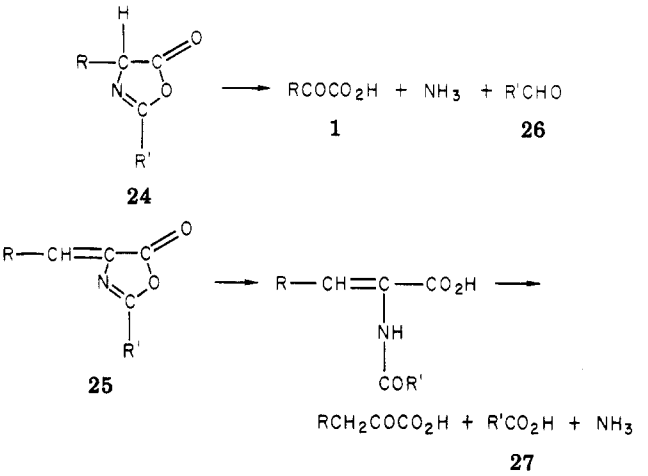
a general method for α-keto formation, there appears to be no publication in which the applicability of the method has been systematically investigated. Indeed, dehydropeptides have occasionally been synthesized from α-keto acids.[24,25] The dehydropeptide method has been more widely utilized for the synthesis of α-keto acid-containing peptides. Thus, pyruvoylglycine (20)[26] and phenylpyruvoylglycine (21)[27] have been synthesized by this method. Pyruvoylglycine (20) can also be prepared from glycylserine methyl ester (22) by treatment with thionyl chloride followed by mild acid hydrolysis of the resulting 3-methylene-2,5-diketopiperazine (23).[28,29]

$H_2NCH_2CONHCH(CH_2OH)CO_2CH_3$ (22) $\xrightarrow[2.\ NH_3]{1.\ SOCl_2}$  (23) $\xrightarrow[H_2O]{H^+}$ $CH_3COCONHCH_2CO_2H$ (20)

Dehydropeptides can be synthesized via reaction of an amino acid (or its ester) with an unsaturated azlactone (section F) or by reaction of an amide (or nitrile) with an α-keto acid. For reviews see Greenstein[24] and Greenstein and Winitz.[25]

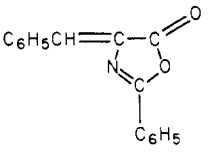
F. Hydrolysis of Azlactones (Oxazolones) (24, 25)

In practice, hydrolysis of either the saturated azlactone (24) or unsaturated azlactone (25) may be used for the synthesis of α-keto acids. In the first case, the



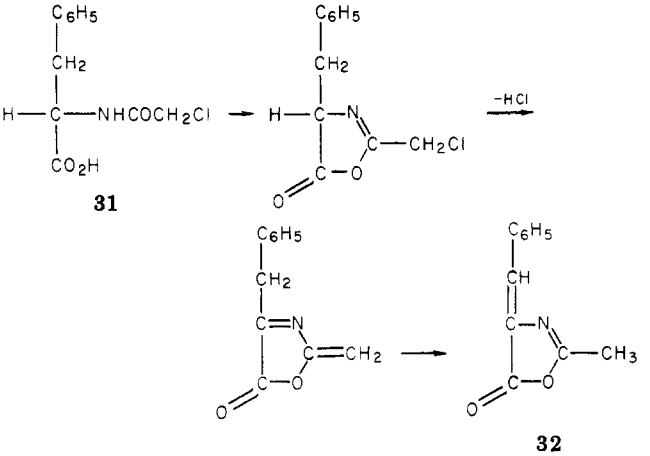
side product is an aldehyde (26) whereas in the second case it is an acid (27).

The first unsaturated azlactone (i.e., 30) was prepared by Plöchl who condensed benzaldehyde (28) with hippuric acid (29).[11] Plöchl thought that the compound

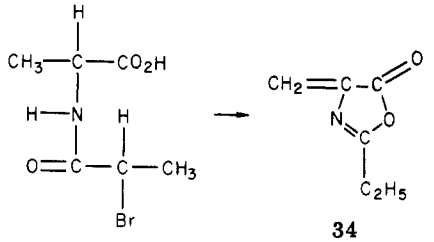
C_6H_5CHO (28) + $C_6H_5CONHCH_2CO_2H$ (29) $\xrightarrow[CH_3CO_2Na]{(CH_3CO)_2O}$  (30)

contained a 3-membered ring but later Erlenmeyer established the true nature of the adduct.[30] Erlenmeyer coined the word (unsaturated) azlactones and initially showed the usefulness of these compounds for the synthesis of α-keto and α-amino acids.[30]

Bergmann and Stern showed that *N*-(chloroacetyl)-phenylalanine (31) could be readily converted to an unsaturated azlactone (α-acetamidocinnamic azlactone 32).[31] Based on some experimental evidence, the authors proposed the following reaction sequence:

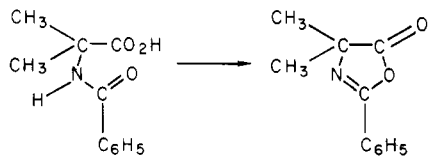


Bergmann and Stern also showed that (α-bromopropionyl)alanine (33) is readily converted to α-propionamidoacrylic azlactone (34).[31]



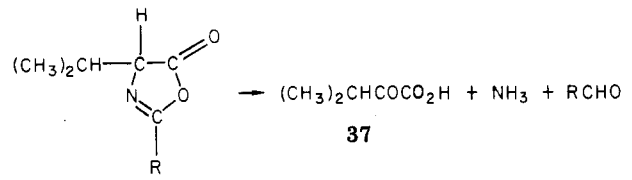
33 34

The first saturated azlactone (**35**) was produced by Mohr and Geis in 1908 by heating *N*-benzoyl-α-aminoisobutyric acid (**36**) with a slight molar excess of acetic anhydride.[32]



36 35

The various methods of synthesis of saturated and unsaturated azlactones and their application to α-keto acid and peptide chemistry have been reviewed by Greenstein and Winitz.[25] We will mention a few examples of α-keto acid synthesis via azlactones. α-Ketoisovaleric acid (**37**) has been synthesized via the appropriate saturated azlactone.[33]



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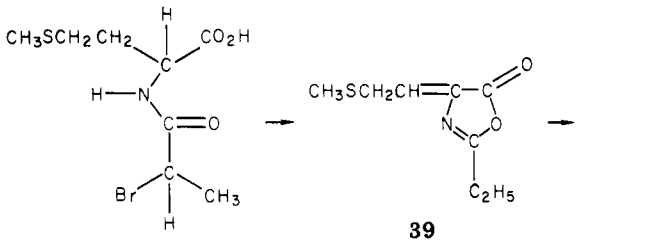
Cahill and Rudolph prepared (α-bromopropionyl)-methionine (**38**) from α-bromopropionic acid and methionine.[34] (α-Bromopropionyl)methionine was con-



38 39

$CH_3CH_2CO_2H + NH_3 + CH_3SCH_2CH_2COCO_2H$

40

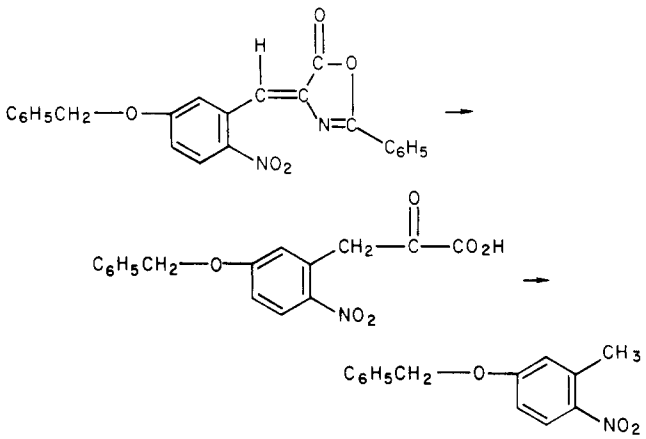
verted to the corresponding azlactone (**39**) which gave α-keto-γ-(methylthio)butyric acid (**40**) on hydrolysis. The keto acid was isolated from the mixture as the 2,4-dinitrophenylhydrazone (42% overall yield) and reconverted to α-keto acid with acetone. Acetone has often been used to generate parent carbonyl-containing compounds from hydrazone derivatives but no general method for reconstituting α-keto acids from the corresponding 2,4-dinitrophenylhydrazones appears to have been published. Therefore, it seems worthwhile to describe the method of Cahill and Rudolph for regenerating α-keto-γ-(methylthio)butyrate[34] as the method may be of wider applicability. The 2,4-dinitrophenylhydrazone was dissolved in 50% acetone–50% water (v/v) and heated in a pressure bottle at 90–92 °C for 4 h; the solution was cooled and concentrated in vacuo. Following storage at 4 °C for 24 h, the unsplit 2,4-dinitrophenylhydrazone and acetone 2,4-dinitrophenylhydrazone were removed by filtration. The barium salt of α-keto-γ-(methylthio)butyrate was precipitated by neutralization with barium hydroxide.

A similar synthesis has been used to prepare phenylpyruvic acid (**6**), except that the starting material is the intermediate derived from the azlactone, i.e., the unsaturated amide. Thus, hydrolysis of α-acetamido-

$$C_6H_5CH{=}C(CO_2H)NHCOCH_3 \xrightarrow{2H_2O/HCl} \underset{41}{} C_6H_5CH_2COCO_2H + NH_4^+ + CH_3CO_2H \underset{6}{}$$

cinnamic acid (**41**) affords phenylpyruvic acid (**6**) in 90% yield.[35] (The authors[35] also provide a survey of methods of synthesis of phenylpyruvic acid up to 1943).

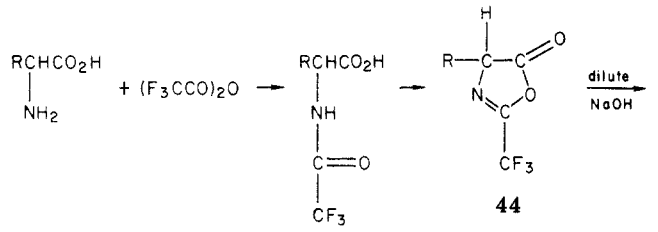
Occasionally, the azlactone method for the synthesis of α-keto acids fails, especially for azlactones of the type **25** in which R = a nitrobenzene derivative. For example, attempts to make 2-nitro-5-(benzyloxy)phenylpyruvic acid via the appropriate azlactone resulted in formation of 2-nitro-5-(benzyloxy)toluene (**42**).[36]



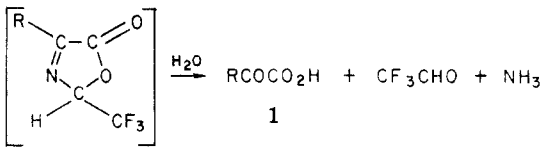
42

The azlactone method has been greatly improved and its scope extended by Weygand et al.[37] The appropriate amino acid is gently heated with trifluoroacetic anhydride to yield the corresponding *N*-(trifluoroacetyl) amino acid (**43**) that rapidly cyclizes to a 4-substituted-2-(trifluoromethyl)-5-oxazolone (**44**).[37] This compound is readily converted to the corresponding α-keto acid via a novel intramolecular oxidation–reduction followed by hydrolysis.

Yields of nine α-keto acids (**4–6, 17, 37, 45–48**) produced by hydrolysis of the oxazolone were good (58–91%). It is interesting to note that Weygand et al. were the first to synthesize L-(+)-α-keto-β-methylvaleric acid (**46**) by a nonenzymatic procedure; the optically active α-keto acid was generated from the corresponding L-isoleucine oxazolone (i.e., from (+)-2-(trifluoromethyl)-4-(2-butyl)oxazolone (**44**, R = $CH_3CH_2(CH_3)CH$)) by controlled hydrolysis in citrate–phosphate buffer (pH 6.8).[37] Previously Meister[38] had prepared L-(+)- and D-(−)-α-keto-β-methylvaleric acids by enzymatic oxidation of L- or D-isoleucine with L- or D-amino acid oxidases, respectively. At the time of this report (1951)[38] the absolute configuration about the β-carbon



43 44



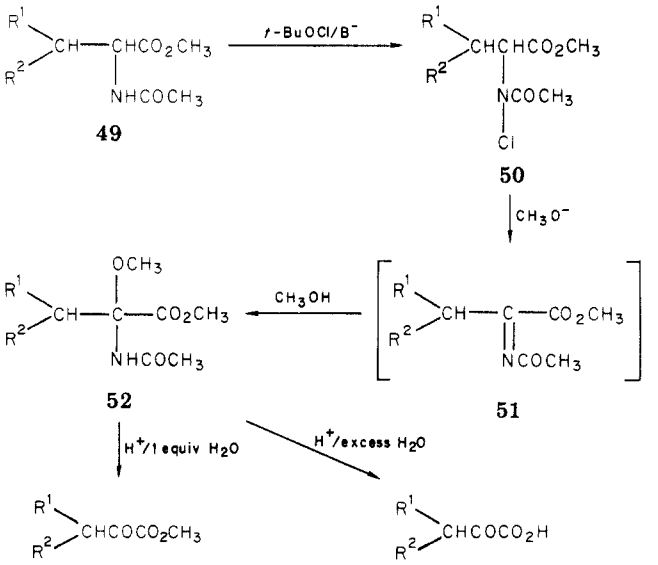
1

R = $(CO_2H)CH_2$ (4)
R = $(CO_2H)CH_2CH_2$ (5)
R = $C_6H_5CH_2$ (6)
R = C_6H_5 (17)
R = $(CH_3)_2CH$ (37)
R = $(CH_3)_2CHCH_2$ (45)
R = $(CH_3CH_2)(CH_3)CH$ (46)
R = p-$CH_3OC_6H_4$ (47)
R = p-$ClC_6H_4CH_2$ (48)

was not known, but in 1954 Trommel and Bijvoet determined the configuration of the β-carbon of isoleucine i.e., the β-methyl is cis to the α-amino group[39] (cf. Greenstein and Winitz[25]). Thus, the α-keto acid analogues of D- and L-isoleucine are D-(−)- and L-(+)-α-keto-β-methylvaleric acid, respectively.

G. Acid-Catalyzed Cleavage of α-Acetylamino α-Methyl Esters (49)[40]

The α-acetylamino α-methyl ester (**49**) is converted to the *N*-chloro derivative (**50**) with *tert*-butyl hypochlorite and catalytic amounts of base, which in the presence of methanol/sodium methoxide undergoes elimination of HCl and spontaneous addition of methanol to yield the α-acetylamino-α-methoxy-α-methyl ester (**52**); **52** can be converted to the α-keto methyl ester (**53–57**) or to the free α-keto acid (**6, 37, 46**). Best



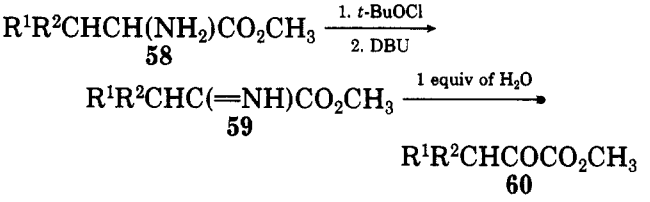
49 50 52 51

53, $R^1 = CH_3$; $R^2 = H$
54, $R^1 = CH_3$; $R^2 = CH_3$
55, $R^1 = (CH_3)_2CH$; $R^2 = H$
56, $R^1 = CH_3CH_2$; $R^2 = CH_3$
57, $R^1 = C_6H_5$; $R^2 = H$

37, $R^1 = (CH_3)_2CH$; $R^2 = H$
46, $R^1 = CH_3CH_2$; $R^2 = CH_3$
6, $R^1 = C_6H_5$; $R^2 = H$

yields (76–87%) of methyl ester were obtained when **52** was treated with a little more than 1 equiv of water in ethereal H_2SO_4 solution.[40] The free α-keto acid (**6, 37, 46**) was obtained in good yield (67–92%) by warming **52** with 1 M HCl.[40] The overall reaction leading to the free branched-chain α-keto acids (**37, 45, 46**) can be carried out in a single reaction flask, without isolation of **52**, provided that the reaction is carried out rapidly enough to prevent dimerization of the α-imino ester (**51**) to an imidazolidone.[40] It is of interest that the sodium salt of α-keto-β-methylvaleric acid (**46**) obtained from the ester derived from L-isoleucine (**49**, $R^1 = C_2H_5$, $R^2 = CH_3$) is racemic free (i.e., of the L-(+)-configuration).[40] This finding excludes an enamine–imine shift in **51** or during the acid hydrolysis of **52**.

Poisel also describes an alternative method for the synthesis of α-keto acid esters (**60**).[40] Thus, the ap-

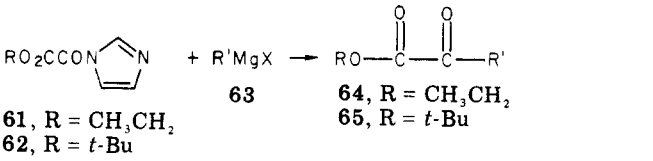


58 59 60

propriate amino acid methyl ester (**58**) is converted to the imino methyl ester (**59**) by the action of *tert*-butyl hypochlorite and 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU). The imino acid ester is then converted to the corresponding α-keto acid ester (**60**) by limited hydrolysis.[40] Yields ranged from 17 to 71%.[40] Both methods of Poisel are advantageous in that the precursors are amino acid derivatives that are available commerically or can be readily synthesized.

H. Reaction of Acylimidazolides (61, 62) with Grignard Reagents (63) Followed by Hydrolysis of the Resulting α-Keto Acid Ester (65)

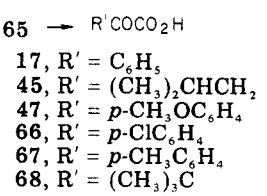
Nimitz and Mosher have recently shown that α-keto acid ethyl and *tert*-butyl esters (**64** and **65**, respectively) may be obtained from acylimidazolides (**61** and **62**) and the appropriate Grignard reagent (**63**).[41] Ethyl α-



61, R = CH_3CH_2
62, R = *t*-Bu

63

64, R = CH_3CH_2
65, R = *t*-Bu



17, R′ = C_6H_5
45, R′ = $(CH_3)_2CHCH_2$
47, R′ = p-$CH_3OC_6H_4$
66, R′ = p-ClC_6H_4
67, R′ = p-$CH_3C_6H_4$
68, R′ = $(CH_3)_3C$

oxo-1*H*-imidazole-1-acetate (**61**) is obtained in excellent yield from the action of ethyl oxalyl chloride and 2 equiv of imidazole in THF.[41,42] Similarly, *tert*-butyl α-oxo-1*H*-imidazole-1-acetate (**62**) is obtained from *tert*-butyl oxalyl chloride (generated in situ) and imidazole.[41,42] Yield of α-keto acid ester (**64** or **65**) was fair when R′ = alkyl (22–26%) and good when R′ = aromatic (54–77%).[41] Six α-keto acids (i.e., **17, 45, 47, 66–68**) were prepared in good yield from the corre-

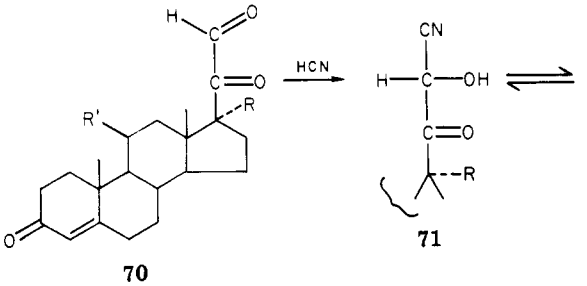
sponding *tert*-butyl ester (65) (54–67%).[41]

I. Oxidation of α-Keto Aldehydes in the Presence of Catalytic Amounts of Cyanide and an Oxidizing Agent

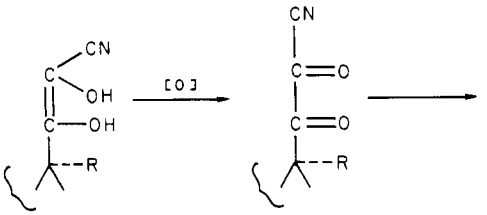
This method was originally used by Mayerhof to convert methylglyoxal (69) to pyruvate (3).[43]

$$\underset{69}{CH_3COCHO} \xrightarrow[O_2]{CN^-} \underset{3}{CH_3COCO_2H}$$

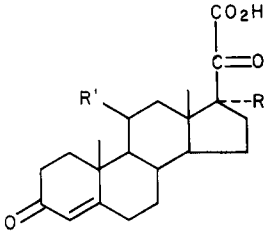
The method has been utilized by Monder[44] to synthesize steroid α-keto acids (substituted glyoxylic acids) (steroidal 20-keto 21-oic acids).



70 71



72 73

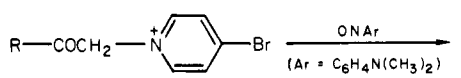


74, R = H; R′ = H
75, R = OH; R′ = H
76, R = H; R′ = OH

Thus, 21-dehydrocorticosteroids (70) in the presence of catalytic amounts of cyanide are oxidized by methylene blue or chromium tetroxide to the corresponding α-keto acids (74–76). Presumably, the reaction sequence involves formation of the cyanohydrin (71) and the unsaturated diol (enediol) (72). The enediol (72) is oxidized under mild conditions to the cyano compound (73) which, in turn, is hydrolyzed to α-keto acid (74–76) with regeneration of cyanide.

J. From α-Keto Aldonitrones (78)

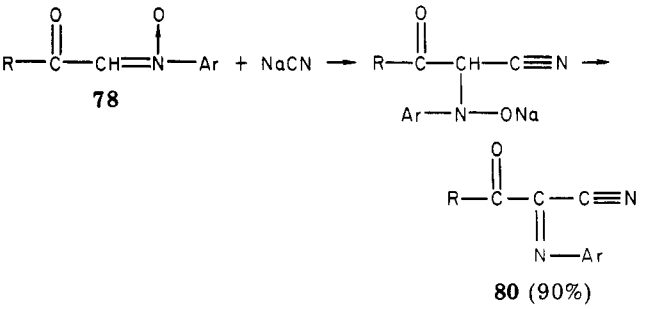
It has long been known that phenacylpyridinium salts (77) are readily converted to α-keto aldehydes (79) via aldonitrone intermediates (78) (See Kröhnke[45] and references quoted therein).



77

$$\underset{78}{RCOCH{=}N(\rightarrow O)Ar} \longrightarrow H_2N(\rightarrow O)Ar + \underset{79}{RCOCHO}$$

Kröhnke has shown that phenacylpyridinium salts may also be used to synthesize α-keto acids in good yield. When phenacylpyridinium salts (77) are reacted with nitrosoaryl compounds using solid alkali cyanides or solutions of alkali cyanides, surprisingly stable crystalline anils (80) are formed instead of the expected aldonitrone (78).[45] Alternatively, the α-keto aldonitrone (78) may be reacted directly with alcoholic solutions of alkali cyanides to produce anils (80) in good yield (~90%).



