Inclusion Compounds. XVII.[1] Catalysis of Decarboxylation by Cyclodextrins. A Model Reaction for the Mechanism of Enzymes

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In homogeneous aqueous solution cyclodextrins catalyze the decarboxylation of methylphenylcyanoacetic acids, substituted acetoacetic acids, and trihalogenated acetic acids. The catalytic acceleration factors lie between 1 and 14.8. Catalysis shows substrate specificity in a high degree and depends mainly on the size of the substituent group. The catalytic reaction is first order; on complete occupation of the catalytic centers, however, a reaction of zero order can be observed. Activation energies, enthalpies, and entropies for catalyzed and noncatalyzed reactions were measured. In the catalysis the activation energies were reduced to a large extent ($\Delta\Delta H$ = 6–8 kcal.), but this has very little effect on the change of the reaction rate because it is compensated by a change of the activation entropy working in the opposite direction.

Introduction

In previous publications we have shown that inclusion compounds of cyclodextrins are stable in solution[2] and react as catalysts in some cases.[3] At that time[4] we pointed out also that certain aspects of enzyme catalysis can be understood on the basis of inclusion catalysis. In this publication we wish to report some results of our work on the inclusion catalysis of decarboxylation reactions.[5]

The kinetic and thermodynamic data of this catalysis resemble those of an enzyme catalysis in many details; the catalytic effectiveness of this model, however, is very inferior to the enzyme catalysis.

(1) Catalysis of Decarboxylation of Carboxylic Acids in the Presence of Cyclodextrin. Reaction Mechanism. A typical reaction curve is shown in Figure 1. The reaction temperature must be close to 60–70°, otherwise the inclusion compounds of the decarboxylated reaction products precipitate. Table I shows the decomposition rates obtained.

In principle there are two methods of interpreting the mechanism of this catalysis. (1) The catalytic effect can be attributed to the basic properties of the cyclodextrin cavity, *i.e.*, to the electron-donor properties of ether and hydroxyl O atoms.[6] (2) The reaction can be interpreted by assuming hydrogen bonding to the OH groups of cyclodextrin.

Investigation of the decarboxylating tendency of various carboxylic acids with a cationic center in the α-position to the carboxyl C atom showed that these acids decarboxylate when the anion is formed.[7] Trichloro-[8–14] and tribromoacetic acids,[15] trinitrobenzoic acid,[16,17] nitroacetic acid,[18] etc., belong to this group of acids. Furthermore, it was observed[10] that in the presence of aniline the decarboxylation rate of the trichloroacetic acid proceeds proportionally to the square of the aniline concentration; *i.e.*, the effect of the base probably does not depend on the binding of acid protons only.[7] α,α'-Dimethyl-γ-pyrone also catalyzes the decarboxylation of trichloroacetic acid[12]; here the electron pair of the ether O atom is considered to be responsible for the base catalysis. A similar assumption can be made regarding cyclodextrins where the ether and hydroxyl O atoms cause the basicity of the cavity.

Therefore, one should assume that carboxylic acids, after inclusion in the cavity of cyclodextrin, are activated by nucleophilic attack of the ether and hydroxyl O atoms at the carboxyl C atom and that their decarboxylation is accelerated. On this basis the mechanism of the reaction of cyclodextrins with cyanoacetic acid and trichloro- and tribromoacetic acid can be explained.

In the decarboxylation of β-keto acids a different mechanism must also be considered. It proceeds best in a weakly acidic medium, and a mechanism[19] (as shown below) is assumed which is characterized by a cyclic transition state having a particularly low energy.

(1) (a) Part XVI: F. Cramer and W. Dietsche, *Chem. Ber.*, **92**, 1739 (1959); (b) this work was supported by Deutsche Forschungsgemeinschaft, Bad Godesberg; Rockefeller Foundation, New York, N. Y.; Verband der Chemischen Industrie, Düsseldorf; and Research Corporation, New York, N. Y.

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(3) F. Cramer, *ibid.*, **86**, 1576 (1953).

(4) For a summary, see F. Cramer, "Einschlussverbindungen," Springer-Verlag, Heidelberg, 1954.

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(14) G. A. Hall and F. H. Verhoek, *ibid.*, **69**, 613 (1947).

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(16) F. H. Verhoek, *J. Am. Chem. Soc.*, **61**, 186 (1939).

