minutes with 50 ml. of 10% aqueous sodium hydroxide, diluted with 50 ml. of water and extracted with ether. The solution was then acidified with 6 *N* sulfuric acid. The oily layer which separated at this point and the aqueous phase were then continuously extracted for 24 hours with 200 ml. of carbon tetrachloride. The cooled carbon tetrachloride extract deposited a crop of white crystals which was removed by filtration. An additional crop was collected from the concentrated mother liquor. The total product, m.p. 142–148°, after recrystallization from benzene, weighed 2.63 g.

Anal. Calcd. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.06. Found: C, 59.54; H, 8.20.

δ-Lactones of 2,3,5-Trihydroxyhendecanoic Acid.—The procedure employed in the hydroxylation of 7.6 g. of ethyl 5-hydroxy-2-hendecenoate was the same as described previously for ethyl 5-cyclopentyl-5-hydroxy-2-pentenoate. A total of 1.76 g. of white crystalline material, m.p. 108–112°, was collected from the carbon tetrachloride extract.

Anal. Calcd. for $C_{11}H_{20}O_4$: C, 61.08; H, 9.32. Found: C, 60.80; H, 9.54.

Fractional Sublimation.—The crude mixtures of isomeric δ-lactones, III (R = cyclopentyl) and III (R = *n*-hexyl) were sublimed at 100–110° (0.5–1.0 mm.) onto a water cooled condenser. The sublimation was interrupted at arbitrary intervals to collect portions of sublimate. Melting points were determined for the various fractions and the less volatile fractions with higher melting points were combined. This process was repeated four or five times until the least volatile fractions melted sharply at a constant value. The melting points of these latter fractions were unchanged by chromatography on columns on Silene EF. No similar constant-melting fractions were obtained from the more volatile fractions.

III, R = cyclopentyl, m.p. 164–165°. *Anal.* Calcd. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.06. Found: C, 59.76; H, 7.92.

III, R = *n*-hexyl, m.p. 124–125°. *Anal.* Calcd. for $C_{11}H_{20}O_4$: C, 61.08; H, 9.32. Found: C, 60.80; H, 9.27.

Chromatography.—The adsorbent employed was Silene EF mixed with Supercel in a 4:1 ratio. A quantity of solute which was 1% of the weight of the adsorbent was dissolved in a quantity of benzene small enough to give a nearly saturated solution. After addition of the solute solution, the column was eluted with additional benzene while successive fractions of eluent were collected and evaporated to dryness. In each case constant-melting products were obtained in the initial fractions of eluent containing solute.

III, R = cyclopentyl, m.p. 157–158.5°. *Anal.* Calcd. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.06. Found: C, 59.86; H, 7.92.

III, R = *n*-hexyl, m.p. 108–109°. *Anal.* Calcd. for $C_{11}H_{20}O_4$: C, 61.08; H, 9.32. Found: C, 61.04; H, 9.27.

Summary

1. A study has been made of the Reformatsky reaction of ethyl γ-bromocrotonate and two aliphatic aldehydes. The structure of the rearranged by-products has been investigated and determined.

2. The Reformatsky reactions of heptaldehyde with ethyl γ-bromocrotonate and its allylic isomer, ethyl α-bromovinylacetate, were found to yield the same pair of isomeric unsaturated hydroxyesters as products.

3. The stereoisomeric mixture of δ-lactones of α,β,δ-trihydroxy acids, obtained as products from the reaction of performic acid with two δ-hydroxy-α,β-unsaturated esters, has been analyzed by the use of fractional sublimation and chromatography. Two apparently homogeneous isomers have been obtained in each case.

NEW HAVEN, CONN. RECEIVED AUGUST 7, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND LABORATORY FOR NUCLEAR SCIENCE AND ENGINEERING, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Decarbonylation of Diphenyl Triketone[1]

BY JOHN D. ROBERTS, DONALD R. SMITH AND C. C. LEE

One of the characteristic reactions of α-polycarbonyl compounds is their tendency to undergo "decarbonylation"[2] with the formation of a compound with one less carbon atom and carbon monoxide or carbon dioxide. An important synthetic example of this type of reaction is the thermal decomposition of oxalylacetic esters which yield the corresponding malonic esters and carbon monoxide. The related thermal decarbonylation of ethyl pyruvate has been studied by Calvin and Lemmon.[3] These workers found that ethyl pyruvate-2-C^{14} gave only non-radioactive carbon monoxide which result indicates that the ester carbonyl group is lost exclusively.

The decarbonylation reactions of diphenyl triketone (diphenylpropane-1,2,3-trione, I) are particularly interesting since they have been reported to be brought about by a wide variety of agents such as bases,[4] acids,[5] cupric acetate,[6] aluminum chloride[5] and sunlight.[7] In the present investigation, a study was made of some of these reactions using C^{14}-labeled diphenyl triketones in order to determine whether the center or side carbonyl groups are lost in the decarbonylation process.