78 80 (90%)

The anils may also be obtained, but in lower yields, by reacting the nitrosyl compound and sodium cyanide with (phenacyl)trimethylammonium bromide (50%), ω-bromoacetophenonone (60%), ω-cyanoacetophenone (40%), or phenacyldiethylamine (35%).[45] The anil (80) may also be prepared from phenyl phenacyl ethers in 75% yield.[45]

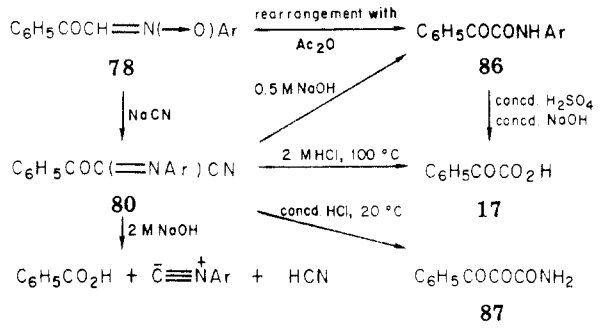
$$C_6H_5COCH_2OC_6H_5 \xrightarrow[2.\ NaCN]{1.\ ONAr} \underset{80}{RCOC(=NAr)CN} + C_6H_5OH$$

The anil (80) is readily converted to the corresponding α-keto acid (1). In most cases it is not necessary to isolate the α-keto aldonitrone; quantitative yields of α-keto acid are obtained by reacting the phenacylpyridinium salt (77) in dilute alcoholic solution with 1 equiv of nitroaryl compound and 2 equiv of alkali cyanide.[45]

$$\underset{78}{RCOCH(=N\rightarrow O)Ar} \xrightarrow{NaCN} \underset{80}{RCOC(CN){=}NAr} \rightarrow ArNH_2 + HCN + \underset{1}{RCOCO_2H}$$

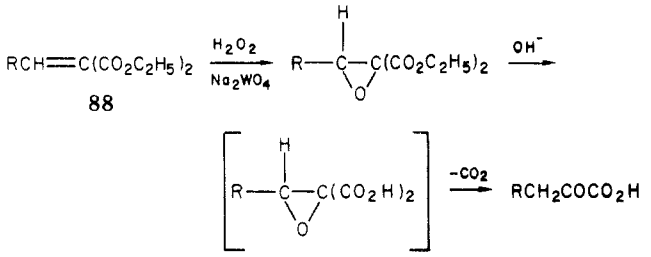
1, R = C_6H_5 (17)
1, R = $CH_3OC_6H_4$ (47)
1, R = *p*-ClC_6H_4 (66)
1, R = *p*-BrC_6H_4 (81)
1, R = *p*-$CH_3CONHC_6H_4$ (82)
1, R = 2-$C_{10}H_7$ (83)
1, R = 2-C_4H_3O (84)
1, R = 2-C_4H_3S (85)

The method has been used to synthesize phenylglyoxylic acid (17) and a number of substituted glyoxylic acid (47, 66, 81–85), including β-naphthyl- (83), 2-furyl- (84), and 2-thienylglyoxylic (85) acids.[45] Kröhnke has also employed α-keto aldonitrones to synthesize α-keto amides (86) and α,β-diketo amides (87).[45] The scheme is as follows:



K. Via Epoxidation of Diethyl Alkylidenemalonates (88)

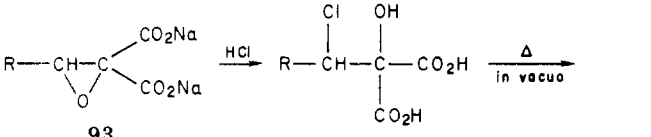
The diethyl alkylidenemalonate (88) is converted by hydrogen peroxide/Na tungstate to the epoxy ester (oxirane).[46] In base, ring opening and decarboxylation



18, R = C_2H_5
45, R = i-C_3H_7
89, R = n-C_3H_7
90, R = i-C_4H_9
91, R = n-C_5H_{11}
92, R = n-C_6H_{13}

occur to yield the α-keto acid in moderate yield (40–60%).

This method appears to be useful since alkylidenemalonates are readily prepared.[47] A variation of this method has been published by Kulkarni and Rao.[48] The epoxidation of the diethyl alkylidenemalonate (88) with H_2O_2 followed by reaction with sodium ethoxide results in formation of the corresponding disodium salt (93). In the presence of dry HCl gas, 93 is converted

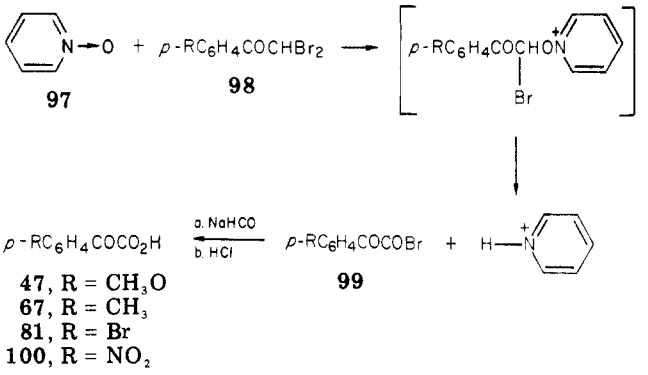


6, R = C_6H_5
8, R = CH_3
48, R = p-ClC_6H_4
92, R = n-C_6H_{13}
95, R = p-BrC_6H_4
96, R = p-$CH_3OC_6H_4$

to the chlorohydroxy acid (94). When 94 is heated in vacuo, HCl and CO_2 are removed to yield the α-keto acid.[48]

L. Via the Reaction of Aromatic *N*-Oxides with Dibromomethyl Aryl Ketones (98)

This method is applicable to the synthesis of para-substituted phenylglyoxylic acids.[49]

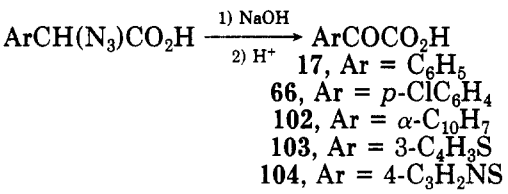


47, R = CH_3O
67, R = CH_3
81, R = Br
100, R = NO_2

The *p*-substituted-phenyl dibromomethyl ketone (98) is reacted with the *N*-oxide of pyridine (97), 2-picoline, 2,4,6-collidine, or quinoline to yield an α-keto acyl bromide (99).[49] Treatment with $NaHCO_3$ and HCl yields the corresponding α-keto acid.[49] Yields of *p*-methoxy-(47), *p*-methyl-(67), and *p*-bromophenylglyoxylic (81) acids were good to excellent (50–95%); yields of *p*-nitrophenylglyoxylic (100) acid were somewhat poorer (20–30%).[49]

M. From α-Azidoarylacetic Acids (101)

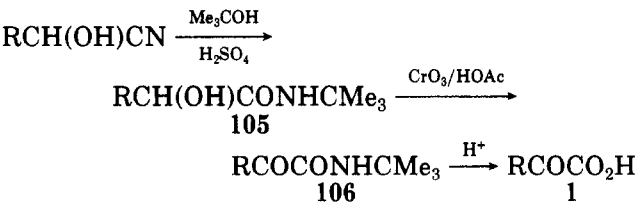
Raap has used this method to synthesize a number of arylglyoxylic acids (17, 66, 102–104), including α-naphthyl-(102), 3-thienyl-(103), and 4-isothiazolylglyoxylic (104) acids, in good yields (56–89%).[50]



17, Ar = C_6H_5
66, Ar = p-ClC_6H_4
102, Ar = α-$C_{10}H_7$
103, Ar = 3-C_4H_3S
104, Ar = 4-C_3H_2NS

N. From α-Hydroxy-*N*-*tert*-butylcarboxamides (105)[51]

The appropriate cyanohydrin is converted to the α-hydroxy-*N*-*tert*-butylcarboxamide (105); 105 is oxidized to the keto amide (106), which in turn is hydrolyzed to α-keto acid in yields of 48–87%.[51]



1, R = $C_6H_5CH_2$ (6)
1, R = C_2H_5 (8)
1, R = C_6H_5 (17)
1, R = n-C_3H_7 (18)
1, R = $(CH_3)_2CH$ (37)
1, R = n-C_4H_9 (89)
1, R = n-C_7H_{15} (92)
1, R = $C_6H_5CH_2CH_2$ (107)
1, R = $(C_6H_5)_2CH$ (108)
1, R = $(C_6H_5)_2CHCH_2$ (109)

O. From *N*-Bromo-α-cyano Amines (111)[52]

N-Bromo-α-cyano amines (111), prepared by addition of BrCN to $RCH{=}NC_6H_5$ (110) gave 71–85% $RCOCO_2H$ on dehydrobromination with Et_3N followed by refluxing with concentrated HCl in acetone.[52]

$$\underset{110}{RCH{=}NC_6H_5} + BrCN \rightarrow \underset{111}{RCH(CN)N(Br)C_6H_5} \xrightarrow[2)\ \text{reflux concd. HCl/acetone}]{1)\ Et_3N} \underset{1}{RCOCO_2H}$$

1, R = C_6H_5 (17)
1, R = *p*-ClC_6H_4 (66)
1, R = *p*-HOC_6H_4 (112)
1, R = $C_6H_5CH{=}CH$ (113)
1, R = *m*-$(NO_2)C_6H_4CH{=}CH$ (114)

P. From Halomagnesium Alcoholates (115, 118)[53,54]

The method is described by Lapkin and Kashinskii.[53]

$$RCH_2MgBr + (CO_2CH_2R^1)_2 \rightarrow \underset{R^1 = H,\ Me}{RCH_2C(OCH_2R^1)(OMgBr)CO_2CH_2R^1}$$

$$\Big|\ \underset{115}{}\ (R^2CO)_2O,\ R^2 = Et,\ Pr$$

$$RCH{=}C(O_2CR^2)CO_2CH_2R^1\ (31\text{–}53\%) \xrightarrow{H_2O} RCH_2COCO_2H$$

92, R = *n*-C_6H_{13}
116, R = *n*-C_7H_{15}
117, R = *n*-C_8H_{17}

These authors also describe an alternative method utilizing halomagnesium alcoholates (118).[54] Gröger

$$RCH_2MgX + (CO_2R^1)_2 \rightarrow \underset{R^1 = CH_3,\ C_2H_5,\ (CH_3)_2CH,\ n\text{-}C_3H_7}{RCH_2C(OR^1)(OMgX)CO_2R^1} \xrightarrow[R^2 = Me,\ Bu,\ Ph]{R^2COCl}$$

$$\underset{118}{} \xrightarrow{H_2O} \underset{119}{RCH{=}C(O_2CR^2)CO_2R^1} \rightarrow RCH_2COCO_2H$$

91, R = *n*-C_5H_{11}
92, R = *n*-C_6H_{13}
116, R = *n*-C_7H_{15}
117, R = *n*-C_8H_{17}
120, R = *n*-C_9H_{19}

and Waldmann[55] have also shown that compounds similar to 119 (i.e., 121) are readily converted to α-keto acids. Thus, 121 is produced via a Claisen cinnamic acid synthesis from a disubstituted benzaldehyde and glycolic ester (where R^2O– = ester or ether linkage).

$$3,4\text{-}RR^1C_6H_3CHO + R^2OCH_2CO_2R^3 \rightarrow \underset{121}{3,4\text{-}RR^1C_6H_3CH{=}C(OR^2)CO_2R^3} \xrightarrow[H_2O]{H^+} 3,4\text{-}RR^1C_6H_3CH_2COCO_2H$$

6, R = H; R^1 = H
122, R,R^1 = $-OCH_2O-$

The method has not yet been shown to be of wide applicability since only phenylpyruvate (R = H, R^1 = H; 6) and 3,4-(methylenedioxy)phenylpyruvate (R,R^1 = $-OCH_2O-$; 122) were synthesized.[55] Moreover, overall yields were poor to moderate (8–50%).

Q. Via the Reaction of Ketones with (2,2-Difluoro-1-(tosyloxy)vinyl)lithium (124)[56]

2,2,2-Trifluoroethyl tosylate (123) is reacted with 2 equiv of lithium diisopropylamide (LDA) in tetrahydrofuran at −78 °C under nitrogen to generate (2,2-difluoro-1-(tosyloxy)vinyl)lithium (124). The reaction

$$\underset{123}{CF_3CH_2\text{–}OTs} \xrightarrow[LDA]{2\ equiv} \underset{124}{CF_2{=}CLi\text{–}OTs} \xrightarrow{RR^1C{=}O} \underset{125}{RR^1C(OH)C(OTs){=}CF_2} \xrightarrow{95\%\ H_2SO_4}$$

$$\underset{126}{RR^1C{=}C(OTs)CO_2H} \xrightarrow[2.\ H^+]{1.\ OH^-} RR^1CHCOCO_2H$$

6, R = C_6H_5; R^1 = H
37, R = CH_3; R^1 = CH_3
46, R = C_2H_5; R^1 = CH_3
92, R = *n*-C_6H_{13}; R^1 = H
127, R = c-C_6H_{11}; R^1 = H

of the appropriate ketone with 124 yields quantitatively the carbinol (125) which, without further purification, is converted to the corresponding unsaturated acid (126) in 95% H_2SO_4; 126 is subsequently converted to α-keto acid. By use of the appropriate ketone or aldehyde the following α-keto acids were made in good yield (89–95%): β-phenylpyruvic (6), α-ketoisovaleric (37), α-keto-β-methylvaleric (46), α-ketononanoic (92), and β-cyclohexylpyruvic (127).[56] The method appears to be of general applicability since many of the ketone precursors are available commercially.

R. From α,β-Unsaturated Esters (128)[57]

The α,β-unsaturated ester (128) is brominated followed by treatment with piperidine to form the mono- or diadduct (129, 130). The piperidine adducts are

$$\underset{128,\ R^3 = Me,\ Et}{R^1R^2C{=}CHCO_2R^3} \xrightarrow{Br_2} R^1R^2CBrCHBrCO_2R^3$$

$$\xrightarrow{piperidine} R^2CH(NC_5H_{10})CH(NC_5H_{10})CO_2R^3$$

129 (R^1 = H)
or $R^1R^2C{=}C(NC_5H_{10})CO_2R^3$
130 (R^1, $R^2 \neq$ H)

$$129 \xrightarrow[\Delta,\ H^+]{H_2O} R^2CH_2COCO_2H$$

$$130 \xrightarrow[\Delta,\ H^+]{H_2O} R^1R^2CHCOCO_2H$$

then hydrolyzed to the corresponding α-keto acids.[57] Bromination of the double bond may be brought about by dissolving 128 and bromine in CS_2 followed by removal of CS_2 by evaporation. The piperidine adduct is formed by extracting the residue with ethanol containing excess piperidine and refluxing for 3 h. The α-keto acid is obtained following removal of ethanol, extraction into ether, and refluxing in 1 M H_2SO_4.

When R^1 = H, the dipiperidine adduct (**129**) is formed; when R^1, $R^2 \neq$ H, the monopiperidine adduct (**130**) is formed. Overall yields of α-keto acid are 40–50% and 18%, respectively. Examples of α-keto acids synthesized by this procedure include **4**, **6**, **8**, **18**, and **107**.

S. Enzymatic Methods

The metabolic relationship between α-amino and α-keto acids has been recognized for many years.[58] In more recent years optically specific amino acid oxidases have been found to oxidize amino acids (**131**) according to the following equations.

$$\underset{\mathbf{131}}{RCH(NH_2)CO_2H} + O_2 \xrightarrow{\text{enzymatic}} \underset{\mathbf{132}}{RC(=NH)CO_2H} + H_2O_2$$

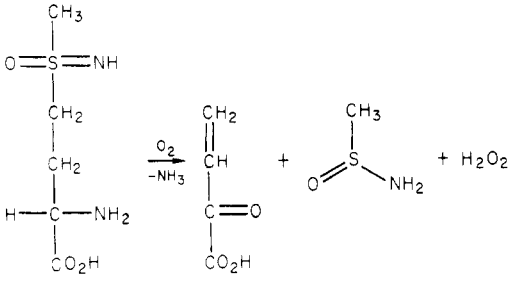
$$\underset{\mathbf{132}}{RC(=NH)CO_2H} + H_2O \xrightarrow{\text{nonenzymatic}} \underset{\mathbf{1}}{RCOCO_2H} + NH_3$$

$$\underset{\mathbf{1}}{RCOCO_2H} + H_2O_2 \rightarrow \underset{\mathbf{133}}{RCO_2H} + CO_2 + H_2O$$

The D- or L-amino acid (**131**) is oxidized by the action of amino acid oxidase to the corresponding α-imino acid (**132**) with the generation of hydrogen peroxide; the α-imino acid (**132**) is spontaneously (nonenzymatically) hydrolyzed, usually within a few seconds, to the corresponding α-keto acid (**1**) and ammonia. The α-keto acid (**1**) is susceptible to oxidative decarboxylation by hydrogen peroxide (section IVD) so that unless hydrogen peroxide is rapidly removed (e.g., by the action of catalase), a considerable amount of carboxylic acid (**133**), containing one less carbon than the parent amino acid, is generated. The relative concentration of α-keto acid (**1**) to carboxylic acid (**133**) in the final product depends on the conditions employed for oxidation of amino acid.[4,5] In the presence of catalase oxidation results in stoichiometric formation of α-keto acid (**1**) and ammonia.

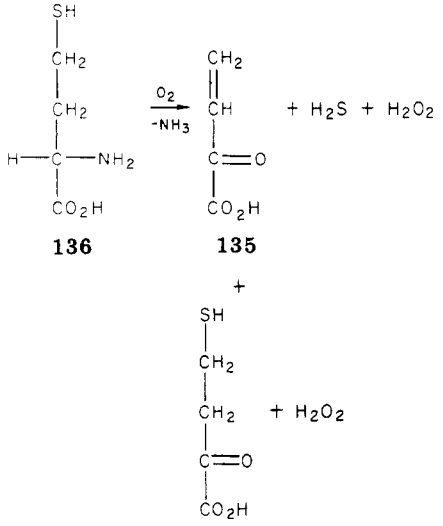
The feasibility of using biological methods for the preparation of α-keto acids was first demonstrated by Krebs in 1933.[59] Krebs showed that kidney slices converted D-amino acids to α-keto acids.[59] Kidney is a rich source of D-amino acid oxidase, and D-amino acid oxidase from hog kidney has been used for the synthesis of α-keto acids.[60] An established procedure is to oxidize an L-amino acid with dialyzed snake (usually rattlesnake, *Crotalus adamanteus*) venom (which contains ~2% L-amino acid oxidase) in the presence of excess crystalline beef heart catalase.[4] Details of the procedure are given by Meister.[4] Briefly, the L-amino acid is oxidized under an atmosphere of O_2. When the reaction is complete protein is removed by dialysis or by ultrafiltration. Ammonia and unreacted α-amino acid are removed on a Dowex 50 (H^+) column. The effluent is taken to pH ~4.0 with sodium hydroxide or barium hydroxide, concentrated, and the α-keto acid salt is precipitated with ethanol. The enzymatic procedures permit preparation of α-keto acids not yet available by organic synthetic methods. Other advantages include ready availability of precursor amino acid and enzymes, simplicity of operation, and good yield. Under certain conditions, α-keto acids can enolize (see section IVC); for those α-keto acids with a β-asymmetric center and a β-proton, such as L-(+)- or D-(−)-α-keto-β-methylvaleric acids, such enolization will result in loss of optical activity. However, for the simple aliphatic α-keto acids, enolization is not favored at neutral pH. Therefore, oxidation of an L-amino acid, with a second asymmetric center at the β-carbon (in addition to the α-amino acid center) and a β C–H bond (e.g., L-isoleucine, L-alloisoleucine), at neutral pH, will result in preservation of the second asymmetric center.[4,38] Indeed, the rate of enolization of L-(+)-α-keto-β-methylvaleric acid is very slow, but greatly increases at pH values above 8.4.[38] Although the enzymatic method is advantageous for the preservation of asymmetry at the β-carbon of the α-keto acid, as noted above, organic synthesis procedures have also been devised which are capable of preservation of the asymmetry at the β-carbon.[37,40]

Although the enzymatic method has been used successfully to oxidize a large number of L-amino acids,[4] dipeptides, and even a tripeptide,[61] apparently anomalous reactions have occasionally been observed. Thus, the oxidation of methionine sulfoximine (**134**), gave vinylglyoxylic acid (**135**), and methanesulfinamide.[62]



134 **135**

Apparently, the β C–H bond is ~10^5 times more reactive in the initially generated imino acid (**132**, R = $CH_2CH_2S(O)(NH)CH_3$) than in the corresponding α-keto acid.[62] Thus the imino acid derived from **134** undergoes very rapid β,γ-elimination before hydrolysis of the imino acid function occurs.[62] The α-keto acid analogue of **134** can be prepared by a transamination reaction and is relatively stable. In another example it was found that oxidation of L-homocysteine (**136**)



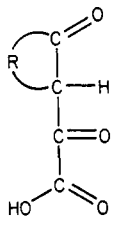
136 **135**

results in formation of some hydrogen sulfide as well as the expected α-keto-γ-mercaptobutyrate.[63]

Enzymatic methods have been adapted for large scale synthesis of α-keto acids. Brodelius et al.[64] showed that immobilized yeast cells (*Trigoropsis variabilis*) containing D-amino acid oxidase could be used to prepare α-keto acids from the corresponding D-amino acid. The authors showed that as much as 700 mg/day of α-keto-γ-(methylthio)butyric acid (**40**) could be generated from methionine using yeast immobilized on a 10 ml calcium alginate column and a recycling system.[64] Some α-keto acids are made industrially via biological methods.

III. Classification

Table I lists α-keto acids, reference to preparation, and the available melting points of their corresponding 2,4-dinitrophenylhydrazone. The α-keto acids are classified generally according to the structure of the R group.[65] Occasionally, an α-keto acid fits two or more categories; such α-keto acids are cross-referenced. The α-keto acid analogues of most of the commonly occurring amino acids have been prepared and are included. Only those aromatic α-keto acids that are closely related to phenylpyruvate, *p*-hydroxyphenylpyruvate, or phenylglyoxylate have been listed. Subsection 9 (Table I) lists a series of α-keto acids that have been synthesized in order to study nitrosation–rearrangement reactions of substituted glyoxylic acids with complex ring structures. Several of these are potential oxaloacetic acid (**4**) or α-ketoglutaric acid (**5**) antagonists, e.g., camphor-, norcamphor-, α-santenone-, and β-santenone-3-glyoxylic acids (**137**). α-Ketohomocamphoric acid (**138**) may be



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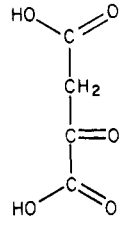

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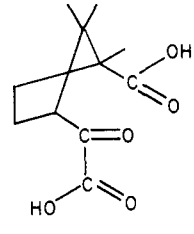

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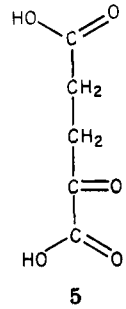

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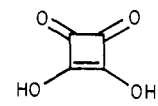
regarded as a substituted α-ketoadipic acid; since the α-ketoadipic acid skeleton is not fully extended, α-ketohomocamphoric acid is a potential α-ketoglutaric antagonist.