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(18) K. J. Pedersen, *Trans. Faraday Soc.*, **23**, 316 (1927); *Acta Chem. Scand.*, **1**, 437 (1947).

(19) (a) F. H. Westheimer and W. A. Jones, *J. Am. Chem. Soc.*, **63**, 3283 (1941). (b) The infrared spectrum of the inclusion compounds of the acids with β-dextrin shows no shift of the CN bands to longer wave lengths, but this is not in contradiction to the mechanism suggested since, *e.g.*, hydrogen bonds in *o*-hydroxybenzonitrile are only noticeable from a shifting of the OH bands (*e.g.*, H. A. Staab, "Einführung in die theoretische organische Chemie," Verlag Chemie, Weinheim, 1959, p. 687).

Table I. Half-Life Time of Decarboxylation Reactions[a-c]

	α-Methyl-α-benzyl-acetoacetic acid	α-Benzyl-acetoacetic acid	α-Methyl-acetoacetic acid	Acetoacetic acid	Tri-bromo-acetic acid	Tri-chloro-acetic acid	Methyl(4-chlorophenyl-cyanoacetic acid	Methyl(2-chlorophenyl-cyanoacetic acid	Methyl-phenylcyano-acetic acid
Temp., °C.	60	60	70	70	60	80	60	70	70
Without cyclodextrin	59.0	92.0	126.0	116.0	43	186	37.0	25.0	32.0
With 1.65×10^{-2} mole/l. of α-dextrin	59.0 (1.00)	92.0 (1.00)	126.0 (1.00)	116.0 (1.00)	35 (1.23)	132 (1.41)	32.5 (1.14)	25.0 (1.00)	30.5 (1.03)
With 1.65×10^{-2} mole/l. of β-dextrin	28.0 (2.11)	30.0 (3.06)	84.0 (1.50)	93.0(1.25)	18 (2.40)	87 (2.14)	8.5 (4.35)	11.5 (2.18)	16.0 (2.0)

[a] Time in min. [b] In parentheses are shown the catalytic acceleration factors assuming that the velocity for the noncatalyzed reaction is one. [c] Concentration of the acids, 5.0×10^{-2} mole/l.

In our investigations, however, acid anions were used (pH 10); this mechanism, therefore, can only be applied to our catalyzed reactions in a modified form. The OH groups of dextrin form hydrogen bonds with the carbonyl or nitrile group of the acids (acid catalysis[19a]), whereas, on the other hand, a donor function is exerted by the oxygen electron pair. Dextrin is a donor and acceptor at the same time here. An "electron pressure" takes place through the base effect and an "electron suction" through hydrogen bonding. This would be an example of *bifunctional catalysis*, which is also very probably exerted by enzymes.[20]

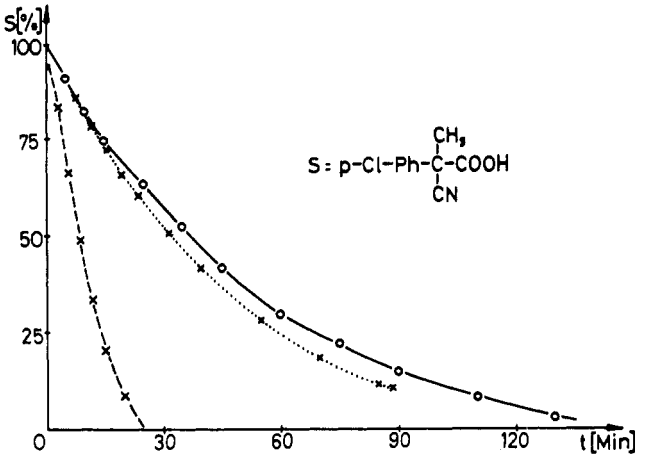

Figure 1. Decarboxylation of methyl(4-chlorophenyl)cyanoacetic acid (sodium salt, 2×10^{-5} mole/l.) at 60°; ordinate: percentage of unreacted carboxylic acid: —, without cyclodextrin; . . ., with α-cyclodextrin (6.6×10^{-6} mole/l.); - - -, with β-cyclodextrin (6.6×10^{-6} mole/l.).

The requirement for the catalytic effect is inclusion. It must be assumed that the molecule is fixed by the inclusion in a certain position which is associated with a sufficient approach of the carbonyl or nitrile group to one of the OH groups. The partial utilization of the free electron pair by hydrogen bonding leads to a shift of the electron charge center, as indicated in Figure 2; splitting off of CO_2 is thus facilitated.