Diphenyl-1,2,3-propanetrione-1-C^{14} (Ia) was synthesized from sodium acetate-1-C^{14} as follows.[8] Acetophenone-carbonyl-C^{14} was prepared by the procedure of Brown and Neville[9] and converted to dibenzoylmethane.[10] Although it involves more steps, the procedure of Kohler and Erickson[11] was found to be preferable to other reported methods[12] for the preparation of I from dibenzoylmethane.

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[1] Supported in part by the joint program of the Office of Naval Research and the Atomic Energy Commission.

[2] In our definition, decarbonylation is the loss of a carbonyl group without reference to whether carbon monoxide or carbon dioxide is formed. Since the carbonyl group might be of any type, the decarboxylation of acids is considered to be included as a special case.

[3] Calvin and Lemmon, THIS JOURNAL, **69**, 1232 (1947).

[4] de Neufville and von Pechmann, *Ber.*, **23**, 3375 (1890).

[5] Schönberg and Azzam, *J. Chem. Soc.*, 1428 (1939).

[6] Blatt and Hawkins, THIS JOURNAL, **58**, 81 (1936).

[7] Schönberg and Mustafa, *J. Chem. Soc.*, 997 (1947).

[8] The sodium acetate-1-C^{14} was obtained from Tracerlab, Inc., on allocation from the United States Atomic Energy Commission.

[9] Brown and Neville, United States Atomic Energy Commission, MDDC 1168.

[10] "Organic Syntheses," Coll. Vol. I, 2nd ed., pp. 78, 205.

[11] Kohler and Erickson, THIS JOURNAL, **53**, 2301 (1931).

[12] (a) Wieland and Bloch, *Ber.*, **37**, 1524 (1904); (b) "Organic Syntheses," Coll. Vol. II, p. 244.

TABLE I

RADIOACTIVITY ANALYSES

Reaction	Compound	Measured activity, counts/min.[a]	Counts/min./mg. of $BaCO_3$/ tagged position[b]
Diphenyl triketone with cupric acetate	Diphenyl triketone	2448 ± 18	12,765 ± 90
	Benzil	2546 ± 6	12,306 ± 28
	Carbon dioxide	−0.3 ± 2	−0.1 ± 0.7
Diphenyl triketone[c] with aluminum chloride	Benzil	2547 ± 8	12,306 ± 42
	Carbon monoxide	25 ± 2	8.6 ± 0.7
Diphenyl triketone hydrate[c] with aluminum chloride	Carbon dioxide	53 ± 6	18 ± 2
	Carbon monoxide	2.3 ± 2	.....[d]
Diphenyl triketone[e] with sodium hydroxide	Benzoin	665 ± 8	3,220 ± 42
	Carbon dioxide	1.8 ± 2	0.6 ± 0.7
p-Methoxyphenyl phenyl triketone with cupric acetate	Triketone	2053 ± 9	11,344 ± 50
	p-Methoxybenzil	2188 ± 24	11,332 ± 124
	Carbon dioxide	5 ± 3	1.8 ± 1
p-Methoxybenzil with alkaline hydrogen peroxide	*p*-Methoxybenzil	2200 ± 20	11,400 ± 114
	p-Anisic acid	4112 ± 26	11,360 ± 72
	Benzoic acid	68 ± 4	161 ± 10
p-Anisamide with sodium hypochlorite	*p*-Anisamide	557 ± 6	1,536 ± 17
	p-Anisidine	2.9 ± 2.5	9.6 ± 8.3

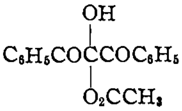
[a] Activities (corrected for background) of "infinitely thick" barium carbonate samples having a cross-sectional area of 2.90 cm.[2] measured with a methane-filled windowless counter ("Nucleometer") manufactured by the Radiation Counter Laboratories. For method of preparation of the samples see Roberts, Bennett, Holroyd and Fugitt, *Anal. Chem.*, **20**, 904 (1948). [b] Corrected for self-absorption, calculated from (specific activity) × (number of carbon atoms)/number of labeled positions in a given molecule. [c] The specific activity of the starting material was the same as that used in the cupric acetate reaction. [d] The sample of barium carbonate was too small for accurate computation of specific activity. [e] Diphenyl triketone of lower specific activity than used in other runs.

The hydrated form of the triketone was obtained by crystallization of the anhydrous material from aqueous acetic acid. 1-(*p*-Methoxyphenyl)-3-phenyl-1,2,3-propanetrione-1-C^{14} (II) was prepared by similar reactions.

Experimental Results

The cupric acetate, aluminum chloride and sodium hydroxide induced decarbonylations of diphenyl triketone were given particular attention since the reagents represent rather different types and preliminary experiments indicated that the reactions proceed readily in reasonable yields. Previous reports[5] of decarbonylations induced by sulfuric and phosphoric acids could not be duplicated. Since earlier workers did not often identify the gaseous reaction products, these were investigated in each case.