A strategy often employed for the design of irreversible inhibitors of pyridoxal 5′-phosphate-containing enzymes is to incorporate a β,γ-double bond into an analogue that mimics the natural amino acid substrate. We note that Table I contains several examples of β,γ-unsaturated α-keto acids (e.g., crotonylidenepyruvic, furfurylidenepyruvic, benzylidenepyruvic) which are potential irreversible inhibitors of transaminases.

It is of interest that squaric acid (1,2-dihydroxycyclobut-1-ene-3,4-dione) (**139**) and semisquaric acid (1-hydroxycyclobut-1-ene-2,3-dione) (**140**) possess a reactive carbonyl α to an acidic =C(–)OH group.[66,67] Therefore, these compounds (**139**, **140**) could be regarded as α-keto acids. However, it seems more appropriate to reserve the term, α-keto acid, for α-keto carboxylic acids. Squaric acid (**139**), semisquaric acid (**140**), and 2-substituted semisquaric acid analogues have been called vinylogous α-keto acids.[67] (The sodium salt of semisquaric acid (**140**) is the highly toxic fungal metabolite, moniliformin).[66,67]



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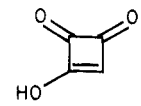

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It should be noted that α-thioketo acids (i.e., analogues in which the α-carbonyl oxygen is replaced by sulfur) are well-known.[68] β-, γ-, and δ-Keto acids and diketo acids are also well known and many (e.g., acetoacetic, levulinic, γ,δ-diketovaleric, and β,δ-diketofumarylacetoacetic acids) are of biological importance. However, discussion of these classes of compounds is beyond the scope of this review.

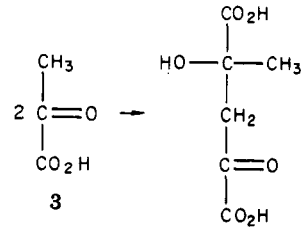
IV. Properties

A. Physical Characteristics

The straight-chain α-keto acids are either liquids or low-melting solids. The branched-chain and phenyl-substituted α-keto acids vary from liquids to high-melting solids. For a discussion on variation of melting point with structure see Waters[2] and Adickes and Andresen.[15] Several α-keto acids ($CH_3(CH_2)_nCOCO_2H$; n = 0, 1, 2, 3, 4, 5, 7) have been subjected, as their sodium salts, to X-ray crystallography.[233] The crystals exhibit orthorhombic symmetry and grow as thin plates parallel to the (100) face from water/*n*-butanol or water/isopropanol mixtures.[233] Jain et al.[233] report interatomic distances and bond angles for sodium α-ketovalerate (n = 2) and α-ketoheptanoate (n = 4).

B. Stability

Some α-keto acids are unstable as the free acid and exhibit a tendency to decarboxylate and polymerize. The majority are relatively stable as salts, of which the sodium and barium salts have most often been prepared. In general, α-keto acids are relatively stable in neutral solution and can be stored frozen at –20 °C with little decomposition, but there are notable exceptions. It is well-known that pyruvic acid (**3**) readily polymerizes on storage in aqueous solution to DL-γ-hydroxy-γ-methyl-α-ketoglutaric acid (**141**)[234] and higher molecular weight compounds. Such polymerization occurs



3 **141**

even in frozen aqueous solutions. (The aldol dimer and aldol trimer of pyruvic acid have been given the names meta- and parapyruvic acids, respectively;[235] see also references quoted by Hughes and Watson.[247])

Table I. Preparation of α-Keto Acids

α-keto acid ($RCOCO_2H$)	R	corresponding amino acid	ref[a]	mp of 2,4-DNP, °C[b]
		1. Aliphatic Side Chain (Unbranched)		
glyoxylic	H	glycine	7, 69	203(h)
pyruvic	CH_3	alanine	1, 12, 13, 23	216(h)
α-ketobutyric	CH_3CH_2	α-aminobutyric	13–15, 23, 48, 51, 57, 70, 71	198(h)
α-ketovaleric	$CH_3CH_2CH_2$	norvaline	14, 15, 23, 46, 51, 57, 71, 72	167(h)
α-ketocaproic	$CH_3(CH_2)_2CH_2$	norleucine	15, 21, 46, 51, 71, 73	153(h)
α-ketoheptylic (α-ketoheptanoic, α-ketoenanthic)	$CH_3(CH_2)_3CH_2$	α-aminoheptylic	15, 71	130(e,l)
α-ketocaprylic (α-ketooctanoic)	$CH_3(CH_2)_4CH_2$		15, 46, 54, 74	137(a)
α-ketononanoic (α-ketopelargonic)	$CH_3(CH_2)_5CH_2$		15, 21, 46, 48, 51, 53, 54, 56	129
α-ketodecanoic (α-ketocapric)	$CH_3(CH_2)_6CH_2$		15, 53, 54, 74	126–128
α-ketoundecanoic (α-ketohendecanoic)	$CH_3(CH_2)_7CH_2$		15, 53, 54	86
α-ketolauric (α-ketododecanoic)	$CH_3(CH_2)_8CH_2$		15, 54	86
α-ketotridecanoic	$CH_3(CH_2)_9CH_2$		15	
α-ketopentadecanoic	$CH_3(CH_2)_{11}CH_2$		15	
α-ketopalmitic[c]	$CH_3(CH_2)_{12}CH_2$		75	
α-ketoheptadecanoic	$CH_3(CH_2)_{13}CH_2$		21	
α-ketostearic	$CH_3(CH_2)_{14}CH_2$		76	
α-ketononadecanoic	$CH_3(CH_2)_{15}CH_2$		21	
crotonylidenepyruvic	$CH_3CH{=}CHCH{=}CH$		77	
sorbinylidenepyruvic	$CH_3(CH{=}CH)_2CH{=}CH$		77	
octatrienylidenepyruvic	$CH_3(CH{=}CH)_3CH{=}CH$		77	
		2. Aliphatic Side Chain (Branched)		
α-ketoisovaleric	$(CH_3)_2CH$	valine	33, 37, 40, 51, 56, 71, 72, 78	196(h)
trimethylpyruvic	$(CH_3)_3C$	*tert*-leucine	41, 79	180(h)
α-ketoisocaproic	$(CH_3)_2CHCH_2$	leucine	15, 21, 37, 46, 71, 80, 81	162(h)
DL-α-keto-β-methylvaleric	$(C_2H_5)(CH_3)CH$	DL-isoleucine (or DL-alloisoleucine)	21, 56, 82–84	169(h)
D-α-keto-β-methylvaleric	$(C_2H_5)(CH_3)CH$	L-isoleucine (or D-alloisoleucine)	37, 38, 40	176(h)
L-α-keto-β-methylvaleric	$(C_2H_5)(CH_3)CH$	D-isoleucine (or L-alloisoleucine)	38	176(h)
α-keto-β-ethylvaleric	$(C_2H_5)_2CH$		21	
α-keto-δ-methylcaproic	$(CH_3)_2CHCH_2CH_2$		85	
α-keto-β,β-dimethylvaleric	$(CH_3)_2(C_2H_5)C$		86	
α-keto-γ,γ-dimethylvaleric	$(CH_3)_3CCH_2$		46, 80	
α-keto-β-methylnonanoic	$CH_3(CH_2)_5C(CH_3)H$		80	
β,β-diisobutylpyruvic	$(i\text{-}C_4H_9)_2CH$		87	
β-cyclohexylpyruvic	$C_6H_{11}CH_2$	β-cyclohexylalanine	56, 71	189(h)
cyclopentylglyoxylic	C_5H_9	2-cyclopentylglycine	88	199(dec)
cyclohexylglyoxylic	C_6H_{11}	2-cyclohexylglycine	88	215–216
2-keto-3-cyclohexenepropionic	$(C_6H_9)CH_2$	3-cyclohexenealanine	88	190
prephenic	$(C_6H_4OH)(CO_2H)CH_2$	pretyrosine	89	

3. Polycarboxylic α-Keto Acids (and Amides and Esters)[k]

mesoxalic	CO_2H	aminomalonic	90	202(hc)
oxaloacetic	$(CO_2H)CH_2$	aspartic	8, 9, 37, 57, 91-95[e]	218(h)
α-ketoglutaric	$(CO_2H)CH_2CH_2$	glutamic	10, 37, 96-98[e]	220(h)
α-ketoadipic	$(CO_2H)CH_2CH_2CH_2$	α-aminoadipic	99	208(h)
α-ketopimelic	$(CO_2H)CH_2(CH_2)_2CH_2$	α-aminopimelic	100	190-191
α-ketosuberic	$(CO_2H)CH_2(CH_2)_3CH_2$	α-aminosuberic	100	173-174
oxalosuccinic	$(CO_2H)CH_2CH(CO_2H)$	α-aminotricarballylic (2-diastereoisomers)	101, 102	
oxaloacetic β-ethyl ester	$(CO_2C_2H_5)CH_2$	aspartic β-ethyl ester	103	
α-ketoglutaric γ-methyl ester	$(CO_2CH_3)CH_2CH_2$	glutamic γ-methyl ester	*f*	
α-ketoglutaric γ-ethyl ester	$(CO_2C_2H_5)CH_2CH_2$	glutamic γ-ethyl ester	71	*h*
α-ketoglutaric γ-benzyl ester	$(CO_2C_6H_5)CH_2CH_2$	glutamic γ-benzyl ester	*f*	
α-ketosuccinamic	$(CONH_2)CH_2$	asparagine	106, 107	173(a,h)
α-ketoglutaramic	$(CONH_2)CH_2CH_2$	glutamine	106, 108	*h*
α-keto-*N*-methylglutaramic	$CONH(CH_3)CH_2CH_2$	glutamic γ-methylamide	106	*i*
α-keto-*N*,*N*-dimethylglutaramic	$CON(CH_3)_2CH_2CH_2$	glutamic γ-dimethylamide	109	*h*
α-ketoadipamic	$(CONH_2)CH_2CH_2CH_2$	α-aminoadipamic (homoglutamine)	109	*h*
α-keto-*N*-methyladipamic	$CONH(CH_3)CH_2CH_2CH_2$	α-amino-N^{γ}-methyladipamic	109	*h*
α-keto-DL-γ-methylgutaric	$(CO_2H)CH(CH_3)CH_2$	DL-γ-methyl-DL-glutamic	109-111	*h*
α-keto-DL-γ-methylglutaramic	$(CONH_2)CH(CH_3)CH_2$	DL-γ-methyl-DL-glutamine	109	180
DL-γ-methyl-γ-hydroxy-α-ketoglutaric	$(CO_2H)C(OH)(CH_3)CH_2$	DL-γ-methyl-γ-hydroxy-DL-glutamate	110	
α-keto-γ-methyleneglutaric	$(CO_2H)C(=CH_2)CH_2$	γ-methyleneglutamic	111, 112	224-228
β-methyloxaloacetic	$(CO_2H)CH(CH_3)$	β-methylaspartic	113, 114	164, 188-184

4. Sulfur- and Oxygen-Containing Side Chain

β-mercaptopyruvic	$HSCH_2$	cysteine	115-119	195-200[95,j] 161-162[97]
β-mercaptopyruvic disulfide	$(CO_2H)COCH_2SSCH_2$	cystine	115, 117	255-265[95,j] 206-207[97]
α-keto-β-mercaptobutyric	$HSCH(CH_3)$		119	
β-(methylthio)pyruvic	CH_3SCH_2	*S*-methylcysteine	119	
β-(*p*-X-phenylthio)pyruvic	$XC_6H_4SCH_2$ (X = CH_3, Cl, I, Br)		119	
S-benzyl-β-mercaptopyruvic	$C_6H_5CH_2SCH_2$		117, 120	150(a)
β-sulfopyruvic	HO_3SCH_2	cysteic	117	210(a)
β-mercaptopyruvic–cysteine mixed disulfide	$(CO_2H)(NH_2)CHCH_2SSCH_2$	cystine	121	
2-thienylglyoxylic	(C_4H_3S)		45, 122	
3-thienylglyoxylic	(C_4H_3S)		50	
β-(2-thienyl)pyruvic	$(C_4H_3S)CH_2$	thienylalanine	123, 124	
4-(2-thienyl)-2-keto-3-butenoic	$(C_4H_3S)CH{=}CH$		445	
4-isothiazolylglyoxylic	C_3H_2NS		50	
α-keto-γ-(methylthio)butyric	$CH_3SCH_2CH_2$	methionine	34, 71, 125	150(h)
α-keto-γ-(ethylthio)butyric	$C_2H_5SCH_2CH_2$	ethionine	71	131(h)
α-keto-γ-(β-hydroxyethylthio)butyric	$HOCH_2CH_2SCH_2CH_2$	ω-hydroxyethionine	62	165(h)
α-keto-γ-(methylsulfoximinyl)butyric	$CH_3S(O)(NH)CH_2CH_2$	methionine sulfoximine	62	
β-hydroxypyruvic	$HOCH_2$	serine	126	162(e)
α-keto-γ-hydroxybutyric	$HOCH_2CH_2$	homoserine	71, 127, 128	

Table I (*Continued*)

α-keto acid ($RCOCO_2H$)	R	corresponding amino acid	ref[a]	mp of 2,4-DNP, °C[b]
DL-α-keto-β-hydroxybutyric	$CH_3C(OH)H$	DL-threonine or DL-allothreonine	126, 129	157–158[106] 198[109]
α-keto-ε-hydroxycaproic	$HOCH_2CH_2CH_2CH_2$	α-amino-ε-hydroxycaproic acid	71	183(h)
2-furylglyoxylic	(C_4H_3O)		45, 130	
β-(2-furyl)pyruvic	$(C_4H_3O)CH_2$		189, 203	
2-furfurylidenepyruvic (α-furalpyruvic)	$(C_4H_3O)CH{=}CH$		131, 445	
	5. Halogen-Containing Side Chain			
β-chloropyruvic	$ClCH_2$	β-chloroalanine	132–135	
β,β-dichloropyruvic	Cl_2CH	β,β-dichloroalanine	132	
β,β,β-trichloropyruvic	Cl_3C	β,β,β-trichloroalanine	136, 137	
β-bromopyruvic	$BrCH_2$	β-bromoalanine	126, 138, 371,	180(d)
β,β-dibromopyruvic	Br_2CH	β,β-dibromoalanine	132, 137, 139, 140, 371	
β,β,β-tribromopyruvic	Br_3C	β,β,β-tribromoalanine	137, 139, 371	
β-chloro-β-bromopyruvic	$ClBrCH$		133, 137	
β,β-dichloro-β-bromopyruvic	Cl_2BrC		137, 141	
β-chloro-β,β-dibromopyruvic	$ClBr_2C$		137	
β-fluoropyruvic	FCH_2	β-fluoroalanine	142	127
β,β,β-triiodopyruvic	I_3C		143	
DL-β-bromo-α-ketobutyric	CH_3CHBr		126	
DL-β-bromo-β-phenylpyruvic	C_6H_5CHBr		144	
DL-β-fluoro-α-ketobutyric	CH_3CHF		145	
DL-β-fluoro-α-ketovaleric	CH_3CH_2CHF		145	
DL-β-fluoro-α-keto-γ-phenylbutyric	$C_6H_5CH_2CHF$		145	
DL-β-fluorooxalacetic	$(CO_2H)CHF$		146	210–214
β,β-difluorooxaloacetic	$(CO_2H)CF_2$		146	200(dec)
DL-α-keto-β-bromoglutarate	$(CO_2H)CH_2CHBr$		232	
	6. Nitrogen in Side Chain (other than Amide)			
α-keto-δ-aminovaleric	$H_2NCH_2CH_2CH_2$	ornithine, proline[d]	147–149	232–242[149] 219[148] 211–212[147]
α-keto-ε-aminocaproic	$H_2NCH_2(CH_2)_2CH_2$	lysine, pipecolic[d]	149	212(h)
α-keto-N^{ϵ}-(chloroacetamido)caproic	$ClCH_2CONHCH_2(CH_2)_2CH_2$	N^{ϵ}-(chloroacetyl)lysine	71	
α-keto-N^{ϵ}-acetamidocaproic	$CH_3CONHCH_2(CH_2)_2CH_2$	N^{ϵ}-acetyllysine	150	230
α-keto-δ-guanidinovaleric	$NH_2C(=NH)NHCH_2CH_2CH_2$	arginine	149, 151	216[149] 267[151]
α-keto-ε-guanidinocaproic	$NH_2C(=NH)NHCH_2(CH_2)_2CH_2$	homoarginine	152	>260(h)
α-keto-δ-carbamidovaleric	$NH_2C(=O)NHCH_2CH_2CH_2$	citrulline	71	190(h)
α-keto-N^{δ}-carbobenzoxyvaleric	$C_6H_5CH_2OCONHCH_2CH_2CH_2$	N^{δ}-carbobenzoxyornithine	149	194(e)
α-keto-ε-N^{ϵ}-carbobenzyoxycaproic	$C_6H_5CH_2OCONHCH_2(CH_2)_2CH_2$	N^{ϵ}-carbobenzoxylysine	149	160(e)
α-keto-δ-nitroguanidinovaleric	$O_2NNHC(=NH)NHCH_2CH_2CH_2$	nitroarginine	149	225(ac)

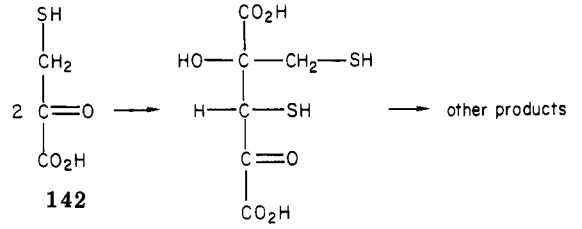
β-imidazolylpyruvic	$(C_3N_2H_3)CH_2$	histidine	151, 153, 154	240(e,l) hydrochloride, 190–192(hc)
β-indolylpyruvic	$(C_8NH_6)CH_2$	tryptophan	155–159	169
		7. Aromatic Side Chain[n]		
phenylglyoxylic (α-ketophenylacetic, benzoylformic)	C_6H_5	phenylglycine (α-aminophenyl acetic)	23, 37, 41, 45, 50–52, 71 160–162	162–164(e–p) 193(h)
β-phenylpyruvic	$C_6H_5CH_2$	phenylalanine	11, 23, 35, 37, 40, 48, 51, 55–57, 71, 163	162–164(h,a) 192–194(e,p)
α-keto-γ-phenylbutyric (benzylpyruvic)	$C_6H_5CH_2CH_2$	α-amino-γ-phenylbutyric	51, 57, 164–166	
α-keto-δ-phenylvaleric	$C_6H_5CH_2CH_2CH_2$	α-amino-δ-phenylvaleric	167	
p-tolylglyoxylic	$CH_3C_6H_4$		41, 49	
β-*p*-tolylpyruvic	$CH_3C_6H_4CH_2$	*p*-methylphenylalanine	168, 194	
α-keto-γ-(*p*-tolyl)butyric	$CH_3C_6H_4CH_2CH_2$		165	
α-keto-δ-(*p*-tolyl)valeric	$CH_3C_6H_4CH_2CH_2CH_2$		167	
2,6-dimethylphenylglyoxylic	$(CH_3)_2C_6H_3$		169	
p-ethylphenylglyoxylic	$C_2H_5C_6H_4$		170	
p-*tert*-butylphenylglyoxylic	$(CH_3)_3CC_6H_4$		171	
β-(*p*-*tert*-butylphenyl)pyruvic	$(CH_3)_3CC_6H_4CH_2$		172	
p-isopropylphenylglyoxylic (cumylglyoxylic)	$(CH_3)_2CHC_6H_4$		173	
β-(*p*-isopropylphenyl)pyruvic (cumylpyruvic)	$(CH_3)_2CHC_6H_4CH_2$		174	
2,4,6-trimethylphenylglyoxylic (mesitylglyoxylic)	$(CH_3)_3C_6H_2$		175–177	
2,3,5,6-tetramethylphenylglyoxylic	$(CH_3)_4C_6H$		177	
2,3,4,6-tetramethylphenylglyoxylic	$(CH_3)_4C_6H$		177	
pentamethylphenylglyoxylic	$(CH_3)_5C_6$		177	
β-(2,4,6-trimethylphenyl)pyruvic	$(CH_3)_3C_6H_2CH_2$		178	
β,β-dimethyl-β-phenylpyruvic	$C_6H_5C(CH_3)_2$		179	157
phthalonic	$(CO_2H)C_6H_4$		175, 180	
3-carboxyphenylglyoxylic	$(CO_2)C_6H_4$	3-carboxyphenylglycine	181, 182	
(3-carboxy-4-hydroxyphenyl)glyoxylic	$(CO_2H)(OH)C_6H_3$	(3-carboxy-4-hydroxyphenyl)glycine	181, 182	
β-(3-carboxyphenyl)pyruvic	$(CO_2H)C_6H_4CH_2$	β-(3-carboxyphenyl)alanine	181–183	
β-(3-carboxy-4-hydroxyphenyl)pyruvic	$(CO_2H)(OH)C_6H_3CH_2$	β-(3-carboxy-4-hydroxyphenyl)alanine	181–183	
4-methoxyphenylglyoxylic	$(CH_3O)C_6H_4$		37, 41, 45, 49, 194	
p-hydroxyphenylglyoxylic	$(OH)C_6H_4$		52	
2,5-dihydroxyphenylglyoxylic	$(OH)_2C_6H_3$		184	
2-(carboxymethyl)phenylglyoxylic	$(CO_2H)CH_2C_6H_4$		185	
α-keto-γ-(4-methoxyphenyl)butyric	$(CH_3O)C_6H_4CH_2CH_2$		165, 186	
β-(*p*-hydroxyphenyl)pyruvic[l]	$(OH)C_6H_4CH_2$	tyrosine	71, 163, 187	178(h)
p-(methylthio)phenylglyoxylic[m]	$CH_3SC_6H_4$		193	
p-bromophenylglyoxylic	BrC_6H_4		45, 49, 193, 194	185–187
p-chlorophenylglyoxylic	ClC_6H_4		41, 45, 50, 52, 193, 194	171–173
p-fluorophenylglyoxylic	FC_6H_4		193	167
p-iodophenylglyoxylic	IC_6H_4		193	189–190
o-bromophenylglyoxylic	BrC_6H_4		193	
m-bromophenylglyoxylic	BrC_6H_4		171, 195	
2,4-dichlorophenylglyoxylic	$Cl_2C_6H_3$		194	
3-fluoro-4-methoxyphenylglyoxylic	$F(CH_3O)C_6H_3$		196	
p-nitrophenylglyoxylic	$(NO_2)C_6H_4$		49, 171	
m-nitrophenylglyoxylic	$(NO_2)C_6H_4$		197, 198	
β-(*p*-bromophenyl)pyruvic	$BrC_6H_4CH_2$	*p*-bromophenylalanine	48, 191, 199	209–210(dec)