A different interpretation of catalytic decarboxylation by cyclodextrins can be derived from results obtained by Hall and Verhoek.[14] In quantitative investigations of the decarboxylation of the trichloroacetate anion in alcoholic solution they found a decrease of the decarboxylation rate when water was added. They supposed that increasing hydration of the trichloroacetate anion owing to the addition of water was the reason for this. Thus, the catalytic effect of the cyclodextrins could be explained by the reduction of the hydration of the carboxylate anions due to the inclusion below.

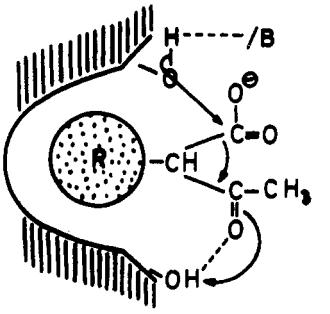

Figure 2. Mechanism of bifunctional cyclodextrin catalysis (schematic representation[21]).

(2) *Substrate Specifity of Catalysis.* It is seen from Table I that the catalysis is substrate specific. β-Dextrin is the better catalyst throughout. For an explanation, mainly the special conditions have to be considered. We shall consider the situation of phenylcyanoacetic acids first. From the Stuart–Calotte model[21] one sees that the three acids are included with their benzene residues lying in the direction of the longitudinal axis of the *α-dextrin cylinder* (axial inclusion, Figure 3); but, apparently, this axial method of inclusion is not sufficient to cause a substantial catalytic effect. For methyl(4-chlorophenyl)cyanoacetic acid this *partial inclusion* is still accompanied by a small catalytic effect.

The situation, however, seems to be different in the case of β-dextrin. Methylphenyl- and methyl[4-chlorophenyl]cyanoacetic acid fit equatorially in β-dextrin very well (*i.e.*, parallel to the diameter of the cavity, Figure 3), and so can interact with the OH groups. In the case of methyl(2-chlorophenyl)cyanoacetic acid, the 2-chloro group interferes with the model, but it cannot prevent the inclusion as is also indicated by the ac-

(20) D. E. Koshland, *Biochem. Biophys. Acta*, **25**, 219 (1957), and further examples therein; see also H. Morawetz and J. Oreskes, *J. Am. Chem. Soc.*, **80**, 2591 (1958).

(21) For figures of more detailed Stuart–Calotte models, see F. Cramer, *Angew. Chem.*, **68**, 115 (1956).

celeration factor. The results with trihalogenated acetic acids are more difficult to interpret. Trichloro- and tribromocyanoacetic acid with a length of the molecular axis (X–C–C–O) of 4.70 and of 4.85 Å. (max.) would fill out the cavity of α-dextrin (with a diameter of 6 Å.) much better than that of β-dextrin (with a diameter of 7–8 Å.). The catalysis should thus be much stronger for α-dextrin than for β-dextrin.

In Table I the substituted *acetoacetic acids* are listed according to their decreasing cavity filling. The catalytic effect is relatively strong for β-dextrin and reaches its maximum with benzylacetoacetic acid which in the Stuart–Calotte model fits in cyclodextrin particularly well. Methylbenzylacetoacetic acid is somewhat too large; on the other hand, unsubstituted and methyl-substituted acids are small and probably not hydrophobic enough to be included in the cavity, since, for the formation of inclusion compounds in solution, hydrophobic bonds might be more important than hydrogen bonds and other short-reaching bonding forces.

The inactivity of *α-dextrin* in the case of the large α-methyl-α-benzylacetoacetic acid, as well as in the case of α-benzylacetoacetic acid, is understandable. The behavior of α-dextrin towards α-methylacetoacetic acid and acetoacetic acid, however, is not clear. From the spatial point of view the two acids fit in the cavity of α-dextrin. One possible explanation is implied in the above-mentioned assumption of an equatorial fixation of the two acids in the dextrin cavity, which is required to obtain a catalytic effect. This is connected with difficulties in the case of α-dextrin, because the longest axis of the acid is 7–8 Å., whereas the diameter of α-dextrin is only 6 Å.