Table I (*Continued*)

α-keto acid ($RCOCO_2H$)	R	corresponding amino acid	ref[a]	mp of 2,4-DNP, °C[b]
β-(*p*-chlorophenyl)pyruvic	$ClC_6H_4CH_2$	*p*-chlorophenylalanine	37, 48, 200, 201	171
β-(*p*-fluorophenyl)pyruvic	$FC_6H_4CH_2$	*p*-fluorophenylalanine	200, 202	
β-(*o*-chlorophenyl)pyruvic	$ClC_6H_4CH_2$		203	
β-(*m*-chlorophenyl)pyruvic	$ClC_6H_4CH_2$		204	
β-(*p*-nitrophenyl)pyruvic	$(NO_2)C_6H_4CH_2$		177, 191	
β-(*m*-nitrophenyl)pyruvic	$(NO_2)C_6H_4CH_2$		203	
β-(*o*-nitrophenyl)pyruvic	$(NO_2)C_6H_4CH_2$		205, 206	
β-[3,5-diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)-phenyl]pyruvic	$(OH)(I)_2C_6H_2OC_6H_2(I)_2CH_2$	thyroxine	207	
β-[3,5-diiodo-4-(4-hydroxyphenoxy)-phenyl]pyruvic	$(OH)C_6H_4OC_6H_2(I)_2CH_2$	thyronine	207, 208	
β-(3-iodo-4-hydroxyphenyl)pyruvic	$(OH)IC_6H_3CH_2$	3-iodotyrosine	209, 210	
β-(3,5-diiodo-4-hydroxyphenyl)pyruvic	$(OH)(I)_2C_6H_2CH_2$	3,5-diiodotyrosine	208, 209	
p-aminophenylglyoxylic	$H_2NC_6H_4$		211	
p-acetamidophenylglyoxylic	$CH_3CONHC_6H_4$		45	
β-(*p*-aminophenyl)pyruvic	$H_2NC_6H_4CH_2$		212	
β-(*p*-dimethylaminophenyl)pyruvic	$(CH_3)_2NC_6H_4CH_2$		203	
β-phenylpyruvic mustard	$(ClCH_2CH_2)_2NC_6H_4CH_2$		212	
α-naphthylglyoxylic	$C_{10}H_7$		50	
β-naphthylglyoxylic	$C_{10}H_7$		45, 213	
4-methyl-1-naphthylglyoxylic	$(CH_3)C_{10}H_6$		214	
β-(1-naphthyl)pyruvic	$C_{10}H_7CH_2$		215	
β-(2-naphthyl)pyruvic	$C_{10}H_7CH_2$		216	
3-pyridineglyoxylic	C_5H_4N		217	
β-benzylidenepyruvic (benzalpyruvic)	$C_6H_5CH{=}CH$		52, 131, 165, 218-221, 445[g]	
anisalpyruvic	$CH_3OC_6H_4CH{=}CH$		222, 223	
β-(*m*-nitrobenzylidine)pyruvic	$(NO_2)C_6H_4CH{=}CH$		52, 223	
β-(*o*-nitrobenzylidine)pyruvic	$(NO_2)C_6H_4CH{=}CH$		223	
β-(*p*-methylbenzylidene)pyruvic	$(CH_3)C_6H_4CH{=}CH$		221, 224	
β-methyl-β-benzylidenepyruvic	$C_6H_5CH{=}CCH_3$		225	
cinnamalpyruvic	$C_6H_5CH{=}CH{-}CH{=}CH$		224	
4,4-bis(*p*-chlorophenyl)2-ketobut-3-enoic acid	$(ClC_6H_4)_2C{=}CH$		226	180-184
β-styrylpyruvic	$C_6H_5CH{=}CHCH_2$		203	
β,β-diphenylpyruvic	$(C_6H_5)_2CH$		51, 227	
β,β,β-triphenylpyruvic	$(C_6H_5)_3C$		228	
α-keto-γ,γ-diphenylbutyric	$(C_6H_5)_2CHCH_2$		51	
	8. Steroid Side Chain[44]			
	9. Side Chain Containing a Non-Aromatic Ring[o]			
camphor-3-glyoxylic	$C_{10}H_{15}$		229, 230	
α-ketohomocamphoric	$C_9H_{15}O_2$		229	
β-santenone-3-glyoxylic	$C_9H_{13}O$		230	
α-santenone-3-glyoxylic	$C_9H_{13}O$		230	
norcamphor-3-glyoxylic	C_7H_9O		230	
7-acenaphthenone-8-glyoxylic	$C_{12}H_7O$		230	
1-oxoindan-2-glyoxylic	C_9H_7O		230, 231	

[a] Space precludes listing of all literature references to the preparation of the common α-keto acids. Literature references to the first preparation (where known) and those quoted in the text are given. [b] DNP = dinitrophenylhydrazone. Letters in parentheses refer to solvent employed where known: a, ethyl alcohol; ac, acetic acid; d, dioxane; e, ethyl acetate; h, water; hc, hydrochloric acid; l, ligroin; p, petroleum ether. [c] Characterized as the methyl ester. [d] α-Keto-δ-aminovaleric acid and α-keto-ε-aminocaproic acid exist in equilibrium with cyclic forms (see the text). Thus, oxidation of D-proline with D-amino acid oxidase or of L-ornithine with L-amino acid oxidase yields α-keto-δ-aminocaproic acid. Similarly, enzymatic oxidation of L-lysine or of D-pipecolic acid yields α-keto-ε-aminocaproic acid (see, for example, A. Meister, D. Wellner, and S. J. Scott, *J. Natl. Cancer Soc.* **1960**, *24*, 31). [e] The following α-keto acid dipeptides have been enzymatically synthesized as the barium salt,[4,61] γ-(α-Ketoglutaryl)glycinate, γ-(α-ketoglutary)-β-alaninate, γ-(α-ketoglutary)-L-alaninate, β-oxalacetylglycinate, and β-oxalacetyl-L-alaninate; the α-ketoglutaryl compounds do not possess a reactive carbonyl and are presumably in a cyclic form; the oxalacetyl compounds possess a reactive carbonyl.[61] [f] The preparation of these α-keto acids has not been described in detail in the literature but they have been prepared in the authors' laboratory by enzymatic oxidation of the corresponding amino acid according to the method of Meister.[4] These α-keto acids have been employed in specificity studies with glutamine transaminase[104] and with ω-amidase.[105] [g] Reimer et al. also prepared β-monobromo, dibromo, *p*-methoxy, *p*-ethoxy, and β-bromo-*p*-ethoxy derivatives, and several bromo-*p*-methoxy derivatives of benzilidenepyruvic acid.[221] Stecher et al. also prepared the 4-chloro-, 2,4-dichloro-, and 3,4-dichloro ring substituted analogues.[445] [h] These 2,4-dinitrophenylhydrazones contained traces of the corresponding α-keto dicarboxylic acid 2,4-dinitrophenylhydrazone. [i] This compound did not form a 2,4-dinitrophenylhydrazone suggesting that it exists predominantly in a cyclic form that does not possess a reactive carbonyl.[106] [j] α-Keto-γ-mercaptobutyric and its disulfide have been shown to be produced in reaction mixtures containing L-amino acid oxidase and L-homocysteine (or L-homocystine). However, the α-keto acids have not yet been isolated; the corresponding 2,4-dinitrophenylhydrazones have been prepared (mp = 153 °C (ea) and 204 °C (e), respectively).[63] [k] (For halogen-containing analogues of oxaloacetate and α-ketoglutarate see subsection 2). [l] The following analogue of β-(*p*-hydroxyphenyl)pyruvic acid have been synthesized (ring substituent, ref): 2-OH(163, 188, 189); 3-OH (163, 189); 2,4-(OH)$_2$ (163); 2,5-(OH)$_2$ (163, 184, 187); 3,4-(OH)$_2$ (163, 190); 2-MeO (163); 3-MeO (163); 4-MeO (48, 174, 192); 5-Me, 2-OH (163); 3-Me, 4-OH (163); 3,4-OCH$_2$O- (55, 163, 174, 192); 2-OH, 3-MeO (163); 3-OH, 4-MeO (163, 203); 3-OH, 4-MeO (163); 2,4-(MeO)$_2$ (163); 3,4-(MeO)$_2$ (163, 192); 3,5-(MeO)$_2$, 4-OH (163). [m] For other sulfur-containing aromatic α-keto acids see subsection 4. [n] For aromatic α-keto acids that contain a non-ring halogen see subsection 5. [o] See also subsection 2.

Other α-keto acids, such as 3-mercaptopyruvic (**142**)[118] and 3-hydroxypyruvic (**143**)[126,236] also readily polymerize.



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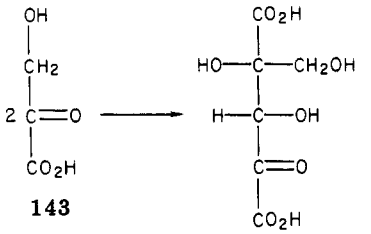
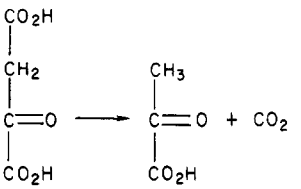


143

Oxaloacetic acid (**4**) and β-substituted oxaloacetic acids readily undergo β-decarboxylation at neutral pH at 25 °C ($t_{1/2}$ ~ 30–60 min). The mechanism of this



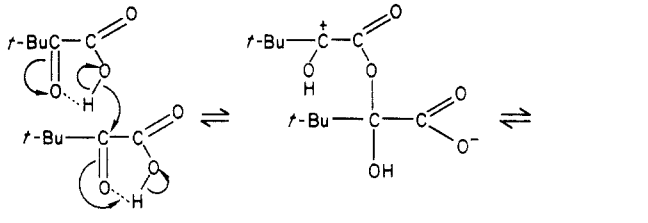
4 **3**

reaction has been extensively studied.[114,237,238] (The article by Kubala and Martel[114] contains an extensive and up-to-date review of the literature on β-decarboxylation of oxaloacetic acid and its β-substituted analogues). Oxaloacetic acid is relatively stable in strongly acidic solutions.

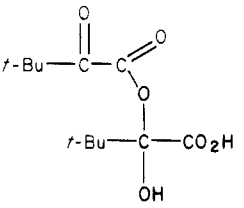
C. Equilibrium with Other Forms

Schellenberger and co-workers have published more than 20 papers under the title "theory of α-keto acids". These authors investigated the physico-chemical properties of α-keto acids and their derivatives in aqueous solution, nonaqueous solution, in the gas phase and in the solid phase.[171,235,239-244] (Only those papers directly concerned with α-keto acids are referenced).

Schellenberger and Oehme noted that the molecular weight of pure trimethylpyruvic acid (**68**) when measured cryoscopically in dioxane up to 0.01 mol fraction was 10% greater than expected.[239] Since association by hydrogen bonding is unlikely, it was concluded that some dimerization occurred. Subsequently, crystalline dimer was obtained from cold samples of trimethylpyruvic acid. (Glücksmann[79] first isolated the dimer from etheral solutions in 1889). It was found that acid stabilized the dimer in aqueous solution but the breakdown to monomer was catalyzed by bases in the order Et_3N > pyridine > dimethylalanine > quinoline. The reaction is dependent on both basicity and steric effects.[239] It was concluded from dipole measurements and from infrared spectroscopic data that the dimer is an "activated ester" (**144**).

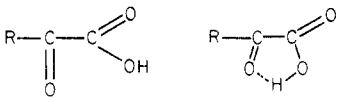


68



144

The tendency of pure samples of α-keto acids to dimerize to "activated ester" was noted for pyruvate (3) and for other higher homologues of pyruvate (i.e., $RCOCO_2H$; R = C_2H_5, 8; R = $CH(CH_3)_2$, 37), with α-ketobutyrate (8) showing the least tendency. Schellenberger et al. have also concluded, from infrared data, that these α-keto acids are to a considerable degree in a "proton chelate" form (1a) in the gas phase between 90 and 180 °C.[242] Other workers have also concluded



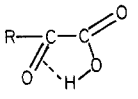
1 1a

from infrared evidence that pure pyruvic acid contains intramolecular hydrogen bonds.[245,246] In the "proton chelates" the carbonyls are in the s-trans configuration.

Two series of α-keto acids were further studied in order to determine their ability to form proton chelates in solution (i.e., ring-substituted glyoxylic acids and β-methyl-substituted pyruvic acids).[171,241,243,244] The ratio of proton-chelate (1a) to open-form (1) was found to depend on concentration, temperature, solvent, and nature of R. For example, for pyruvic acid and β-methyl-substituted pyruvic acids (3, 8, 37, 68), "proton-chelates" are important contributions in nonpolar solvents (e.g., CCl_4) and probably in acid aqueous solution but are unimportant in polar solvents such as dioxane. For both series of α-keto acids, the chelates cause a major bathochromic shift of the n→π* bands of the carbonyl group compared to the nonchelated carbonyl. The intensity of the n→π* transition of various α-keto acids was in the order pyruvate (3) < α-ketobutyrate (8) > α-ketoisovalerate (37) > trimethylpyruvate (68). The special position of pyruvate (3) may be due to hyperconjugation.[244] Schellenberger et al.[244] point out that the *tert*-butyl group of trimethylpyruvate should favor the "proton-chelate" structure by increasing the electron density at the neighboring carbonyl group; however, the trans position of both carbonyls are sterically hindered. Such steric hindrance may explain the significant amount of open-chain form noted even with trimethylpyruvate (68) in CCl_4.[244]

The phenylglyoxylic acids were shown to form proton chelates in both nonpolar (CCl_4) and polar solvents (water).[244] The infrared frequencies of the α-carboxyl and hydroxyl bands of the substituted glyoxylic acids are strongly dependent on the nature of the ring substituents.[171] Both conjugative and inductive effects were found to influence these frequencies; however, the finding that these frequencies exhibited by mesitylglyoxylic acid are similar to those observed for aliphatic α-keto acids suggests that the former effect is more important. Substitution in both the *m*- and *p*-positions does not affect the CO frequency of the carboxyl but does influence the frequency of α-CO and OH groups.[243] Interestingly, the frequency of the OH band in the "proton chelate" is affected by ring substituents in a different fashion from that of the open-form. Thus, the frequency correlates well with the σ^+ values of ring substituents of the "proton chelate" whereas the frequency correlates with the Hammett σ function in the open form.[171,243,244]

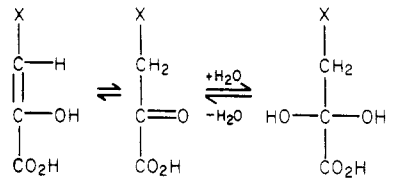
Schellenberger et al. make the controversial suggestion that the hydrogen bond between the carboxyl OH and the α-carbonyl in the "proton chelate" is not due to an interaction with the free electron pair on the oxygen but to an interaction with the π electron pair (1c).[171,244]



1c

Whereas supporting evidence was presented for this conclusion, the authors also state that the bond angles (calculated from literature values on model compounds) deviate considerably from those necessary for optimal π ← H hydrogen bond formation.

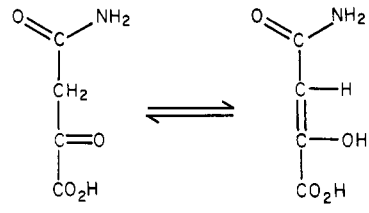
It is well-known that α-keto acids (145) can form enols (146) or add water to form hydrates (*gem*-diols) (147).



146 145 147

When X is a simple aliphatic group or is linked through one or more methylenes (e.g., as in α-ketoglutarate, 5) the enol is quantitatively unimportant in neutral or acidic solutions. Evidence for this conclusion is as follows: (a) Bromine was found to add relatively smoothly to solutions of pyruvic acid (3) to yield β-monobromopyruvic acid, but there was no initial rapid addition as would be expected if enol was present in solution.[247] (b) Similar bromination experiments with α-keto-β-methylvaleric acid (46) suggested no enol at pH 6.1 and < 1% at pH 0.9.[248] (c) Solutions of D-α-keto-β-methylvalerate (46) do not lose optical activity at pH values below 8.4 at an appreciable rate; above pH 8.4, the rate of loss of optical activity is proportional to OH^- concentration.[38] (Presumably, loss of optical activity is mediated by enol formation, but even in basic solution the enol contribution must be small since alkaline solutions of D-α-keto-β-methylvaleric acid do not absorb strongly in the UV region of ~300 nm). (d) Solutions of α-keto acids (145, X = H, CH_3, CH_2R) exhibit stable NMR spectra in neutral or acidic solu-

tions of D_2O; appreciable enol formation would result in rapid loss of signal.[249] On the other hand, if X (in structure 145) is electron-withdrawing (e.g., CO_2H (4), C_6H_5 (6), $CONH_2$ (148), indolyl (149)), the enol is an



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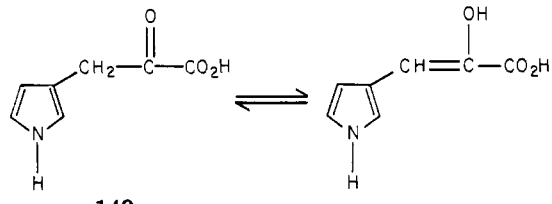

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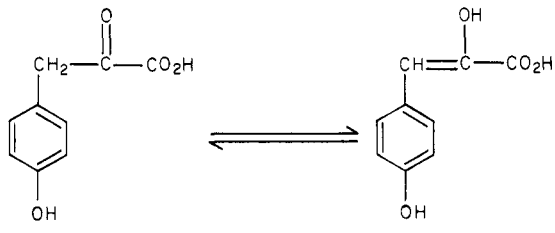
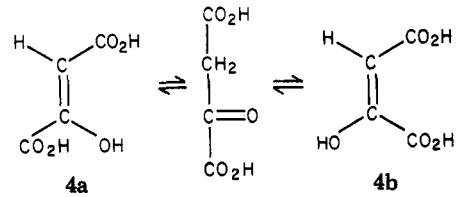


150 150a

important contribution, particularly in alkaline solutions.[106,237,238] These α-keto acids absorb strongly in the UV region (~300 nm) in base due to conjugation of enol with carboxyl carbonyl.

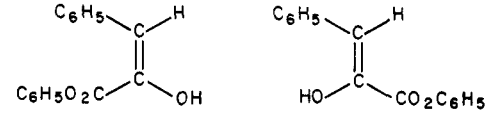
Some evidence has been presented that β-indolylpyruvic acid (149)[250] and β-(*p*-hydroxyphenyl)pyruvic acid (150)[251] crystallize as the enol (149a, 150a, respectively). Under anaerobic conditions two forms of β-indolylpyruvic acid can be detected by paper chromatography, presumably the keto (149) and enol (149a) forms.[252] Pure oxaloacetic acid (4) is also probably in the enol form,[253,254] while in solution it contains both cis and trans enolic forms,[238,253-255] i.e., hydroxyfumaric acid (4a) and hydroxymaleic acid (4b). The mechanism



4a 4 4b

of the interconversion between keto and enol forms of oxaloacetic acid (4) has been extensively studied.[256]

The cis and trans isomers of phenylpyruvic acid enol have not yet been obtained, but the cis and trans isomers of the ethyl ester of phenylpyruvic acid enol have been isolated.[257]



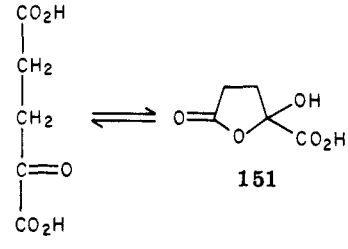
Although β-arylpyruvic acids ($ArCH_2COCO_2H$) readily enolize in strong base, at physiological pH values the concentration of the enol form is generally low. However, in the presence of borate buffer (~ pH 8.0), enolization is promoted via formation of an enol–borate complex. Attainment of equilibrium is slow as determined by monitoring of the characteristic UV absorbance of the enol.[258] Interestingly, glutathione-dependent arylpyruvate keto–enol tautomerase activity is widespread in mammalian tissue and the enzyme has been extensively purified from lamb kidney and studied.[258] The enzyme catalyzes rapid interconversion between keto and enol forms of phenylpyruvate (6), *p*-hydroxyphenylpyruvate (150), various ring substituted phenylpyruvates, and, to a lesser extent, β-indolylpyruvate (149); the simultaneous presence of tautomerase is thus useful for the spectroscopic determination of enzymatically generated aromatic α-keto acids in borate buffer.[258]

Generally, for those α-keto acids in which an enol contribution is unimportant, the percentage of the *gem*-diol is also low in solution at neutral pH (5–10%).[249] However, when the α-carboxyl is protonated, the percentages of the *gem*-diol increases (≥ 60%).[249] (Rates of hydration and dehydration are relatively slow so that the two forms are easy to distinguish using ^1H NMR techniques[249]). Interestingly, glyoxylate is ~95% hydrated in D_2O at pH 6.0 and glyoxylic acid (2) is ≥99% hydrated at pH 0.5.[249]

$$\underset{\mathbf{2}}{HCOCO_2H} \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} HC(OH)_2CO_2H$$

The degree of hydration of an α-keto acid correlates well with the inductive effect of the substitutents adjacent to the carbonyl (see Cooper and Redfield[249] and references quoted therein). The kinetics of the hydration reaction of oxaloacetate (4) have recently been studied in detail.[259] Interestingly, Bellamy and Williams showed that although pure pyruvic acid (3) exists in the keto form, the lithium monohydrate salt is in the *gem*-diol form (i.e., $CH_3C(OH)_2CO_2Li$) and is not a true hydrate (i.e. $\neq CH_3COCO_2Li{\cdot}H_2O$); similarly, β-hydroxypyruvic acid exists in the pure form as the keto acid but the lithium hydrate has the *gem*-diol form.[245]

α-Ketoglutaric acid (5) exhibits an anomalous ^1H NMR spectrum in D_2O at pH 0.5.[249] Cooper and



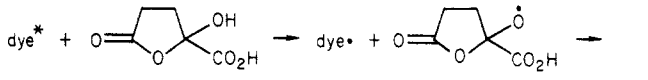
151

5

Redfield attributed this anomaly to equilibrium between α-keto acid and lactol (151).[249] No such anomaly was noted for α-ketoadipic acid[249]

Evidence that α-ketoglutaric acid (5) is in equilibrium with a lactol structure (151) was obtained by Jefford et al. who showed that in acetonitrile/pyridine solvent, α-ketoglutaric acid (5) is decarboxylated in yields of up to 90%, by the dye, rose bengal, in the absence of ox-

ygen; at the same time the dye is bleached.[260] Simple



151 151a

other products

aliphatic α-keto acids (e.g., pyruvic (3), α-ketobutyric (8), and α-ketovaleric (18) acids) are much less susceptible to decarboxylation under these conditions.[260]

Subsequent reaction of the initially formed lactol radical (151a) would account for the formation of a number of initially unexpected products (lactic, malic, and oxalic acids).[260] More recently, evidence for the lactol structure (151) in aqueous solutions of α-ketoglutaric acid (5) was obtained by Viswanathan et al. using a ^{13}C-NMR technique.[261] These authors found that, at neutral pH, the α-keto acid predominates with small amount of lactol and 7% *gem*-diol; protonation of the γ-carboxylate increases the lactol to 20% with little increase in *gem*-diol; protonation of the α-carboxylate results in an equilibrium mixture of 35% α-keto acid, 30% lactol, and 35% *gem*-diol.[261] These data agree closely with those of Cooper and Redfield who found that, under slightly different conditions at pH 0.5 in D_2O, the values are 31%, 16%, and 53%, respectively.[249]

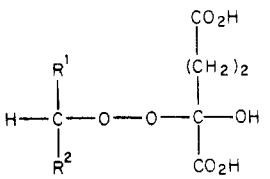
D. Oxidative Decarboxylation

It is well-known that many α-keto acids are metabolized via enzyme-catalyzed oxidative decarboxylation (i.e. via pyruvate, α-ketoglutarate, and branched-chain α-keto acid dehydrogenases).[262] It has also been known for many years that α-keto acids are rapidly and quantitatively decarboxylated by such mild oxidizing reagents as ceric sulfate,[263,264] hydrogen peroxide,[106,265-268] peroxyphthalic acid,[268] potassium permanganate,[265] and lead tetraacetate.[269] Decarboxylation by hydrogen peroxide is base catalyzed[264,266] and is accelerated in the presence of Fe^{2+} (but not other cations), and this reaction has been studied in detail.[270]

Interestingly, a group of enzymes known as dioxygenases (hydroxylases; e.g., butyrobetaine hydroxylase, proline hydroxylase) has recently been described,[271,272] which utilize α-ketoglutarate (5), virtually exclusively, as a cofactor. Substrate (SH) is converted to oxidized substrate (SOH); 1 mol of α-ketoglutarate is consumed for each mol of succinate (152) and hydroxylated product (SOH) formed:

$$SH + O_2 + \underset{5}{(CO_2H)CH_2CH_2COCO_2H} \rightarrow SOH + \underset{152}{(CO_2H)CH_2CH_2CO_2H} + CO_2$$

Linblad et al. proposed that for each molecule of O_2 utilized, one atom of oxygen is incorporated into SOH



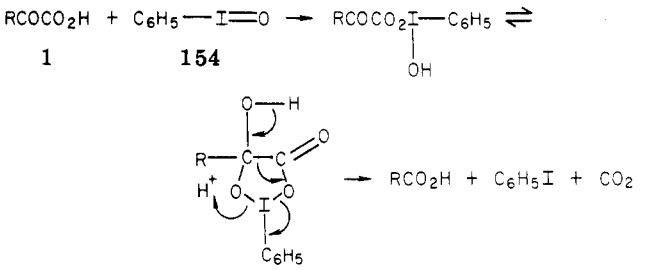
153

and one into succinate (152).[273] In support of this idea, label from $^{18}O_2$ was found to be incorporated into succinate in the reaction catalyzed by butyrobetaine hydroxylase.[273] Lindblad et al.[273] suggest an α-hydroxy-α-peroxy acid of the type 153 as an intermediate (In this example S = CHR^1R^2–).

On the other hand, Jefford et al.[274] suggest a peroxy acid as intermediate.

$$\underset{5}{(CO_2H)CH_2CH_2COCO_2H} + O_2 \rightarrow (CO_2H)CH_2CH_2COCO_3H \xrightarrow{SH} SOH + \text{succinate} + CO_2$$

In order to understand the dioxygenase reaction in more detail, a large number of model reactions involving oxidative decarboxylation of α-keto acids has been investigated. Oxidants utilized include (a) photoactivated mixtures of dye[260,274-276] or diazo compounds,[276] (b) iodosobenzene,[277] (c) superoxide.[278] The oxygen atom transfer from iodosobenzene (154) is thought to proceed as follows:



It was originally thought that in UV-activated dye mixtures, singlet oxygen ($^1O_2$) arising from dye sensitization is responsible for the decarboxylation of α-keto acids ($^3O_2$ is unreactive toward α-keto acids).[260,274,275] Somewhat later, Davidson[279] presented evidence to suggest that the sensitized dye (triplet-excited dye) is responsible for the decarboxylation, but he could not rule out the participation of some $^1O_2$ in this reaction. Following this lead, Jefford et al. found that the excited dye could indeed effect α-keto acid decarboxylation but suggested that singlet oxygen in systems containing sensitized dye could also compete with the sensitized dye to decarboxylate α-keto acids, presumably via a peroxy acid.[274]

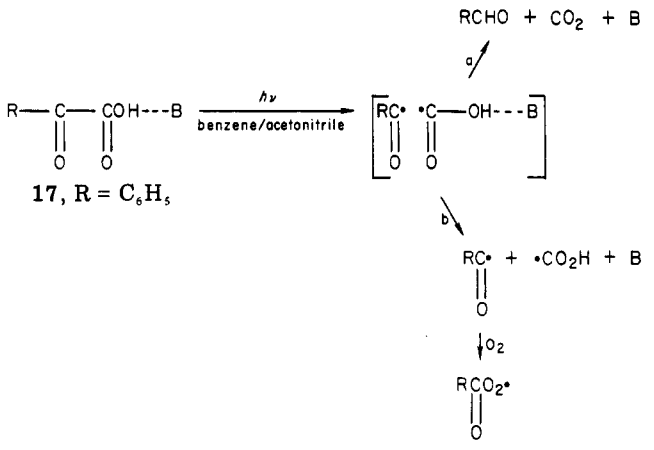
$$\underset{1}{RCOCO_2H} \xrightarrow{^1O_2} RCO_3H + CO_2$$

$$RCO_3H + RCOCO_2H \rightarrow 2RCO_2H + CO_2$$

Davidson et al., by measuring solvent isotope effects and the effect of $^1O_2$ quenchers, concluded that $^1O_2$ has little or no role in the direct decarboxylation of α-ketoglutaric (5), α-ketoisocaproic (45), or phenylpyruvic (6) acids.[280] The authors also showed that pyruvic acid (3) is readily decarboxylated in the presence of UV light and O_2 and that more than 1 mol of CO_2 is produced per mol of pyruvate consumed.[281] Peroxy acid formation probably occurs via direct addition of O_2 to $CH_3CO\cdot$.

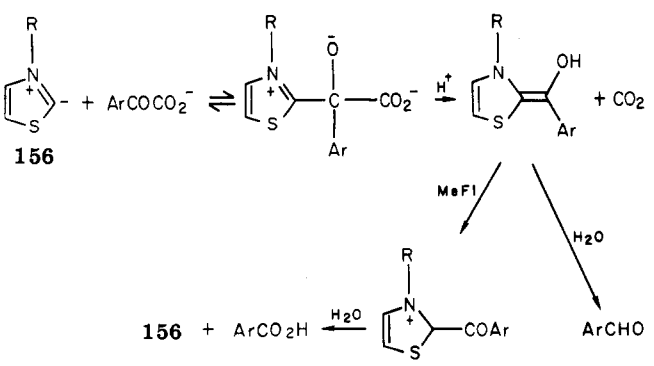
$$\underset{3}{CH_3COCO_2H} \xrightarrow[O_2]{h\nu} CH_3CO_3H \rightarrow CO_2 + \text{other products}$$

Jefford et al. have recently shown that phenylglyoxylic acid (17) is completely unreactive toward $^1O_2$.[282] Recently, Sawaki and Ogata also reported that photooxidation of phenylglyoxylic acid (17) is not due to singlet oxygen but to a photochemical cleavage leading to an acylperoxy radical (155); the reaction is not sensitized by methylene blue and is catalyzed by pyridine.[283] The authors proposed the following mechanism where the square brackets represent a "solvent cage":

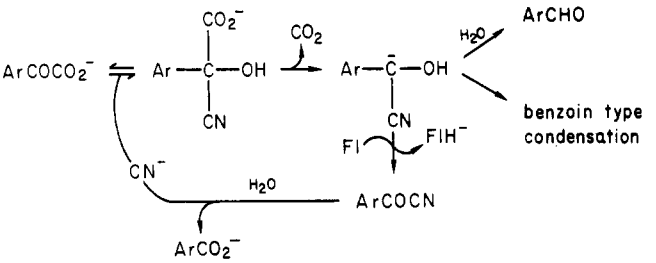


Pathway a predominates when water is present whereas pathway b predominates in the absence of water. Thus, photolysis of phenylglyoxylic acid (17) at wavelengths >320 nm and 20 °C in the presence of oxygen and benzene yields peroxybenzoic acid in 80% yield (and H_2O_2, 35%, and phenylbenzoate, 10%).[283] When α-methylstyrene was added to the photooxidation mixture, the yield of phenylbenzoate was increased and α-methylstyrene was converted to the corresponding epoxide (and to a lesser degree, to acetophenone, by –C–C– bond scission). These findings are fully explicable in terms of reactions of peroxybenzoic acid radical (155).[283] Clearly much work remains to elucidate the mechanism of the photooxidation–decarboxylation reaction and the dioxygenase reaction, but it now seems clear that singlet oxygen does not play a direct role in the oxidative decarboxylation of α-keto acids.

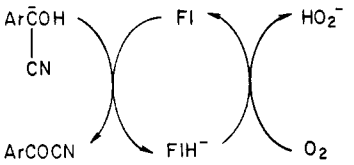
Thiamine pyrophosphate (TPP) is an important cofactor in the following enzyme systems: (a) in the oxidative decarboxylation of α-keto acids, (b) decarboxylation–condensation of pyruvate to acetoin, (c) the nonoxidative decarboxylation of pyruvate to acetaldehyde, and (d) the transketolase reaction. Model reactions that mimic some of these enzymatic reactions have been the subject of much research.[284,285] Recently, Shinkai et al.[286] described the first model of a *combined* flavin–TPP system. In a micellar system, the thiazolium ion (hexadecylthiazolium bromide, 156) and a flavin (3-methyltetra-*O*-flavin, MeFl) efficiently catalyze the oxidation of aldehydes and α-keto acids.[286] The authors suggest that this reaction may provide useful insights into the mechanism of the pyruvate dehydrogenase reaction.



Shinkai et al.[287] also describe another model system for the oxidative decarboxylation of α-keto acids, and suggest that this system may provide useful information concerning the interaction of flavins and carbanions in enzyme reactions.



The reaction is enhanced if carried out in micelles. Apparently, oxygen does not readily react with free reduced flavin (FlH^-) but does so in the micelles.[287]



E. Thermal and Photolytic Decarboxylation

Generally these reactions result in the formation of an aldehyde containing one less carbon atom than the original α-keto acid or of the corresponding aldol adduct. When pyruvic acid (3) is heated in the presence of osmium, palladium, or ruthenium catalysts it is cleanly decarboxylated to acetaldehyde (157); osmium is most effective.[288]

$$\underset{3}{CH_3COCO_2H} \rightarrow \underset{157}{CH_3CHO} + CO_2$$

On the other hand, heating pyruvic acid (3) in the presence of a copper catalyst has been reported to yield acetaldehyde, biacetyl, and pyruvaldehyde, together with smaller amounts of methylglyoxime, dimethylglyoxime and methylsuccinic acid.[289] Schellenberger and Selke report that Cu^{2+} and Al^{3+} catalyze the conversion of pyruvic acid (3) to acetoin (158).[290] α-Ketobutyric (8) acid also yields a similar condensation product.

$$\underset{3}{2CH_3COCO_2H} \xrightarrow{Cu^{2+},\ Al^{3+}} \underset{158}{CH_3COCH(OH)CH_3} + 2CO_2$$

No net oxidation occurred even in the presence of oxygen, but if Mn^{2+} was used as catalyst in place of Cu^{2+}, oxidative decarboxylation of pyruvic acid to acetic acid was noted.[290] Para- and metapyruvic acids are also catalytically decarboxylated by Cu^{2+}.[235]

When phenylglyoxylic acid (17) is refluxed in 50% ethanol with CN^- as catalyst, benzoin (159) is produced.[291]

$$\underset{17}{2C_6H_5COCO_2H} \rightarrow \underset{159}{C_6H_5COCH(OH)C_6H_5} + 2CO_2$$

In the vapor phase, in the presence of UV light, pyruvic acid undergoes photodecomposition (photolysis) to acetaldehyde and CO_2.[292,293]

In the aqueous phase, phenylglyoxylic acid (17) undergoes photolysis to benzaldehyde (28) and CO_2.[292]

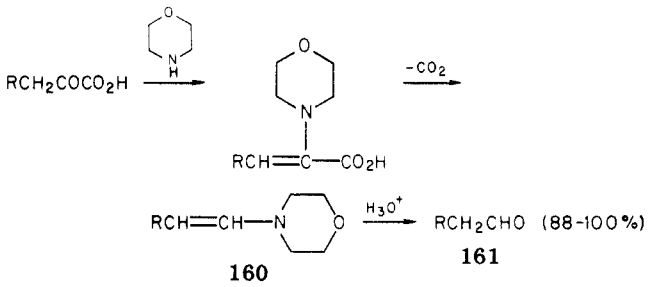
$$\underset{17}{C_6H_5COCO_2H} \rightarrow \underset{28}{C_6H_5CHO} + CO_2$$

Under the same conditions pyruvic acid (3) yields acetoin (158).[292]

$$\underset{3}{2CH_3COCO_2H} \rightarrow \underset{158}{CH_3COCH(OH)CH_3} + 2CO_2$$

Steenken et al. have carried out a detailed investigation of the photofragmentation of several α-keto acids (glyoxylic, pyruvic, α-ketobutyric, α-ketoglutaric, and α-ketoisocaproic acids) using EPR techniques.[294] Decarboxylation occurs via substitution of acyl radicals.[294]

Stamos has shown that α-keto acids with enolizable hydrogens are easily converted to aldehydes with one less carbon atom by boiling with a secondary amine in benzene.[295] Azeotropic removal of water leads to formation of an enamine (160). Decarboxylation and hydrolysis yield the aldehyde (161).[295]



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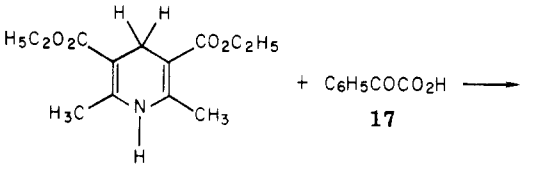
F. Reduction

Waters[2] states that α-keto acids can be reduced by hydrogen to the corresponding α-hydroxy acid, but we have found no systematic description of this process. It is well known that pyruvate (3) can be enzymatically reduced to lactate. It has been known for more than 30 years that lactate dehydrogenase is nonspecific and that it catalyzes the reduction of a large number of α-keto acids to the corresponding α-hydroxy acids (162); some of these α-keto acids are reduced at rates similar to that found with pyruvate.[296]

$$\underset{1}{RCOCO_2H} + NADH + H^+ \rightarrow \underset{162}{RCH(OH)CO_2H} + NAD^+$$

In the presence of a suitable electron donor, pyruvic acid (3) can be reduced to lactic acid (162, R = CH_3). Thus, Mauzerall and Westheimer[297] showed that 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (163) (a Hantzsch compound) can bring about this reduction. Later, Abeles and Westheimer investigated the reaction of this Hantzsch compound and found that phenylglyoxylic acid (17) is reduced to mandelic acid (164) in dimethoxyethane/methanol.[298] Although not a strict mimic of an enzymatic NADH reduction, the reaction is interesting in that a direct H transfer occurs.



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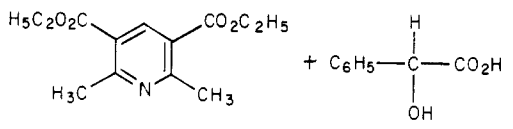

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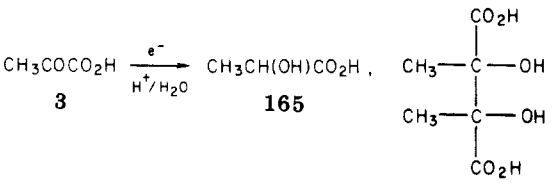
The cathodic reduction of pyruvic (3) and phenylglyoxylic (17) acids using Hg or Hg–Cu electrodes has been described.[299] The products vary according to the



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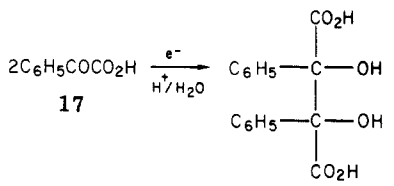


17 167

conditions, but generally in the case of pyruvic acid (3), a major product is lactic acid (165) with smaller amounts of dimethyl tartaric acid (166). In the case of phenylglyoxylic acid (17), a major product is *meso*-diphenyltartaric acid (167).

Pyruvic acid (3) and phenylglyoxylic acid (17) can also be reduced to dimethyl- (166) or diphenyltartaric acids (167), respectively, by vanadate(II) ion.[300] For each mol of pyruvic or phenylglyoxylic acid reduced 1 mol of V^{II} is oxidized to V^{III}.[300] It is of interest that UV irradiation of pyruvic (3) or phenylglyoxylic (17) acids in suitable organic solvents results in photoreduction to dimethyltartaric (166) and diphenyltartaric acid (167), respectively; electrons are apparently furnished by the solvent.[292,301] (If the photolysis of pyruvic acid (3) is carried out in methanol some α-methyl-2,3-dihydroxypropanoic acid is also produced.[292])

G. Free-Radical-Induced Decomposition

In addition to the decarboxylation reactions mentioned above, other fragmentation reactions of α-keto acids are also known. Thus, α-ketodecanoic (α-ketocapric) acid (116) is split to 1-heptene (168) and pyruvic acid (3) on photolysis (a Norrish type II elimination).[302]

$$\underset{116}{CH_3(CH_2)_7COCO_2H} \xrightarrow[C_6H_6]{h\nu} [CH_3(CH_2)_7COCO_2H]^* \rightarrow CH_3(CH_2)_4\dot{C}HCH_2CH_2\dot{C}(OH)CO_2H \rightarrow \underset{168}{CH_3(CH_2)_4CH{=}CH_2} + \underset{3}{CH_3COCO_2H}$$

α-Ketooctanoic acid (91) also yields an olefin and pyruvate on photolysis.[281]

In another example, hydroxyl radicals (furnished, for

example by the autoxidation of 6-aminodopamine) will react with α-keto-γ-(methylthio)butyrate (40) to yield ethylene (169).[303] The reaction is complex but the finding that ethylene production can be supressed by catalase or by superoxide dismutase suggests that both hydrogen peroxide and superoxide are important intermediates in OH· production.[303]

$$\underset{\mathbf{40}}{CH_3SCH_2CH_2COCO_2H} + OH\cdot \rightarrow \underset{\mathbf{169}}{CH_2{=}CH_2} + \text{other products}$$

H. Transamination

It has long been known that amino acids undergo oxidative deamination in the presence of compounds containing reactive carbonyls such as alloxan, isatin, quinones, and ninhydrin; α,β-dicarbonyls are also known to be active. In each case the α-amino nitrogen is released as ammonia; in the case of the ninhydrin reaction, ammonia reacts further (see review by Herbst[304] for an account of this early work). The first transamination reaction between an amino acid and an α-keto acid was described by Herbst and Engel in 1934.[305] As in the reaction with other reactive carbonyls, the α-carbon of the α-amino acid is oxidized to >C=O but the nitrogen is not released as ammonia. Herbst and Engel[305] showed that α-aminophenylacetic acid (phenylglycine; 170) and pyruvic acid (3) when heated together in aqueous solution gave rise to almost quantitative benzaldehyde (28), alanine (171), and carbon dioxide.

$$\underset{\mathbf{170}}{C_6H_5CH(NH_2)CO_2H} + \underset{\mathbf{3}}{CH_3COCO_2H} \rightarrow \underset{\mathbf{28}}{C_6H_5CHO} + \underset{\mathbf{171}}{CH_3CH(NH_2)CO_2H} + CO_2$$

Herbst et al. also showed that many amino acids could effectively replace phenylglycine (170), but α-amino acids that did not possess an α-C–H bond (e.g., α-aminoisobutyric ($(CH_3)_2C(NH_2)CO_2H$), α-acetylaminophenylacetic acid) were inactive. The authors correctly concluded that the reaction takes place in several stages as shown below.[305,306]

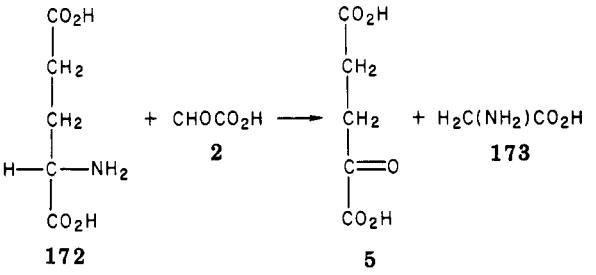
$$RCOCO_2H + R'CH(NH_2)COH \rightarrow RC(CO_2H){=}NCHR'CO_2H \rightarrow RCH(CO_2H)N{=}CR'CO_2H \rightarrow RCH(NH_2)CO_2H + R'CHO + CO_2$$

Herbst and Engel[305] speculated that this amino transfer might be of biological importance, but it was not until three years later that Braunstein and Kritzmann[307] described the first enzyme-catalyzed transamination between an α-amino acid and α-keto acid.

$$R^1CH(NH_2)CO_2H + R^2COCO_2H \rightleftarrows R^1COCO_2H + R^2CH(NH_2)CO_2H$$

Unlike the nonenzymatic reaction no decarboxylation occurs and all known enzyme-catalyzed transamination reactions require pyridoxal 5′-phosphate as cofactor. It is now known that transaminases play a crucial role in amino acid metabolism (see Cooper and Meister[308] for a recent review).

A number of workers have investigated nonenzymatic transamination reactions. In an early model system, Brewer and Herbst showed that transamination occurs without scission of an α-C–C bond when the ethyl esters of pyruvic acid (3) and phenylalanine are heated together.[309] It is worth noting that glyoxylic acid (2) is particularly effective in nonenzymatic transamination.[310-312] Glyoxylic acid (2) undergoes transamination with many amino acids, but especially with glutamine and glutamate at physiological temperature and pH.[310] In the reaction with glutamate (172), the products are α-ketoglutarate (5) and glycine (173); decarboxylation does not occur.