(3) *Alteration of Rate Constant during Decarboxylation of Methyl-4-chlorophenylcyanoacetic Acid.* The decomposition curve of methyl(4-chlorophenyl)cyanoacetic acid in the presence of β-dextrin (3:1; see Figure 1) is exactly linear up to a decomposition of ~50%. The evaluation of this series of measurements, *i.e.*, the determination of the rate constants according to the first-order rate law, gives an unsatisfactory result. In this case a transition from first order to zero order of the catalytic reaction takes place. With a tenfold excess of the substrate a reaction of zero order can be observed also.

When the reaction is carried out with a dextrin:substrate ratio (1:1) under the same conditions, decomposition follows exactly the first-order rate law. For the noncatalyzed reaction the first-order rate law is obeyed within the limits of experimental error (see Table II). The decarboxylation represents a monomolecular decomposition reaction, and thus should follow the first-order rate law (see Table III).

Table II. Calculation of the First-Order Rate Constant for the Noncatalyzed Decarboxylation of Methyl(4-chlorophenyl)cyanoacetic Acid[a]

Time, min.	k^1, min.$^{-1}$	Time, min.	k^1, min.$^{-1}$
0	0.0	25	0.0186
5	0.0190	35	0.0188
10	0.0193	45	0.0194
15	0.0193	60	(0.0202)

[a] 10^{-3} *M* acid; L_0 = 10.4 ml. of 0.1 *N* NaOH; L_t milliliters of NaOH used during time *t*.

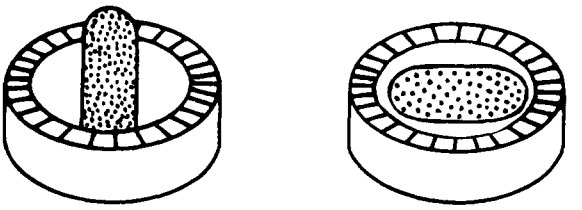

Figure 3. Axial inclusion (left) and equatorial inclusion (right) in cyclodextrins (schematic representation).

For explanation of these phenomena it should be remembered that the cyclodextrin cavity can be considered as a "microheterogeneous phase"[4]; *i.e.*, catalysis by cyclodextrins represents formally a heterogeneous catalysis with well-defined active centers as are also present in enzymes. Consequently, the theory of *heterogeneous catalysis* must be applicable to the cyclodextrin catalysis also. According to this, acid S and dextrin D join first to form a complex SD (inclusion compound); the acid becomes activated and decomposes, whereby the reaction products (P) and the free cavity of dextrin are formed again (eq. 1). Be-

$$S + D \longrightarrow SD \longrightarrow P + D \qquad (1)$$

cause of the relatively small catalytic effect of cyclodextrin, the decomposition outside the cyclodextrin cavity must be considered also; thus, the following, idealized equation for the decomposition rate (v) is obtained

$$v = k_1[S_{fr}] + k_2[SD] \qquad (2)$$

where $[S_{fr}] + [SD]$ corresponds to the respective over-all substrate concentrations.

The reaction order of the "heterogeneous" inclusion catalysis is now determined (1) by the adsorption (inclusion) equilibrium between substrate and cyclodextrin, and (2) by the ratio of the two constants k_1 and k_2. In the case of a small filling of the cavity, the rate of the catalyzed decomposition is proportional to this filling density and therefore independent of the yet unreacted amount of substrate; the first-order rate law follows from this. In the case of a relatively large cavity filling, a diminishing substrate concentration alters the amount of adsorbed material initially only slightly, *i.e.*, [SD] remains constant at first, and the catalyzed decomposition is of zero order. The two quantities in reaction 1 can overlap, and in general, mixed reactions can be observed. An approximate zero order can be expected therefore only in the case

Table III. Calculation of the First- and Zero-Order Rate Constants for the Decarboxylation of Methyl-(4-chlorophenyl)cyanoacetic Acid in the Presence of Cyclodextrin[a]

Time, min.	k^1, min.$^{-1}$	$k^0(L_0-L_t)$, ml./min.
0	...	...
3	0.0604	0.103
6	0.0684	0.105
9	0.0786	0.105
12	0.0909	0.103
15	0.106	0.0995
20	0.123	0.0855

[a] 10^{-3} *M* acid; 0.33×10^{-3} *M* β-dextrin.

Table IV. Calculation of Rate Constants[a] and Acceleration Factors[b]

Temp., °K.	—Methyl(2-chlorophenyl)-cyanoacetic acid— A	B	C	—Methylphenyl-cyanoacetic acid— A	B	C	—Methyl(4-chlorophenyl)cyanoacetic acid— A	B	C	—α-Benzylaceto-acetic acid— A	B	C
303	0.00520	0.036	5.9	...	...	...	...	...	...	...	...	...
313	0.0346	0.168	4.85	0.0331	0.135	4.08	0.0923	1.37	14.85	...	...	...
318	...	...	...	...	...	...	0.224	2.47	11.02	...	...	...
323	0.187	0.582	3.4	0.153	0.512	3.35	0.485	4.12	8.50	0.162	0.64	3.95
328	...	...	...	...	...	...	...	...	...	0.413	1.11	2.69
333	0.864	2.03	2.35	0.524	1.48	2.82	...	...	...	0.728	2.27	3.11
338	...	...	...	...	...	...	...	...	...	1.41	3.99	2.83
343	...	...	...	2.16	4.57	2.11	...	...	...	...	...	...
ΔH^* (cal.)	33,400	26,800		30,300	24,600		30,300	21,400		30,400	25,800	
$\Delta\Delta H^*$ (cal.)		6,600			5,700			8900			4600	

[a] $k^I \times 10^{-2}$, min.$^{-1}$ [b] A, without cyclodextrin; B, with β-cyclodextrin; C, acceleration factors.

of a large cavity filling, when $k_2 >> k_1$. This seems to be the case for methyl(4-chlorophenyl)cyanoacetic acid. Here apparently a threefold excess of substrate is already sufficient to fill out the cavity of cyclodextrin relatively well.

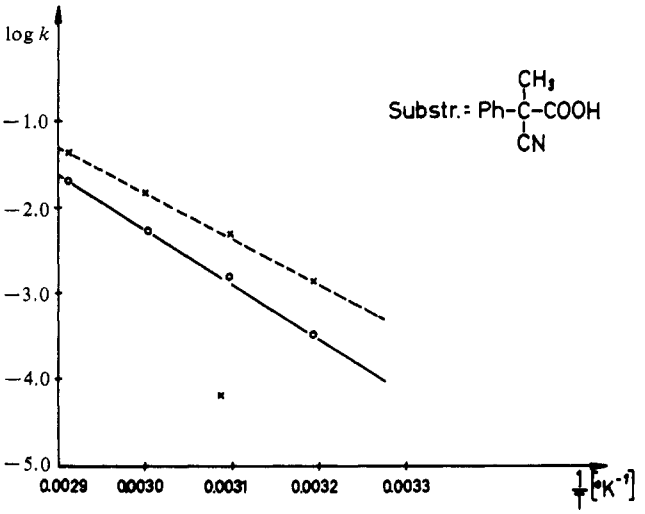

Figure 4. Temperature dependency of decarboxylation rate for methylphenylcyanoacetic acid: —○—○—, without β-dextrin; - - -×- - -×- - -, with β-dextrin.

These considerations are limited by the phenomenon of product inhibition. A complete reformation of the cavity after decomposition probably does not occur. At not too high temperatures one must at least reckon with a partial filling of the cavities by more hydrophobic reaction products. Thus the observed reaction of approximately zero order changes to a reaction of higher order because [SD] is no longer constant.

(4) *Activation Energy and Entropy of Catalysis.* In order to calculate the change in activation energy for reactions catalyzed by cyclodextrins, the rates of decarboxylation at different temperatures were measured. The reaction conditions correspond to those described above. Only for the activation energy of methyl(4-chlorophenyl)cyanoacetic acid in the presence of β-dextrin was the β-dextrin substrate ratio changed from 1:3 to 1:1 because of the different reaction order. At lower temperatures the reaction course could be recorded only from the start to 30% reaction, since after some time the inclusion compounds of the reaction products with β-dextrin precipitated. The rate constant resulting from these measurements are summarized in Table IV. The activation energies were obtained graphically from the slopes of the straight lines in the log k *vs.* $1/T$ plot. A typical diagram is shown in Figure 4.