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Metal ions such as Cu^{2+} and Al^{3+} are particularly effective catalysts for nonenzymatic transamination reactions. Pyridoxamine, pyridoxal, pyridoxal 5′-phosphate, and pyridine have been employed as cocatalysts.[312-314] However, if glyoxylate (2) is heated with an appropriate amino donor, Al^{3+} and pyridoxamine, considerable β-hydroxyaspartic acid (174) is generated in addition to glycine (173), presumably due to addition of glyoxylic acid (2) to glycine (173).[313] β-Hydroxyaspartate (174) is also generated if the reaction is carried out in the presence of pyridine and Cu^{2+}.[312]

$$\underset{\mathbf{2}}{CHOCO_2H} + \underset{\mathbf{173}}{CH_2(NH_2)CO_2H} \xrightarrow[\text{pyridoxamine}]{Al^{3+}} \underset{\mathbf{174}}{(CO_2H)(OH)CHCH(NH_2)CO_2H}$$

Shemin and Herbst[315] showed that transamination reactions can be utilized to synthesize dipeptides, although more efficient procedures are now in use. Thus, alanylalanine (175) was synthesized from pyruvoylalanine.

$$CH_3COCONHCH(CH_3)CO_2H + \underset{\mathbf{170}}{C_6H_5CH(NH_2)CO_2H} \rightarrow \underset{\mathbf{175}}{CH_3CH(NH_2)CONHCH(CH_3)CO_2H} + \underset{\mathbf{28}}{C_6H_5CHO} + CO_2$$

I. Reductive Amination

It is well-known that many enzymes catalyze reductive amination of α-keto acids. Thus, L-glutamate dehydrogenase,[316] L-alanine dehydrogenase,[317] and L-leucine dehydrogenase[318] catalyze the reductive amination of α-ketoglutarate (5), pyruvate (3), and α-ketoisocaproate (45), respectively, to the corresponding L-amino acids.

α-Keto acids can be converted to stable derivatives of the type $RC(=NX)CO_2H$ (see section V). As early as 1910 it was recognized that such derivatives could be used to synthesize amino acids. Thus, Fischer and Groh showed that alanine (171) could be produced by

reduction of pyruvic acid phenylhydrazone (176) with aluminum amalgam.[319]

$$\underset{176}{CH_3C(=N\text{-}NHC_6H_5)CO_2H} \xrightarrow{[H]} \underset{171}{CH_3CH(NH_2)CO_2H}$$

Later, Knoop and Oesterlin[164] showed that α-keto acids could be converted to amino acids (131) (or *N*-methyl amino acids, 177) by catalytic reduction in the presence of ammonia (or methylamine).

$$\underset{1}{RCOCO_2H} + R'NH_2 \xrightarrow{[H]} \underset{\substack{131,\ R' = H\\ 177,\ R' = CH_3}}{RCH(NHR')CO_2H}$$

More recently, amino acids have been generated from α-keto acids by electrolytic reduction in aqueous ammonia solutions with Hg, Pt/C, or Pd/C electrodes.[320] A particularly useful mild reducing agent is sodium cyanoborohydride which was first described by Borch et al. in 1971.[321] These authors showed that pyruvate can be readily reduced to alanine in the presence of sodium cyanoborohydride and ammonia.[321]

N-Hydroxyglycine was first prepared in 1896.[322] Recently, however, more general methods for the mild reduction of α-keto acid oximes (178) to *N*-hydroxy amino acids (179) have been described.[323-325] Under

$$\underset{1}{RCOCO_2H} + NH_2OH \rightleftarrows \underset{178}{RC(=NOH)CO_2H} \xrightarrow{[H]}$$

$$\underset{179}{RCH(NHOH)CO_2H} \xrightarrow{[H]} \underset{131}{RCH(NH_2)CO_2H}$$

more rigorous conditions, the oximes are further reduced to amino acids (131).

Sodium cyanoborohydride is particularly effective in reducing α-keto acid oximes to the *N*-hydroxy compounds.[325] *N*-Hydroxy amino acids have been known for several years as components of various naturally occurring substances but have not yet been found free in nature (cf. discussion by Cooper and Griffith[325] and Møller and Conn[326]). The *N*-hydroxy amino acids inhibit pyridoxal 5′-phosphate-containing enzymes presumably via formation of a stable nitrone with the cofactor.[325] The *N*-hydroxy acids (179) are substrates of D- and L-amino acid oxidase.[325]

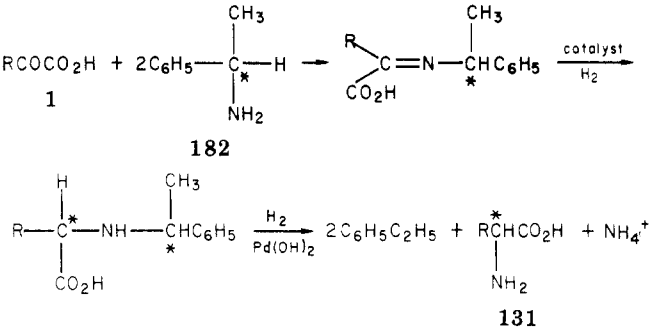
$$\underset{179}{RCH(NHOH)CO_2H} \xrightarrow{O_2} \underset{178}{RC(=NOH)CO_2H} + H_2O_2$$

α-Keto acid semicarbazones (180) are readily reduced to substituted semicarbazides (181) in the presence of Na/Hg catalyst.[213,327] (See also section VD.)

$$\underset{180}{RC(=NNHCONH_2)CO_2H} \xrightarrow{[H]} \underset{181}{RCH(NHNHCONH_2)CO_2H}$$

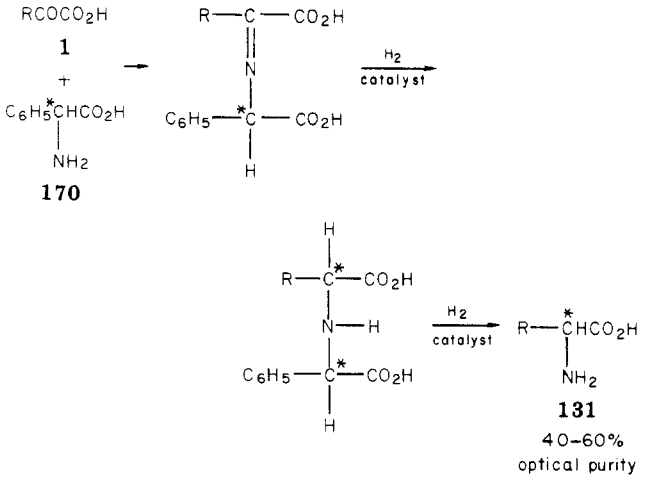
Towers et al. have investigated the suitability of using the reduction of various α-keto acid derivatives for the purpose of identifying α-keto acids in biological materials.[328] They concluded that, of those studied, 2,4-dinitrophenylhydrazones were the best derivatives for this purpose.[328] Several authors have also investigated the reductive amination of α-keto acid 2,4-dinitrophenylhydrazones to α-amino acids as a possible aid in the detection and estimation of α-keto acids.[3,38,329-332] Meister and Abendschein[3] have listed the hydrogenation products of over 30 α-keto acid 2,4-dinitrophenylhydrazones. In most cases, a single ninhydrin-positive spot corresponding to the amino acid analogue was noted. In some cases, where further reduction or hydrolysis is possible, more than one product was noted. Menckes noted that catalytic hydrogenation of β-phenylpyruvate 2,4-dinitrophenylhydrazone yielded some β-(cyclohexyl)alanine in addition to phenylalanine.[333] Catalytic hydrogenation of the β-(*p*-hydroxyphenyl)-pyruvate derivative yielded mostly β-(4-hydroxycyclohexyl)alanine, some tyrosine, and some β-(cyclohexyl)alanine.[333] In the case of 3-mercaptopyruvic acid 2,4-dinitrophenylhydrazone, only alanine was detected following catalytic reduction.[3,4]

Some workers have investigated the possibility of using reductive amination of α-keto acids to produce optically active α-amino acids. Thus, reduction of α-keto acids in the presence of L- or D-α-methylbenzylamine (182) results in amino acids (131) with varying degrees (56–91%) of optical purity.[334] The technique

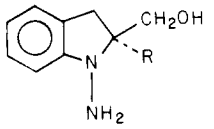


has been used to produce alanylalanine (175) from pyruvoylalanine with a fair degree of optical purity.[335] D-α-Methylbenzylamine yields predominantly a D-amino acid while L-α-methylbenzylamine yields predominantly an L-amino acid.[334]

Matsumoto and Harada[336] also showed that hydrogenation of D- and L-methyl- (and D- and L-ethyl-) benzylamines in the presence of α-keto acids results in an amino acid product with low to good optical purity (2–77%). Harada[337] has introduced a novel modification of the reductive amination technique which he describes as "hydrogenolytic asymmetric transamination", i.e., an optically active α-amino acid is used as nitrogen donor in place of an amine.

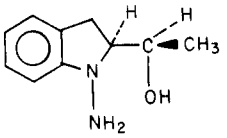


Corey et al. have obtained optical purities of an impressively high degree following reductive amination of α-keto acids.[338,339] These authors used the chiral reagents, *N*-amino-2-hydroxymethylindolines, (*S*)-9 (**183**) and (*S*)-16 (**184**).[338]



183, (*S*)-9, R = H
184, (*S*)-16, R = CH_3

With α-keto acids these compounds form hydrazino lactones which on reduction followed by, N–N hydrolysis and ester hydrolysis yield the chiral amino acid. Alanine and α-aminobutyrate were obtained with this method, in 80% and 90% optical purity, respectively. With another chiral reagent, 1-amino-2-(*S*)-[1(*R*)-hydroxyethyl]indoline (**185**), even greater optical purity was obtained.[339]



185

Thus, D-alanine, D-α-aminobutyrate, D-valine, and D-isoleucine were obtained in 96, 97, 97, and 99% optical purity, respectively.[339]

Model reactions of ferredoxin-dependent bacterial CO_2 fixation have been described which apparently involve an α-keto acid intermediate.[340]

$$CH_3C(O)SC_8H_{17} \xrightarrow[\text{Schrauzer's complex, } Na_2S_2O_4, NaHCO_3, \text{ in THF-MeOH-}H_2O]{CO_2}$$

$$CH_3C(O)CO_2H \xrightarrow[NH_4^+ \text{ or } Na_2S_2O_4]{\text{pyridoxamine, } Zn^{2+}} CH_3CH(NH_2)CO_2H$$
3 **131**

It has been shown that on heating glyoxylic acid (**2**) at pH 4.0 with ammonia (or methylamine) some *N*-oxalylglycine (**186**) (or *N*-oxalylsarcosine, **187**) is produced.[341] Similarly, pyruvic acid (**3**) and ammonia yield

$$2CHOCO_2H + NH_4^+ \rightarrow HO_2CCONHCH_2CO_2H$$
2 **186**, ~20%

$$2CHOCO_2H + CH_3NH_3^+ \rightarrow HO_2CCON(CH_3)CH_2CO_2H$$
2 **187**, ~12%

$$2CH_3COCO_2H + NH_4^+ \rightarrow CH_3CONHCH(CH_3)CO_2H + CO_2$$
3 **188**, ~40%

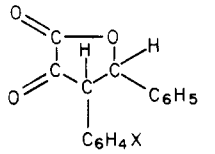
some *N*-acetylalanine (**188**).[341] Formal reductive amination of one carbonyl occurs.

J. Reactions with Aldehydes and Ketones

It has been noted above (section IVA) that α-keto acids can undergo aldol condensation with another molecule of α-keto acid. The condensation can also be brought about with aldehydes or ketones. Phenylpyruvic acid (**6**) forms a crystalline aldol product (**190**) with benzyl methyl ketone (**189**).[342] With benzaldehyde (**28**) (or *p*-substituted benzaldehydes), phenylpyruvic acid (**6**) (or ring-substituted analogues) yields a lactone (**191**).[191,343,344]

$$C_6H_5CH_2COCO_2H + C_6H_5CH_2COCH_3 \rightarrow C_6H_5CH_2C(OH)(CO_2H)CH_2COCH_2C_6H_5$$
6 **189** **190**

$$XC_6H_4CH_2COCO_2H + C_6H_5CHO \rightarrow$$
28



191

Refluxing of **190** in acetic acid/HCl yields the unsaturated lactone (**192**), which can be converted to benzaldehyde (**28**), unsaturated acid (**193**), or α,γ-diketo acid (**194**).[345] Phenylpyruvate (**6**) condenses with acetone in alkaline solution.[346] The product can be

$$C_6H_5C{=}CCH{=}C(CH_2C_6H_5)CO \quad (\textbf{192})$$

$$\xrightarrow{KMnO_4} C_6H_5CHO + \textbf{6}$$
28

$$\xrightarrow{KOH/EtOH} C_6H_5CH{=}C(CO_2H)CH_2COCH_2C_6H_5$$
193

$$\xrightarrow[NaHCO_3]{KMnO_4} C_6H_5CH_2COCH_2COCO_2H$$
194

$$C_6H_5CH_2COCO_2H + (CH_3)_2CO \rightarrow$$
6

$$C_6H_5CH_2C(CH_2COCH_3)(OH)CO_2H \xrightarrow[H^+]{-H_2O}$$

$$C_6H_5CH{=}C(CH_2COCH_3)CO_2H \xrightarrow{[O]}$$

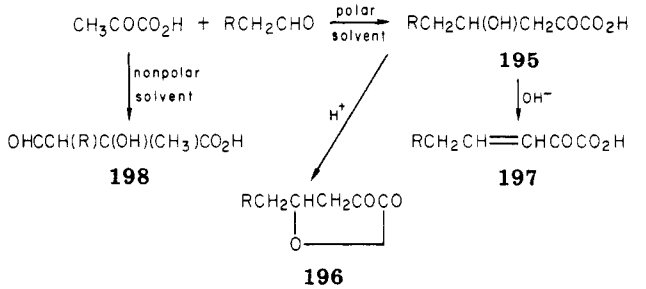
$$C_6H_5CH_2C(=CHCO_2H)CO_2H$$

readily converted to benzylmaleic acid by acid dehydration followed by oxidation with NaOCl or NaOBr.[346]

A similar reaction is the condensation of benzaldehyde (**28**) with pyruvic acid (**3**) to yield benzalpyruvic acid (**113**), a β,γ-unsaturated α-keto acid.[131,165,218-221,445]

$$C_6H_5CHO + CH_3COCO_2H \xrightarrow{-H_2O} C_6H_5CH{=}CHCOCO_2H$$
28 **3** **113**

Pyruvic acid (**3**) reacts with aliphatic aldehydes to



yield an α-keto-γ-hydroxy acid (**195**) that cyclizes to a lactone (**196**) in acid, but which dehydrates in alkali to an α-keto-β,γ-unsaturated acid (**197**). In a nonpolar anhydrous solvent, condensation occurs via the carbon adjacent to the carbonyl of the aldehyde to yield an α-hydroxy-α-methyl-β-formyl acid (**198**).[347]

A condensation-product lactone is produced when phenylpyruvate is allowed to react with formaldehyde.[19,128] Hift and Mahler describe an enzyme that they termed "formaldehyde–pyruvate carboligase". The enzyme catalyzes the addition of formaldehyde to pyruvate (**3**) to produce α-keto-γ-hydroxybutyrate (**199**) which can be isolated as the enol lactone (**200**).[128]

$$\underset{\mathbf{3}}{CH_3COCO_2H} + HCHO \rightarrow \underset{\mathbf{199}}{HOCH_2CH_2COCO_2H} \rightarrow \underset{\mathbf{200}}{CH_2\text{-}CH{=}C(OH)\text{-}CO\text{-}O}$$

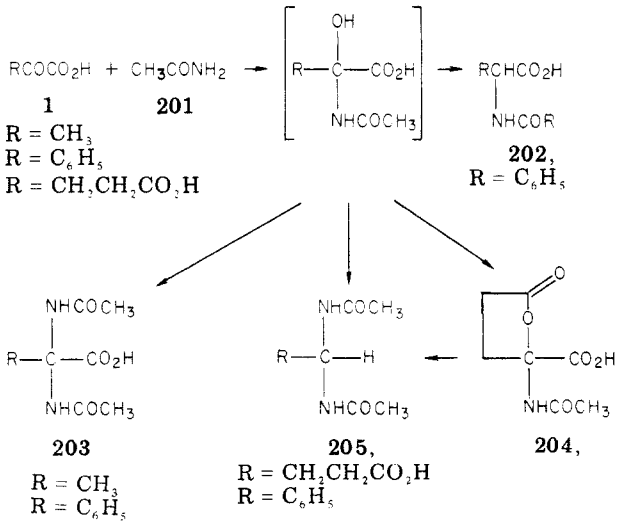
If pyruvate is allowed to react with formaldehyde in H_2SO_4/HAc, at least seven products are detectable, including mono-, di-, and tricyclic lactones.[348]

Glyoxylate (**2**) reacts with acetophenone (or its analogues) to yield 2,2-disubstituted acetic acids.[349]

$$XC_6H_4COCH_3 + \underset{\mathbf{2}}{CHOCO_2H} \xrightarrow{OH^-} (XC_6H_4COCH_2)_2CHCO_2H$$

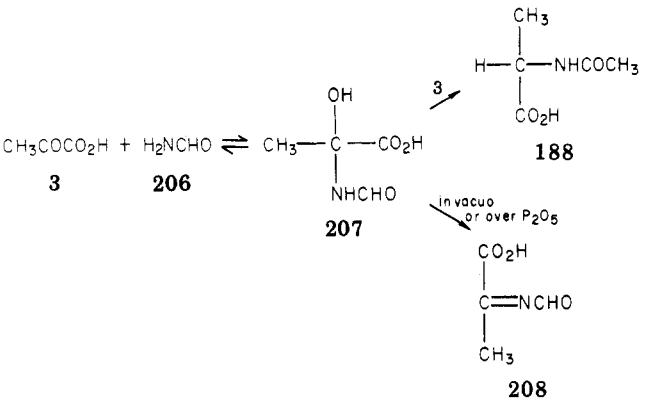
K. Reactions with Amides

Although, amide nitrogen is generally unreactive, it can add to carbonyl carbon under certain conditions. The reaction of pyruvic (**3**), α-ketoglutaric (**5**), and phenylglyoxylic (**17**) acids with acetamide (**201**) has been investigated.[350] The results are summarized as follows:



When R = C_6H_5 the main product was α,α-diacetamidophenylacetic acid (**203**, R = C_6H_5) with smaller amounts of benzylidenediacetamide (**205**, R = C_6H_5) and α-benzylaminophenylacetic acid (**202**, R = C_6H_5); when R = CH_3, the main product was α,α-diacetamidopropionic acid (**203**, R = CH_3); when R = $CH_2CH_2CO_2H$ the main product was the lactone (**204**) or on further reaction—decarboxylation, γ,γ-diacetamidobutyric acid (**205**, R = $CH_2CH_2CO_2H$).[350]

Formamide (**206**) condenses with pyruvic acid (**3**) to yield α-hydroxy α-formamidopropionic acid (**207**) which on standing or on reacting with another mol of pyruvic acid (**3**) yields *N*-acetylalanine (**188**).[351] Storage of **207**



in vacuo or over P_2O_5 yields α-formimidopropionic acid (**208**).[351]

Martell and Herbst showed that benzyl carbamate (**209**) can be condensed with α-keto acids.[352] The resulting dicarbamate (**210**) can be reduced to an amino acid (**131**).[352] The method was successful in synthesizing alanine (**131**, R = CH_3), β-phenylalanine (**131**, R = $C_6H_5CH_2$), but not glutamate (**131**, R = $CH_2CH_2CO_2H$).[352]

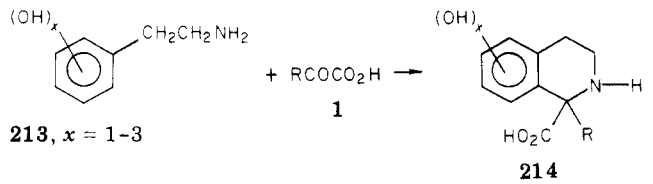
$$\underset{\mathbf{1}}{RCOCO_2H} + \underset{\mathbf{209}}{2C_6H_5CH_2OCONH_2} \xrightarrow{-H_2O} \underset{\mathbf{210}}{RC(NHOCOCH_2C_6H_5)_2CO_2H} \xrightarrow{[H]} \underset{\mathbf{131}}{RCH(NH_2)CO_2H}$$

L. Condensation with Aromatic Amines

Aromatic amines with unreactive hydrogens in the ring (e.g., phenethylamine, **211**) condense with α-keto acids followed by decarboxylation to yield the imine (**212**).[353]

$$\underset{\mathbf{211}}{RCH_2COCO_2H} + C_6H_5CH_2CH_2NH_2 \rightarrow C_6H_5CH_2CH_2NHC(OH)(CO_2H)CH_2R \rightarrow C_6H_5CH_2CH_2N{=}C(CO_2H)CH_2R \xrightarrow{\text{spontaneous}} \underset{\mathbf{212}}{C_6H_5CH_2CH_2N{=}CHCH_2R}$$

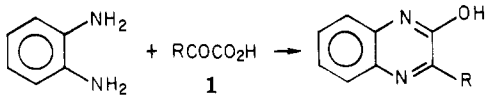
However, phenylethylamines with active ring hydrogens (**213**) yield tetrahydroisoquinoline-1-carboxylic acids (**214**) (via a Bischler–Napieralski reaction) if the α-keto acid contains enolizable hydrogens.[353]



α-Keto acids that are not capable of enolizing (e.g. triphenylpyruvic, **1**, R = $(C_6H_5)_3C$; phenylglyoxylic, **17**) do not react. Tryptamine also reacts with α-keto acids in a similar fashion to phenylethylamine.[353]

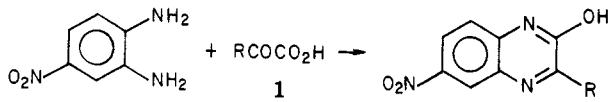
It has been known since 1887 that α-keto acids con-

dense with *o*-phenylenediamine (**215**) to yield very stable quinoxalinols (**216**).[354]



215 + RCOCO$_2$H (**1**) → **216**

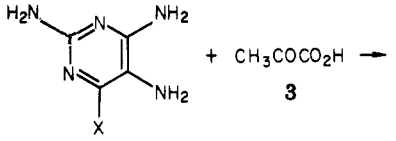
This reaction has recently become very important for the estimation of α-keto acids. Wieland and Fischer[355] showed that α-keto acids could be detected as fluorescent spots on paper chromatograms by spraying with *o*-phenylenediamine reagent. Alternatively, the α-keto acids may be derivatized prior to chromatography.[356,357] Other workers have used the 4-nitro derivative (**217**)[358,359] which produces highly colored products (**218**) that can be separated by paper chromatography or on alumina columns.



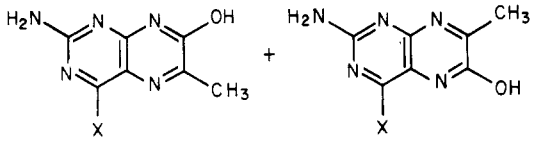
217 + RCOCO$_2$H (**1**) → **218**

Mowbray and Ottaway have investigated the quinoxalinol formation between α-keto acids and *o*-phenylenediamine (or the 4-chloro or 4-fluoro analogue).[360] A sensitive gas-chromatography method for the estimation of α-keto acids based on the detection of the trimethylsilyl(TMS)quinoxalinol derivative is now used by several laboratories (see section VII).

A similar reaction to the quinoxalinol reaction is the formation of 6- and 7- hydroxypteridines (**220, 221**) from 4,5-diaminopyrimidines (**219a,b**).[361] The pro-



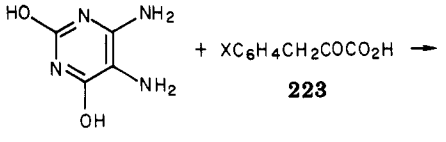
219a, X = OH
219b, X = NH$_2$ + CH$_3$COCO$_2$H (**3**) →



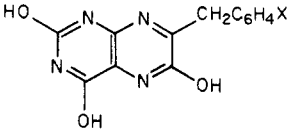
220 + **221**

portion of 6- and 7-positional isomers depends on pH; for example **220** is strongly favored in 1 M H_2SO_4.[361]

Ring-substituted phenylpyruvic acids (**223**) react with 2,6-dihydroxy-4,5-diaminopyrimidine (**222**) to form 2,4,6-trihydroxy-7-benzylpteridines (**224**) of high



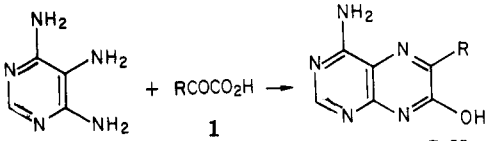
222 + XC$_6$H$_4$CH$_2$COCO$_2$H (**223**) →



224

melting point in 70–75% yield.[362]

Fissekis et al. have shown that cycloalkylglyoxylic acids (C_6 and C_5 rings) readily react with 4,5,6-triaminopyrimidine (**225**) in strong acid to yield 4-amino-6-cycloalkyl-7-hydroxypteridines (**226a,b**).[88]

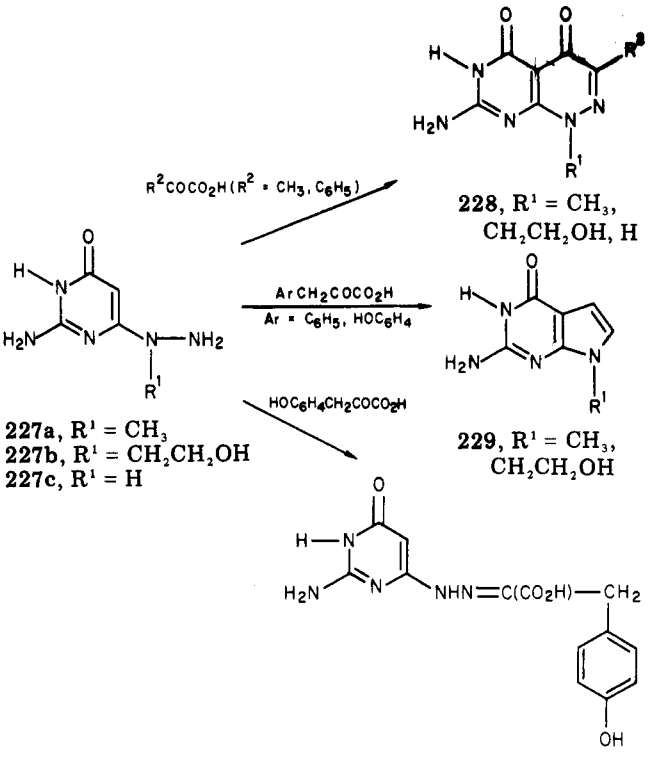


225 + RCOCO$_2$H (**1**) → **226a**, R = C_5H_9; **226b**, R = C_6H_{11}

R = C_5H_9
R = C_6H_{11}

These compounds have some bacteriocidal properties. If the reaction is carried out at pH 5.0, the major product is the 7-cycloalkyl-6-hydroxy product.

α-Keto acids and their esters can also react with 6-(1-alkylhydrazino)isocytosines (**227a,b,c**) to yield bicyclic compounds.[363] A summary of the findings is as follows.



227a, R^1 = CH$_3$
227b, R^1 = CH$_2$CH$_2$OH
227c, R^1 = H

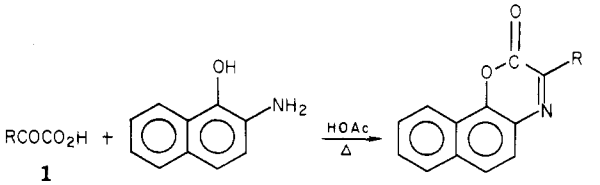
228, R^1 = CH$_3$, CH$_2$CH$_2$OH, H

229, R^1 = CH$_3$, CH$_2$CH$_2$OH

230, R = H

Depending on R^1 and α-keto acid the product is a pyrimido[4,5-*c*]pyridazine-4,5(1*H*,6*H*)-dione (**228**), a pyrrolo[2,3-*d*]pyrimidine (**229**), or a 6-hydrazinoisocytosine hydrazone (**230**). The formation of the pyrrolopyrimidine (**229**) is interesting in that the reaction requires a rapid decarboxylation, loss of nitrogen, and rearrangement; the mechanism is unknown.[363]

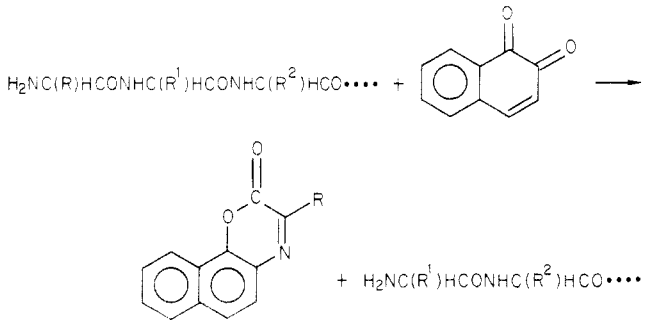
A very recently described reaction that promises to be highly sensitive for the estimation of α-keto acids is the formation of naphtho[1,2-*b*]oxazin-6-ones (**232**)



RCOCO$_2$H (**1**) + **231** → **232**

from 2-amino-1-naphthol (**231**).[364] The [2,1-*b*] isomer (prepared from 1-amino-2-naphthol) appears to be equally promising.[364] When fluorescence techniques and a variety of chromatographic procedures were used picomolar amounts of α-keto acid (**1**) were detected.

The discovery of these derivatives resulted from the development of a sensitive probe for electron impact fragmentation analysis of peptides with *o*-naphthoquinone.[365]



232

M. Other Reactions

α-Keto acids produce characteristic (although usually unstable) colors with dilute ferric chloride solutions. Those acids with aliphatic side groups, such as pyruvic (**3**) and α-ketobutyric (**8**), exhibit yellow color, but other α-keto acids, such as α-keto-γ-(methylthio)butyric (**40**)[104] and phenylpyruvic (**16**),[366] exhibit different colors. This property has occasionally been used to estimate certain transaminase reactions.[104,367] Since many ketones form stable bisulfite addition compounds it has been assumed that α-keto acids also behave similarly,[2] but we have found no systematic study. Occasionally, bisulfite addition compounds have been used to remove carbonyl-containing compounds from organic solvents, and Schreiber has shown that α-ketoheptadecanoic acid (**1**, R = n-$C_{15}H_{31}$) and α-ketononadecanoic acid (**1**, R = n-$C_{17}H_{35}$) can be removed from ether solution as the corresponding bisulfite addition product.[21] α-Keto acids reduce ammoniacal silver nitrate solutions.[2] A number of α-keto acids, particularly aromatic α-keto acids, can be readily halogenated. For example, β-bromo-β-phenylpyruvic acid is readily prepared from β-phenylpyruvic acid,[144] and Reimer et al. have shown that benzilidenepyruvates are readily brominated.[221] Schellenberger et al. have studied the halogenation of several α-keto acids and found that the reaction is accelerated by several metal ions with Al^{3+} the most effective.[368]

The addition of benzenes to the α-carbon of pyruvic acid (**3**) was first noted by Böttinger a hundred years ago.[369] Later, Bistrzycki and Mauron showed that various benzene derivatives react with phenylpyruvic acid (**6**) in cold concentrated sulfuric acid to yield the corresponding α,α-diaryl-β-phenylpropionic acids (**233a–c**); under certain conditions **233** was found to lose CO.[370]

$$\underset{}{ArH} + \underset{6}{C_6H_5CH_2COCO_2H} \rightarrow \underset{\begin{array}{l}\textbf{233a},\ Ar = p\text{-}CH_3C_6H_4\\ \textbf{233b},\ Ar = p\text{-}C_2H_5C_6H_4\\ \textbf{233c},\ Ar = 2,4\text{-}(CH_3)_2C_6H_3\end{array}}{C_6H_5CH_2C(Ar)_2CO_2H}$$

Wegmann and Dahn found that benzene can add to pyruvic acid (**3**) in cold benzene via a two-step mechanism.[371] With phenylpyruvic acid (**6**), the reaction was

$$\underset{3}{CH_3COCO_2H} + C_6H_6 \xrightarrow{AlCl_3}$$

$$CH_3C(OH)(C_6H_5)CO_2H \xrightarrow[C_6H_6]{AlCl_3} CH_3C(C_6H_5)_2CO_2H$$

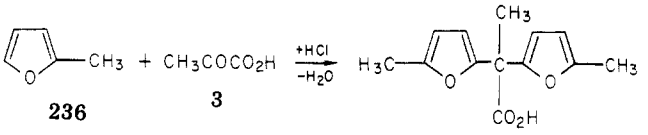
more complex but some α,β,β-triphenylpropionic acid was formed.

Patai and Dayagi found that the diaryl addition compounds with pyruvate (**234**) can be dehydrated and decarbonylated (cf. ref 370) to yield an olefin (**235**).[372]

$$\underset{3}{CH_3COCO_2H} + 2ArH \rightarrow \underset{234}{CH_3C(Ar)_2CO_2H} \rightarrow \underset{235}{Ar_2C{=}CH_2} + H_2O + CO$$

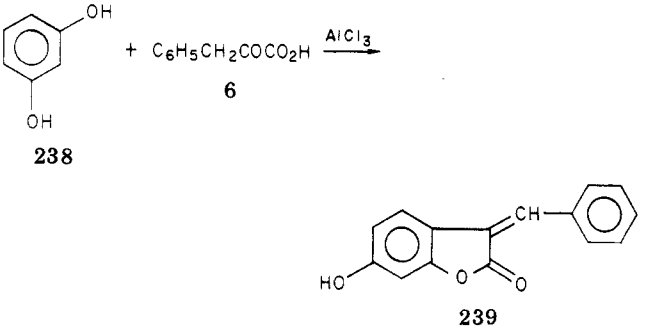
This reaction is very useful for the preparation of 1,1-diarylethylenes and in some cases is the preferred procedure. The mechanism is thought to be an electrophilic aromatic substitution.[372]

A related reaction is the addition of sylvan (2-methylfuran) (**236**) to pyruvic acid (**3**) to yield α,α-bis(5-methyl-2-furyl)propionic acid (**237**).[373]



236 3 237

Phenylpyruvic acid (**6**) can be made to react with resorcinol (**238**) to yield 6-hydroxy-3-benzilidene-2-(3*H*)-benzofuranone (**239**).[374] The applicability of this



238 6 239

reaction to other α-keto acids is unknown.

α-Keto acids react with benzyl cyanide (**240**) to yield addition compounds (**241**).[375] These compounds may

$$\underset{240}{C_6H_5CH_2CN} + \underset{1}{RCOCO_2H} \rightarrow \underset{241}{C_6H_5(CN)CHCR(OH)CO_2H} \rightarrow \text{unsaturated compounds}$$

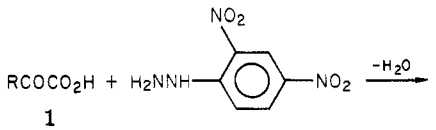
be used to prepare a number of derivatives (e.g., half amide of substituted malonic acids, substituted maleic anhydrides, etc.).[375]

V. Derivatives

A. 2,4-Dinitrophenylhydrazones

As with other compounds containing a reactive carbonyl, α-keto acids form a large number of crystalline

derivatives. Certainly, the most useful derivatives have been the 2,4-dinitrophenylhydrazones (242). However,



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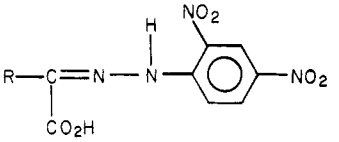
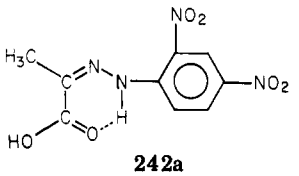


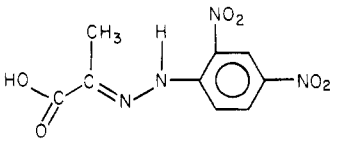
242

phenylhydrazones, *p*-nitrophenylhydrazones, oximes, and semicarbazones have also been useful at times. The melting point of the 2,4-dinitrophenylhydrazones where known is given in Table I.

The 2,4-dinitrophenylhydrazones can theoretically exist in cis and trans forms,[376] and, indeed, crystallization from different solvents sometimes yields different products. For example, crystallization of the phenylpyruvic acid derivative from water or alcohol yields a product with a melting point of 192–193 °C, whereas crystallization from ethyl acetate or petroleum ether yields a product with a melting point of 160–162 °C.[377] Cavallini et al. first suggested in 1949 that paper chromatography of 2,4-dinitrophenylhydrazone derivatives could be useful in identifying α-keto acids in biological samples.[378] Since that time, several systems for the identification and semiquantitative analysis of α-keto acids by paper or thin layer chromatography have been published.[3,329-332,379-385] However, glyoxylic (2), pyruvic (3), oxaloacetic (4), and α-ketobutyric acid 2,4-dinitrophenylhydrazones were all shown to yield two spots (only the α-ketoglutarate (5) derivative consistently yielded a single spot).[379-381] Stewart suggested that the two spots found with the glyoxylate and α-ketobutyrate derivatives were due to cis and trans isomers.[380] Later, Isherwood and Jones isolated two forms of the 2,4-dinitrophenylhydrazone of pyruvic acid which, from infrared evidence, they regarded as cis (**242a**) and trans (**242b**) forms.[386]



242a

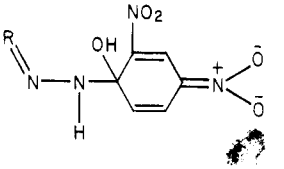


242b

The cis form melts at 208 °C (decomp) whereas the trans form melts at 210 °C (decomp); **242a** yields an intense color in base, while **242b** does not.[386] Similarly, cis (mp 145 °C decomp) and trans (mp 215 °C) forms of α-ketoglutaric acid 2,4-dinitrophenylhydrazones were also prepared.[386] Katsuki et al. have developed a specific color test for the cis isomer; if zinc powder is added to $NiCl_2$ in 70% aqueous ethanol and the *cis*-α-keto acid 2,4-dinitrophenylhydrazone is present, a red color develops. No red color develops with the trans isomer.[387]

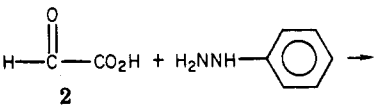
The kinetics of the isomerization reaction of *cis*-α-keto acid 2,4-dinitrophenylhydrazones have been investigated.[388]

The 2,4-dinitrophenylhydrazones are generally stable in acid and, as noted above, yield intensely colored products in base. This color reaction is very useful for the estimation of α-keto acids (see, for example Lu[389] and Friedemann[390]). According to Isherwood and Jones the species responsible for the color in base is probably a quinoid type structure.[386]



B. Other Phenylhydrazones

p-Nitrophenylhydrazones[390] and phenylhydrazones[319] of α-keto acids have been known for about 70 years. It is worth pointing out that glyoxylic acid phenylhydrazone (**243**) is oxidized to a highly colored product with potassium ferricyanide in the presence of excess phenylhydrazine.[392] This reaction is not shown by other α-keto acids and is thus useful for estimation of glyoxylic acid (2). The colored product is now known to be 1,5-diphenylformazan (**244**).[392]



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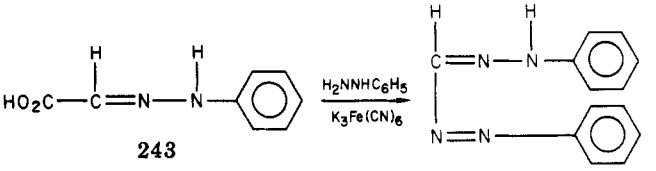
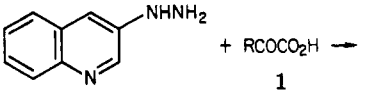


243

244

Robins et al. have shown that 3-quinolylhydrazine (**245**) forms stable hydrazones (**246**) with α-keto acids that exhibit intense absorbance in the UV region.[393]



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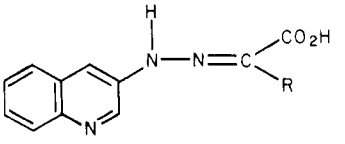
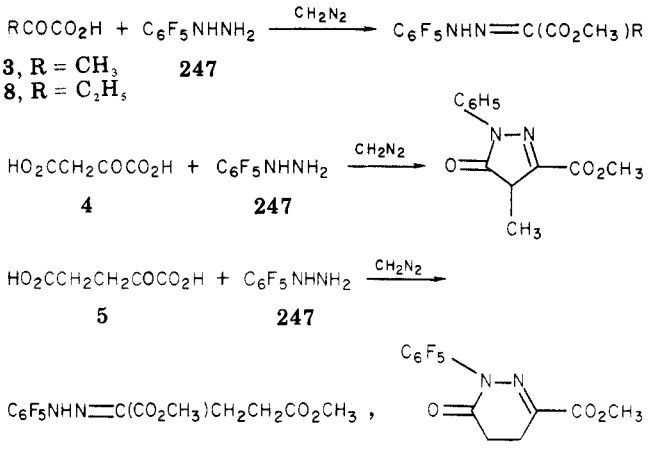


246

This property allows α-keto acids to be detected even in the presence of other carbonyl-containing compounds. Other carbonyl hydrazones absorb at lower wavelengths. This absorption disappears with time.[393]

Ohmori et al. have recently introduced a GC method for determination of α-keto acids based on the reaction with pentafluorophenylhydrazine (**247**) followed by reaction with diazomethane to produce the methyl ester.[394] Pyruvic acid (3) and α-ketobutyric acid (8) yielded the expected cis and trans products but, oxaloacetic acid (4) and α-ketoglutaric acid (5) yielded cyclic products.[394] In the case of oxaloacetic acid, the



product also contained an additional methyl group on the ring.

C. Oximes

α-Keto acids form characteristic crystalline oximes (also known as α-oximino acids (248)) with hydroxylamine; in a few cases, the two geometrical isomers of 248 have been separated and characterized.[395]

$$RCOCO_2H + H_2NOH \rightarrow \underset{248}{RC(=NOH)CO_2H}$$

α-Keto acid oximes have been extensively reviewed by Ahmad and Spenser;[395] so, only a few points will be made here. As noted in section IVI these derivatives are converted to *N*-hydroxy amino acids on mild reduction. The oximes are moderately stable in aqueous solution, particularly under alkaline conditions. With hydrogen peroxide (pH > 4.0 < 6.0), oximes (248) are converted to hydroxamic acids (249), but the reaction does not appear to have been well characterized.[395]

$$\underset{248}{RC(=NOH)CO_2H} \xrightarrow{H_2O_2} \underset{249}{RC(O)NHOH} \xrightarrow{O_2} NO_2^-$$

Under vigorous conditions, oxidation of oxime (248) yields nitrite.[395]

Hydroxamic acids (249) are somewhat unstable. In base they undergo Lossen rearrangement to alkyl isocyanates (RC=C=O).[395] Under carefully controlled, anaerobic conditions, α-keto acid oximes (248) when heated give rise to alkyl cyanides and this appears to be a general reaction.[395]

$$\underset{248}{RC(=NOH)CO_2H} \rightarrow RC{\equiv}N + CO_2 + H_2O$$

In aqueous solution, the reaction appears to involve a trans elimination; however, pyrolytic cleavage is thought to proceed via a cis cyclic internal structure.[395] The production of cyanide via 248 may be of biological importance. Thus, *Chlorella* and spinach leaves have recently been shown to possess an enzyme that converts glyoxylate oxime (248, R = H) to hydrogen cyanide.[396] It is also of interest that, in addition to α-oximino acids, the corresponding α-imino acid ($RCH_2C(=NH)CO_2H$) may also act as a precursor of HCN under certain conditions. Thus, aromatic amino acids (and especially histidine) when incubated in the presence of L-amino acid oxidase and horseradish peroxidase yield HCN in addition to the expected α-keto acid.[397] The reaction is complex but may be formally expressed as

$$RCH_2C(=NH)CO_2H + H_2O_2 + 2O_2 \rightarrow RCHO + HCN + CO_2 + 2O_2^{-}\cdot + H_2O + 2H^+$$

D. Semicarbazones, Thiosemicarbazones, and Related Derivatives

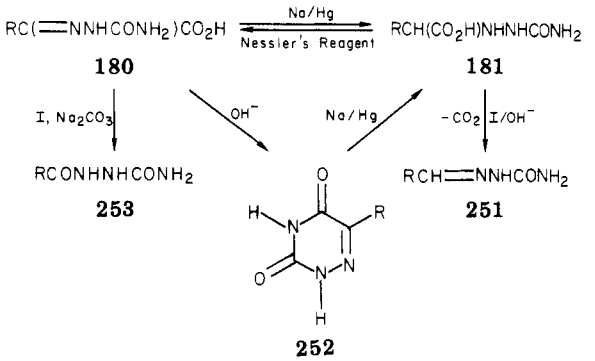
With semicarbazide (250) α-keto acids (1) form well-defined semicarbazones (180).

$$\underset{1}{RCOCO_2H} + \underset{250}{NH_2NHCONH_2} \underset{+H_2O}{\overset{-H_2O}{\rightleftharpoons}} \underset{180}{RC(=NNHCONH_2)CO_2H}$$

The mechanism of this reaction with various carbonyl-containing compounds has been extensively studied.[398,399] The reaction with α-keto acids is general acid catalyzed, and the rate of semicarbazone formation (a complicated function of pH), reaches a maximum at slightly acidic pH values.[400] At pH close to neutral, equilibrium lies far to the right but in strong acid, equilibrium moves far to the left. Since most of the α-keto acid semicarbazones exhibit strong UV absorption ($\lambda_{max} \cong 255$–260 nm; $\epsilon_{max} = 1$–1.1×10^4) they have occasionally been employed in biochemical studies. For example, Olson developed a technique in which the product of the isocitrate lyase reaction was trapped as the semicarbazone.[400] Hafner and Wellner found that semicarbazide is a useful reagent for following the course of the L-amino acid oxidase reaction.[401] Thus, at pH 7.0, semicarbazide (250) reacts more rapidly by a factor of $\sim 10^4$ with the imine derived from oxidation of an L-amino acid (132) than does the corresponding α-keto acid.

$$\underset{132}{RC(=NH)CO_2H} + \underset{250}{NH_2NHCONH_2} \xrightarrow{-NH_3} \underset{180}{RC(=NNHCONH_2)CO_2H}$$

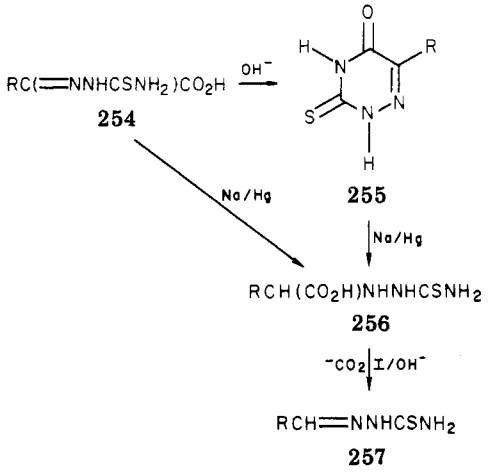
The semicarbazones (180) are readily reduced to the corresponding semicarbazide (181) which in turn may be (a) converted to a semicarbazone of an aldehyde (251) or (b) converted back to 180 with Nessler's reagent.[213,327] α-Keto acid semicarbazones (180) readily cyclize to diketotriazines (252) in base.[213,327] (The diketotriazines have also been called 6-alkyl-*as*-triazine-3,5-diones and 6-alkyl-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazines). The α-keto acid semicarbazone (180) is also readily converted to an acylsemicarbazide (253).[402]



The cyclization reaction (180 → 252) has received much attention since the discovery that 6-azauracil (252,

R = H) has antitumor and narcotic activity. 6-Azathymine (**252**, R = CH_3) is even more potent. (See references quoted by Chang[403] and Tišler and Vrbaški).[404]

Chang prepared the α-keto acid semicarbazones of oxaloacetic (**4**), α-ketoglutaric (**5**), α-ketovaleric (**18**), α-ketoheptanoic (**1**, R = $CH_3(CH_3)_3CH_2$), α-ketononanoic (**92**), and phenylpyruvic acid (**6**).[403] He showed that the corresponding crystalline 5-substituted-6-azauracil (**252**, R = CH_3, C_2H_5, C_3H_7) could be obtained in moderate yield (51–63%) by treatment with ethylene glycol, sodium, and ethanol.[403] α-Keto acid thiosemicarbazones (**254**) readily cyclize to yield 3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazines (**255**); **254** and **255** are also readily converted to thiosemicarbazide (**256**) and thiosemicarbazone of an aldehyde (**257**).[327,405]



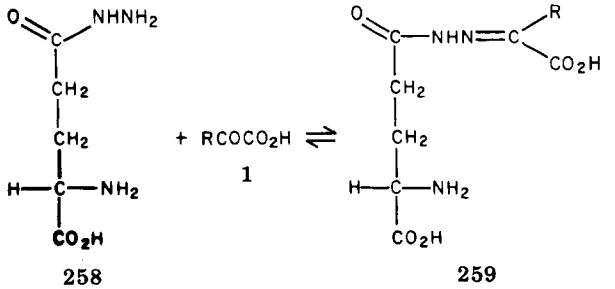
With appropriate substitution on the semicarbazide precursor, 2,6-, 4,6-, or 2,4,6-substituted-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazines may be prepared.[402] The 2,6- and 4,5- and 2,4,6-substituted thio analogues may also be prepared in a similar fashion. (See references quoted by Tišler and Vrbaški[404].)