The tables show, first of all, that the acceleration factors obtained initially at relatively high temperatures could be increased considerably. This increase is especially obvious for methyl(4-chlorophenyl)cyanoacetic acid. When the temperature is reduced from 50 to 40° the catalytic rate is increased from 8.50 to 14.85. In all these cases the activation energy is lowered by the presence of β-dextrin. The largest effect is observed for methyl(4-chlorophenyl)cyanoacetic acid, where it is lowered by 30% for about 8900 cal. In comparison with enzymes it should be mentioned that the activation energy for H_2O_2 cleavage (18 kcal.) is lowered by colloidal platinum to 11.7 kcal. and by liver catalase to 5.5 kcal. Invertase lowers the cane sugar inversion (acid catalysis $\Delta H^* = 25.6$ to 9.4 kcal.[22]). Thus $\Delta\Delta H^*$ for the inclusion catalysis is relatively small in comparison with enzyme catalysis.

When ΔH^* values are split into the values for *activation entropy* and *activation enthalpy*[23] there results a surprising fact (see Table V).

Table V. Activation Enthalpies and Entropies

	ΔH^*, cal.	ΔS^*, cal./deg.	G^*, cal.
Methyl(4-chlorophenyl)-cyanoacetic acid	30,300[a]	25.0	22,300
	21,400[b]	1.5	21,000
Methyl(2-chlorophenyl)-cyanoacetic acid	33,400[a]	32.1	22,900
	26,800[b]	14.2	22,200
Methylphenylcyano-acetic acid	30,300[a]	22.3	23,100
	24,600[b]	7.2	22,300
α-Benzylacetoacetic acid	30,400[a]	22.7	23,100
	25,800[b]	11.3	22,200

[a] Without β-dextrin. [b] With β-dextrin.

In this case a lowering in activation entropy occurs in the same direction, whereas the free activation energy remains almost constant. This entropy term is essentially responsible for the small extent of the acceleration factor in β-dextrin catalysis, formed in the Arrhenius equation, which otherwise according to the Arrhenius

(22) Values from H. Netter, "Theoretische Biochemie," Springer-Verlag, Heidelberg, 1959, p. 564.
(23) H. Eyring, *J. Chem. Phys.*, 3, 107 (1935).

equation would be in the order of 10^6. In enzyme catalysis, besides simultaneous lowering of activation enthalpy, an increase, as well as a decrease, of the entropy term is obtained. In most cases, however, it is found that the activation entropy is small or negative; this is interpreted as an increase in polarity during the process of activation.[24] In any case, in this unusual change of the entropy term for inclusion catalysis, it is shown that the reaction makes great demands on the steric and spatial arrangement of the reaction partners on one side, and of the catalyst on the other side, in the cyclodextrin cavity. Recent results[25] indicate also that a difference in hydration of the substrate molecule may contribute considerably to the catalytic phenomenon.

The model system described here exhibits the following characteristics: catalysis in microheterogeneous phase, substrate specifity, and unambiguous kinetics. Admittedly, we are dealing with a model which does not yet explain completely the mechanism of enzyme action. Above all, the model lacks a high catalytic specifity (acceleration factors of enzymes $\geq 10^4$) since it does not possess any really active groups except the hydrogen bond forming OH groups of the dextrin. Protein, with all its amino, imidazolyl, or thiol groups, has by far more possibilities for chemical catalysis by "active centers," whereas our model illustrates more the physicochemical aspects of enzyme catalysis, *i.e.*, the role of the apoenzyme. Once the cavity is equipped with functional groups, a higher catalytic effect should be produced. Such experiments are being undertaken.

Experimental

A. Starting Materials. (1) Preparation and Separation of Cyclodextrins. The three cyclodextrins were formed together by degradation of a starch solution with amylase from *Bacillus macerans* in the autoclave. They were separated[26] and dried over P_2O_5 for 48 hr.

(2) Methylphenylcyanoacetic Acid. Methylphenylcyanoacetate, prepared according to Horning and Finelli,[27] was methylated according to Widequist.[28] The free acid, so far not described, was prepared in the following way. The ester (4 g.) was shaken with 60 ml. of 1 *N* NaOH at room temperature. After 4 hr. the solution, still alkaline, was extracted three times with 20 ml. of ether. On acidification with 2 *N* H_2SO_4 the acid separated as an oil. It was extracted by shaking with three 20-ml. portions of ether. The ethereal solution was dried over Na_2SO_4 and the ether was carefully evaporated. The oily residue crystallized on scratching, yielding 3.1 g. (90%). Recrystallization from excess cyclohexane or solution in benzene followed by careful precipitation with petroleum ether (b.p. 60°) yielded the product with m.p. 98–99°.

Anal. Calcd. for $C_{10}H_9NO_2$ (mol. wt., 175.0): C, 68.5; H, 5.15; N, 8.00. Found: C, 68.4; H, 5.40; N, 8.05.

(3) Ethyl 4-Chlorophenylcyanoacetate. The preparation was carried out according to Hunger, *et al.*[29]

(4) Ethyl Methyl(4-chlorophenyl)cyanoacetate. To a mixture of 12.3 g. (0.18 mole) of sodium ethylate, 100 ml. of dry ethanol, and 40.0 g. (0.18 mole) of ethyl (4-chlorophenyl)cyanoacetate was added dropwise 260 g. (0.18 mole) of methyl iodide under anhydrous conditions. After refluxing for 1 hr. the ethanol was distilled off *in vacuo*. To remove the separated sodium iodide, water was added and the ester was taken up in ether. The ethereal layer was washed subsequently with 1 *N* NaOH, water until neutral, 2 *N* H_2SO_4, and again with water. The organic layer was dried over $MgSO_4$. After removal of the ether *in vacuo* the residue was fractionated, b.p. 166–167° (14 mm.), $n^{20}D$ 1.5122, yield 23.0 g. (53.8%).

Anal. Calcd. for $C_{12}H_{12}ClNO_2$ (mol. wt., 237.68): C, 60.70; H, 5.09; Cl, 14.92; N, 5.90. Found: C, 59.73; H, 5.09; Cl, 15.03; N, 5.99.

(5) Methyl(4-chlorophenyl)cyanoacetic Acid. Ethyl methyl(4-chlorophenyl)cyanoacetate (5 g.) was shaken with 60 ml. of 1 *N* NaOH. After 1-hr. work-up as above, 4 g. of crude material was recrystallized by dissolving in benzene and careful precipitation with petroleum ether (b.p. 60°), m.p. 81–82°.

Anal. Calcd. for $C_{10}H_8ClNO_2$ (mol. wt., 209.5): C, 57.3; H, 3.8; Cl, 16.9; N, 6.7. Found: C, 57.3; H, 4.0; Cl, 17.1; N, 6.5.

(6) Ethyl (2-Chlorophenyl)cyanoacetate. A solution of sodium (4.6 g., 0.2 g.-atom) in 80 ml. of dry ethanol prepared under anhydrous conditions was freed of excess ethanol *in vacuo*. The flask was equipped as quickly as possible with a stirrer, dropping funnel, and distilling head with a drying tube. Then 120 ml. of dry diethyl carbonate, 24 ml. of dry toluene, and 30.3 g. (0.2 mole) of 2-chlorobenzyl cyanide were added and the solution was heated. Once the distillation began, toluene was added dropwise at the same rate, *i.e.*, approximately 100 ml. in 2 hr. After cooling, 200 ml. of water was added to the mixture. After acidification with 26 ml. of glacial acetic acid the layers were separated. The aqueous layer was extracted with three 40-ml. portions of ether. The combined organic solutions were washed with water and dried over $MgSO_4$. The low-boiling solvents were distilled off under normal pressure and the residue was fractionated *in vacuo*, b.p. 103° (0.25 mm.), m.p. 47–48°, yield 37.0 g. (82.8%).

Anal. Calcd. for $C_{11}H_{10}ClNO_2$ (mol. wt., 223.55): C, 59.20; H, 4.41; Cl, 15.88; N, 6.27. Found: C, 59.18; H, 4.47; Cl, 15.67; N, 6.42.

(7) Ethyl Methyl(2-chlorophenyl)cyanoacetate. Sodium (2.5 g., 0.108 g.-atom) was dissolved in 50 ml. of dry ethanol under anhydrous conditions and 22.4 g. (0.1 mole) of ethyl 2-chlorophenylcyanoacetate was added. Then 15.6 g. (0.11 mole) of methyl iodide was added dropwise. The mixture was refluxed for 1 hr. The subsequent work-up was carried out according to method 4, b.p. 96–96.5° (0.15 mm.), $n^{18}D$ 1.5190, yield 14.2 g. (60.0%).

Anal. Calcd. for $C_{12}H_{12}ClNO_2$ (mol. wt., 237.68): C, 60.70; H, 5.09; Cl, 14.92; N, 5.90. Found: C, 60.74; H, 5.19; Cl, 14.11; N, 6.13.