Although the cyclic forms of α-keto acid semicarbazones (and thiosemicarbazones) have been known for more than 70 years, there has been controversy about the exact structure. Mono- and dienol structures have been postulated, but more recent evidence suggests that the dioxo (i.e., **252**) and the oxo–thioxo (i.e., **255**) forms are the preferred forms.[404]

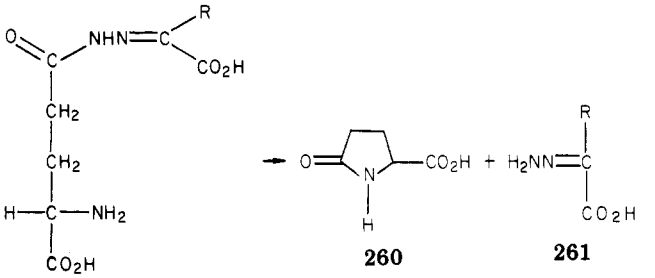
Semicarbazones have occasionally been used for detection of α-keto acids. Thus, the 4-(*p*-azophenylphenyl)thiosemicarbazone and 4-(*p*-azophenylphenyl) semicarbazone derivatives of α-keto acids have been used in paper chromatography studies.[406]

E. γ-Glutamylhydrazones

α-Keto acids form readily crystallizable γ-glutamylhydrazones (**259**) with γ-glutamylhydrazide (**258**).[407]



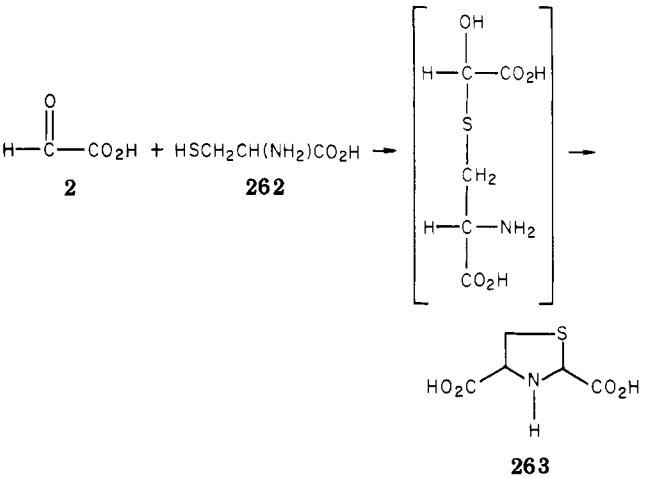
The reaction is reversible in strong acid. The derivatives have characteristic melting points and are readily separable on paper chromatography. The derivatives can be detected on paper with ninhydrin or by quenching under UV light.[407] In aqueous solution, the compounds slowly break down to 5-oxoproline (2-pyrrolidone-5-carboxylic acid, pyroglutamic acid; **260**) and corresponding α-keto acid hydrazone (**261**).[408]



γ-Glutamylhydrazones are substrates of L-amino acid oxidase and of rat liver glutamine transaminase,[407,408] and are inhibitors of γ-glutamyl transpeptidase.[409]

F. Addition Compounds with Thiols

It is well-known that sulfhydryl-containing compounds can add to carbonyl groups to form hemithioketals (–S–C(<)–OH). In some cases, α-keto acid hemithioketals can be isolated. Glyoxylic acid (**2**) reacts with cysteine (**262**) to yield thiazolidine-2,4-dicarboxylic acid (**263**).[410] Pyruvic acid (**3**)[411] and longer chain α-



keto acids[118] yield the corresponding hemithioketals (**264**).

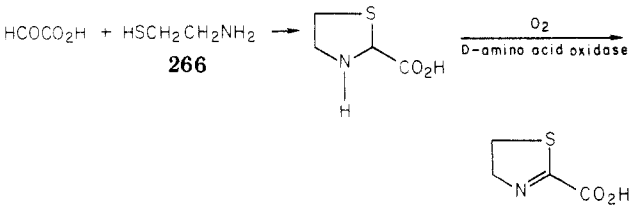
$$\underset{\mathbf{1}}{RCOCO_2H} + \underset{\mathbf{262}}{HSCH_2CH(NH_2)CO_2H} \rightarrow \underset{\mathbf{264}}{RC(OH)(CO_2H)SCH_2CH(NH_2)CO_2H}$$

Glyoxylic acid homocysteine hemithioacetal and pyruvic acid homocysteine hemithioketal have been crystallized and characterized.[63] α-Keto acid cysteine (and homocysteine) hemithioketals are readily separable by paper chromatography and are readily detected with ninhydrin.[63,118]

Many acyclic –SH addition compounds (**265**) with glyoxylic acid are substrates of L-α-hydroxy acid oxidase.[412]

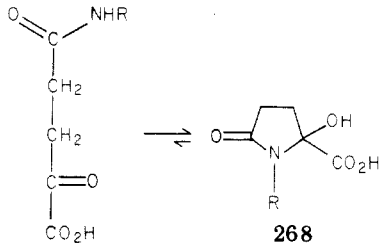
$$\underset{\mathbf{265}}{RSCH(OH)CO_2H} \rightarrow RSCOCO_2H$$

On the other hand, when cyclization occurs (e.g., with cysteamine, **266**) the product may be a substrate of D-amino acid oxidase.[413,414]



VI. α-Keto Acids with Unusual Properties

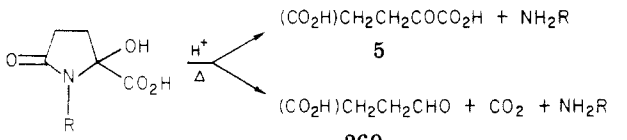
α-Ketoglutaramic acid (**267**, R = H) and *N*-methyl-α-ketoglutaramic acid (**267**, R = CH_3), the α-keto acid analogue of glutamine and glutamic acid γ-methylamide, respectively, exist overwhelmingly in the corresponding cyclic lactam forms (**268**).[61,105,106,415]



267, R = H, Me

268

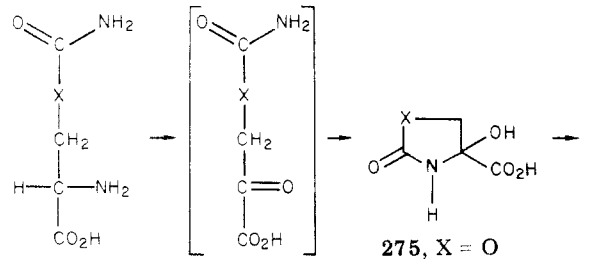
α-Ketoglutaramic acid slowly forms a 2,4-dinitrophenylhydrazone, whereas its *N*-methyl derivative does not form a hydrazone.[106] Acid hydrolysis of α-ketoglutaramic acid (or its methyl analogue) yields a mixture of α-ketoglutaric acid (**5**) and succinic semialdehyde (**269**); the mechanism of this reaction has been extensively studied.[415]



268, R = H, Me

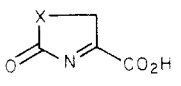
269

The glutamine analogues, L-albizziin (**270**), *S*-carbamyl-L-cysteine (**271**), and *O*-carbamyl-L-serine (**272**) are substrates of glutamine transaminase and of L-amino acid oxidase, but the α-keto acids cannot be isolated because they readily cyclize to 2-oxo-



270, X = NH
271, X = S
272, X = O

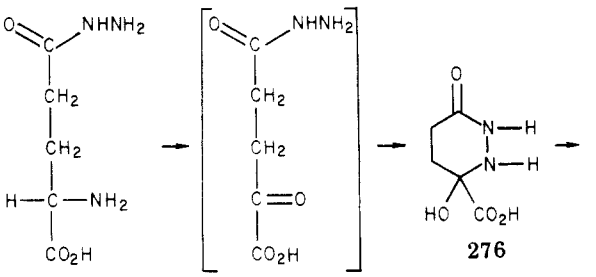
275, X = O



273, X = NH
274, X = S

imidazoline-4-carboxylic acid (**273**), 2-oxothiazoline-4-carboxylic acid (**274**), and 4-hydroxy-2-oxooxazolidine-4-carboxylic acid (**275**), respectively.[416] The mechanisms involved have been investigated.[416]

Enzymatic oxidation of γ-glutamylhydrazide (**258**) to the corresponding α-keto acid results in formation of a cyclic structure with an internal hydrazone linkage, i.e., 1,4,5,6-tetrahydro-6-oxopyridazine-3-carboxylic acid (**277**).[61,407] The reaction appears to proceed via 3-



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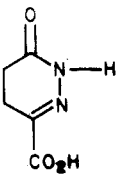
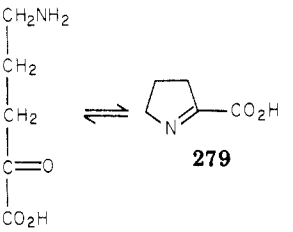
276



277

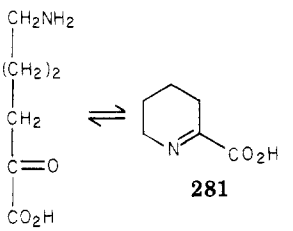
hydroxytetrahydro-6-oxopyridazine-3-carboxylic acid (**276**).[407]

The α-keto acid analogue of ornithine (α-keto-δ-aminovaleric acid, **278**) forms a 2,4-dinitrophenylhydrazone, yet is reduced to proline.[147-149] It may



278

279

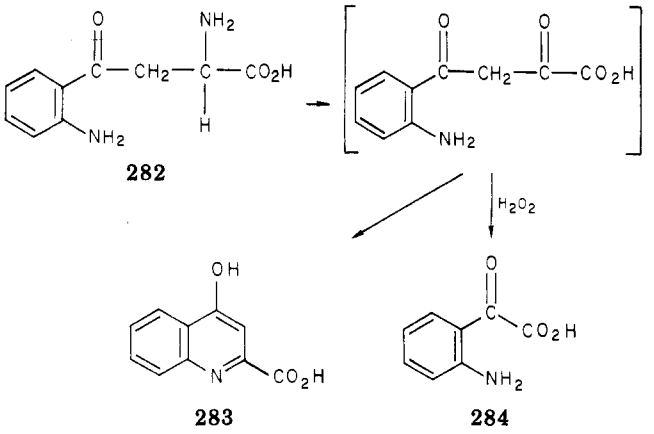


280

281

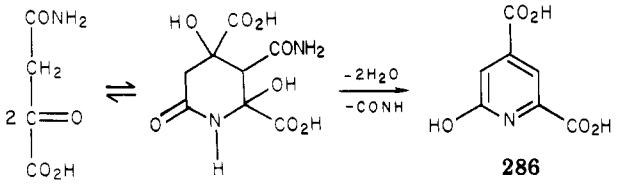
therefore be concluded that the α-keto acid is in equilibrium with Δ^1-pyrroline-2-carboxylic acid (**279**).[147-149] Similarly, the α-keto acid analogue of lysine (α-keto-ε-aminocaproic acid, **280**) is in equilibrium with Δ^1-piperideine-2-carboxylic acid (**281**).[149]

Interestingly, kynurenine (**282**) can undergo enzymatic transamination and oxidation, but the corresponding α-keto acid cannot be isolated due to formation of kynurenic acid (**283**). If the L-amino acid oxidase reaction is carried out in the presence of H_2O_2, *o*-aminophenylglyoxylic acid (**284**) is produced, suggesting that the free α-keto acid is an intermediate.[149]



282 283 284

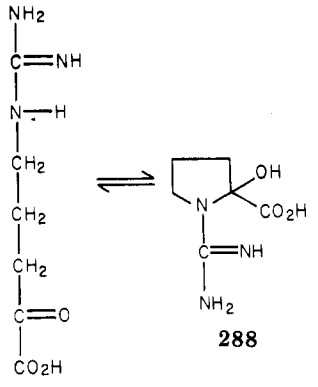
α-Ketosuccinamic acid (148) may exist in an alternative dimeric form which is unreactive to carbonyl reagents.[61,106] The unreactive dimer has been shown to be 5-carbamoyl-4,6-dihydroxy-2-oxopiperidine-4,6-dicarboxylic acid (285).[417] The dimer is readily converted



148 285 286

to 2-hydroxypyridine-4,6-dicarboxylic acid (286).

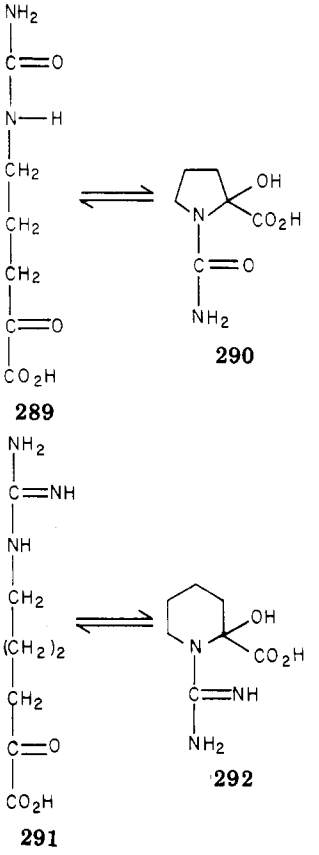
The α-keto acid analogue of arginine (α-keto-δ-guanidinovaleric acid, 287), citrulline (α-keto-δ-carbamidovaleric acid, 289), and homoarginine (α-keto-ε-guanidinocaproic acid, 291) have also recently been shown to exist in equilibrium with their respective cyclic forms: 1-amidino-2-hydroxypyrrolidine-2-carboxylic acid (288), 1-carbamyl-2-hydroxypyrrolidine-2-carboxylic acid (290), and 1-amidino-2-hydroxypiperidine-2-carboxylic acid (292).[152]



287 288

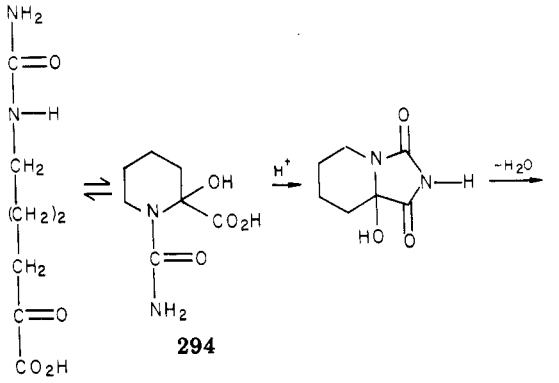
The cyclization reactions explain the previous observations that 287 and 289 react more slowly with 3-quinolylhydrazine[393] and with γ-glutamylhydrazide[407] than acyclic α-keto acids at comparable concentrations.

The α-keto acid analogue of homocitrulline (α-keto-ε-carbamidocaproic acid, 293) cyclizes to 1-carbamyl-2-hydroxypiperidine-2-carboxylic acid (294), but further ring closure and dehydration occurs so that the isolated

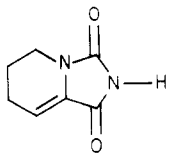


289 290 291 292

product is 2*H*,5*H*,7*H*-imidazo[1,5-*a*]pyridine-1,3-dione (295).[152,418]



293 294



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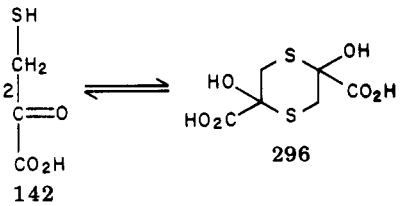
It has been known for some time that 3-mercaptopyruvic acid (142), the α-keto acid analogue of cysteine, exhibits anomalous behavior, previously ascribed to its enol form.[419] However, recent evidence suggests that



142 296

3-mercaptopyruvic acid (142) exists in aqueous solution in equilibrium with a cyclic dimer (internal hemithioketal), 2,5-dihydroxy-1,4-dithiane-2,5-dicarboxylic acid (296).[118]

VII. Analysis of α-Keto Acids in Biological Samples

Enzymatic (spectrophotometric and fluorometric) methods for the determination of pyruvic, oxaloacetic, and α-ketoglutaric acids have been in routine use for many years.[420] More recently *B. subtilis* leucine dehydrogenase has been used to estimate total branched-chain α-keto acids in rat tissues.[421] Analytical methods based on formation of fluorescent products with a number of reagents have been developed over the last 20 years. Such reagents include *o*-phenylenediamine,[422] pyridoxamine + zinc,[423] and 4′-hydrazino-2-stilbazole.[424] These methods, which are sensitive, have been used to estimate total α-keto acid or simple mixtures of pyruvate (3), oxaloacetate (4), and α-ketoglutarate (5). With the development of HPLC, GC, and GC–mass spectrometric techniques, many methods have become available for the simultaneous estimation of numerous α-keto acids in biological materials.

Almost all HPLC techniques require precolumn derivatization. However, a cation exchange HPLC technique that relies on spectrophotometric and amperometric detection has recently been described.[425] A post column derivatization with *N*-methylnicotinamide chloride has been used for fluorometric determination of α-ketoglutaric (5) and pyruvic acids (3).[426] A technique for the measurement of α-ketoisocaproic acid (45) using an isotope tracer and isocratic HPLC has been described.[427] A precolumn derivatization with naphthalene-2,3-diamine has been used to estimate phenylpyruvic acid (6) as the fluorescent product, 3-benzyl-2-hydroxybenzoquinoxaline;[428] the procedure is specific for phenylpyruvic acid.[428] Grushka et al. have shown that glyoxylic (2), pyruvic (3), α-ketobutyric (8), oxaloacetic (4), and α-ketoglutaric (5), acids can be separated on a C_{18} column by reversed-phase HPLC as the 4-(bromomethyl)-7-coumarin derivatives.[429] As noted in section VA, many thin-layer and paper chromatography techniques have been published for the separation of α-keto acid 2,4-dinitrophenylhydrazones. Often the procedures are not ideal; R_f values are sometimes difficult to reproduce, the chromatographic behavior of different hydrazones are often similar, and many hydrazones yield two spots (probably cis and trans isomers).[379–386] Recently, Hemming and Gubler reported that a seven-component homologous series of α-keto acid 2,4-dinitrophenylhydrazones of increasing carbon-chain length are easily separable by HPLC.[430] In some cases, resolution of cis and trans forms is possible.[430] The method was used to detect pyruvic acid (3) in biological samples.[430]

Hayashi et al. have used a reversed-phase HPLC technique coupled with spectrophotometric detection to estimate α-keto acid quinoxalinol derivatives;[431] the authors have also used a novel hydrazide gel to purify the α-keto acids from biological materials.[432]

Since 1965, a large number of GC methods for the determination of α-keto acids have been developed. Gas chromatographic analysis relies on conversion of the compounds of interest to volatile and/or stable derivatives. The first derivatives to be employed for the estimation of α-keto acids were the carboxyl methyl esters, and these have been employed to estimate various acids of the tricarboxylic acid cycle.[433] Other derivatives have included *O*-methyl-,[434,435] *O*-ethyl-,[435] *O*-benzyl-,[435] *O*-(trimethylsilyl)-,[435] and *O*-(2,3,4,5,6-pentafluorobenzyl)oximes.[436] The oxime of the α-keto acid trimethylsilyl derivative has also been employed.[437] A method for the GC separation of 2,4-dinitrophenylhydrazone methyl esters has been published.[438] Cooper et al. reported that α-ketoglutaramic acid can be determined in human cerebrospinal fluid as the tris(trimethylsilyl) derivative of the cyclic lactam.[439] Probably, the most widely used GC method is that initially developed by Langenbeck and colleagues[440] in which the *O*-(trimethylsilyl)quinoxalinol derivatives are quantitated by postcolumn mass spectrometry. The technique has been particularly useful for the measurement of branch-chain α-keto acids in biological materials.[441,442] Various mass spectrometric detection systems have been described; i.e., electron impact (EI)[440] and chemical ionization (CI) with methane[443] and ammonia.[444] Rocchiccioli et al. report that the sensitivity of a capillary GC–mass spectrometric technique (CI, ammonia) utilizing *O*-(trimethylsilyl)quinoxalinol derivatives of branch-chain α-keto acids is <50 ng.[444]

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