(8) Methyl(2-chlorophenyl)cyanoacetic Acid. The ester (5 g.) was shaken with 60 ml. of 1 *N* NaOH at room temperature. After 6 hr. of work-up as above,

(24) See ref. 22, p. 605.
(25) F. Saenger and C. Spatz (Göttingen), unpublished work.
(26) F. Cramer and F. M. Henglein, *Chem. Ber.*, **91**, 308 (1958).
(27) E. C. Horning and A. F. Finelli, *J. Am. Chem. Soc.*, **71**, 3204 (1949).
(28) S. Widequist, *Svensk Kem. Tidskr.*, **55**, 125 (1943).
(29) A. Hunger, J. Kebrle, A. Rossi, and K. Hoffmann, *Helv. Chim. Acta*, **43**, 1727 (1960).

Table VI

Product	Expt.	Acid, g.	0.1 *N* NaOH, ml.	Water, ml.	—Dextrin, g.— α-	β-	Bath temp., °C.
Phenylmethylcyanoacetic acid	a	0.175	10.3	10			70, 60, 50, 40
	b	0.175	10.3	10		0.375	70, 60, 50, 40
	c	0.175	10.3	10	0.324		70
Methyl(2-chlorophenyl)cyanoacetic acid	a	0.2095	10.3	10			70, 60, 50, 40, 30
	b	0.2095	10.3	10		0.375	70, 60, 50, 40, 30
	c	0.2095	10.3	10	0.324		70
Methyl(4-chlorophenyl)cyanoacetic acid	a	0.2095	10.3	10			60, 50, 40, 30
	b_1[a]	0.2095	10.3	30		1.134	60, 50, 40, 30
	b_2[b]	0.2095	10.3	10		0.375	60, 50,40, 30
	b_3[c]	0.2095	10.3	30		0.113	55
	c	0.2095	10.3	10	0.324		60
Trichloroacetic acid	a	0.1635	10.3	10			80
	b	0.1635	10.3	10		0.375	80
	c	0.1635	10.3	10	0.324		80
Tribromoacetic acid	a	0.2967	10.3	10			60
	b	0.2967	10.3	10		0.375	60
	c	0.2967	10.3	10	0.324		60
α-Methyl-α-benzylacetoacetic acid	a	0.206	10.3	10			60
	b	0.206	10.3	10		0.375	60
	c	0.206	10.3	10	0.324		60
α-Benzylacetoacetic acid	a	0.192	10.3	10			65, 60, 55, 50
	b	0.192	10.3	10		0.375	65, 60, 55, 50
	c	0.192	10.3	10	0.324		60
α-Methylacetoacetic acid	a	0.116	10.3	10			70
	b	0.116	10.3	10		0.375	70
	c	0.116	10.3	10	0.324		70
Acetoacetic acid	a	0.102	10.3	10			70
	b	0.102	10.3	10		0.375	70
	c	0.102	10.3	10	0.324		70

β-Dextrin:acid. [a] 1:1. [b] 1:3. [c] 1:10.

the crude material (4 g.) was recrystallized as described under method 5, m.p. 125–126° dec.

Anal. Calcd. for $C_{10}H_8ClNO_2$ (mol. wt., 209.5): C, 57.3; H, 3.8; Cl, 16.9; N, 6.7. Found: C, 57.8; H, 3.9; Cl, 16.5; N, 6.9.

The three acids described are fairly stable, but decompose slowly, however, over a period of several months. Therefore, after long storage they should be recrystallized again before use.

(9) *β-Keto Acids.* All the keto acids were prepared by shaking the ethyl esters with 2.5% aqueous KOH for 12–18 hr. according to Ceresole.[30] The acids should be stored at −20°.

B. Decarboxylation Reactions. To measure the rate of decarboxylation we proceeded as follows. The sodium salts of the acids were decarboxylated at pH 9 and constant temperature, and samples were withdrawn at intervals. By addition of a defined amount of 0.1 *N* HCl the carbonate formed was destroyed and the remaining acid was titrated back to pH 5. The reaction was followed titrimetrically.

The conditions under which the decarboxylations were carried out are shown in Table VI.

(30) M. Ceresole, *Chem. Ber.*, **15**, 1327 (1882).