Diphenyl Triketone and *p*-Methoxyphenyl Phenyl Triketone with Cupric Acetate in Acetic Acid.—The decarbonylation of diphenyl triketone with cupric acetate in refluxing acetic acid yields benzil, *carbon dioxide* and cuprous oxide. No carbon monoxide could be detected. Diphenyl triketone hydrate gives the same products. It is likely that in each case the same or a similar reacting species is involved which might be the hydrate or the corresponding acetic acid complex



$$C_6H_5CO\overset{OH}{\underset{O_2CCH_3}{C}}COC_6H_5$$

The stoichiometry of the simple decarbonylation of the hydrate (or acetic acid complex) is such that the reaction would be expected to yield benzoin (or benzoin acetate) and carbon dioxide or benzil, carbon monoxide and water (or acetic acid). Oxidation of benzoin in the first event or carbon monoxide in the second would account for the observed products. No reaction was detected between carbon monoxide and cupric acetate in acetic acid, although benzoin was rapidly and completely oxidized to benzil under the same conditions. While no other proof was obtained, it appears that the decarbonylation initially yields benzoin and carbon dioxide. The benzoin is then oxidized to benzil. The presence of cupric ion is essential since no reaction was observed on long refluxing of the triketone in glacial acetic acid.

The decarbonylation of the C^{14}-labeled triketone (Ia) by cupric acetate gave radioactive benzil and essentially inactive carbon dioxide (Table I). Thus, the cupric acetate induced decarbonylation results with loss of the *center* carbonyl group. With *p*-methoxyphenyl phenyl triketone labeled with C^{14} at the carbonyl group closest to the *p*-methoxyphenyl group, a similar result was obtained (*cf.* Table I).

Diphenyl Triketone with Aluminum Chloride.—It has been reported that the decarbonylation of I with aluminum chloride[5] or of diphenyl triketone hydrate in benzene solution at 60° with aluminum chloride[13] gives benzil. The identities of the gaseous products apparently were not determined.

We find that heating the pure anhydrous ketone with freshly sublimed aluminum chloride gives benzil and carbon monoxide. In the presence of even small amounts of moisture more or less benzoin and a corresponding quantity of carbon dioxide are formed. Starting with the triketone hydrate about 80% of the reaction produced benzoin and carbon dioxide. The products were analyzed by their infrared spectra which are shown in Figs. 1 and 2. The decarbonylation of the C^{14}-labeled triketone (Ia) with freshly sublimed aluminum chlo-

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(13) Wegmann and Dahn, *Helv. Chim. Acta*, **29**, 1247 (1946).

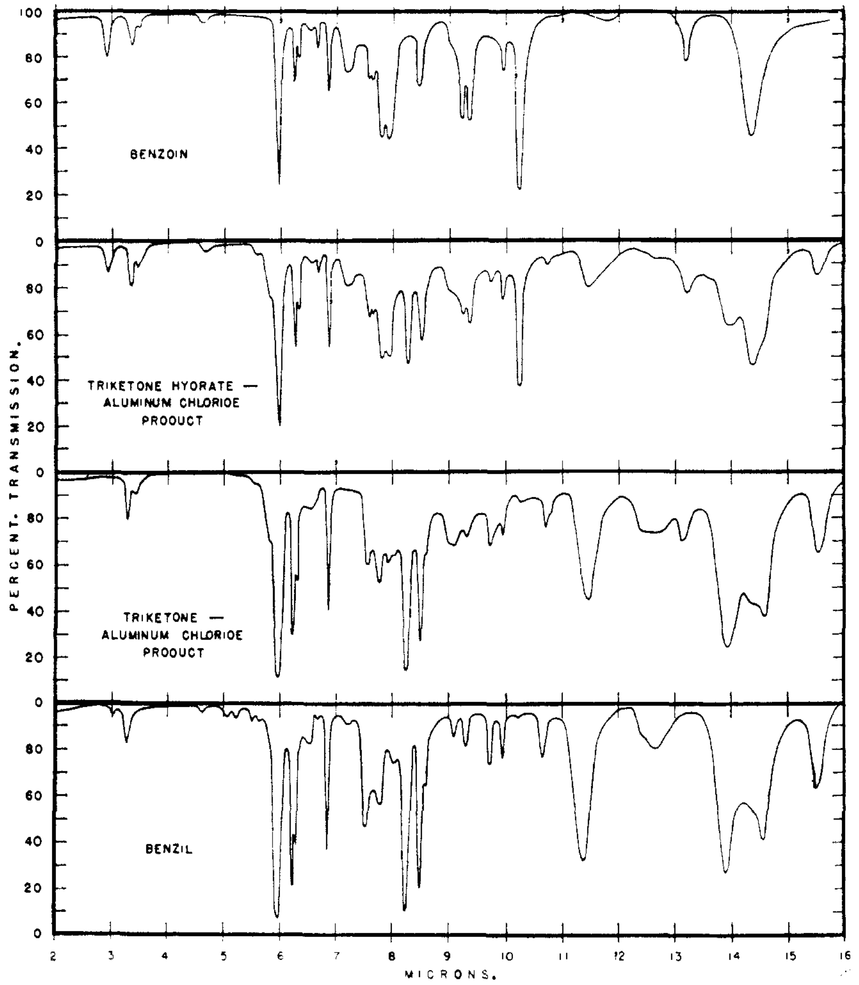


Fig. 1.—Infrared spectra.

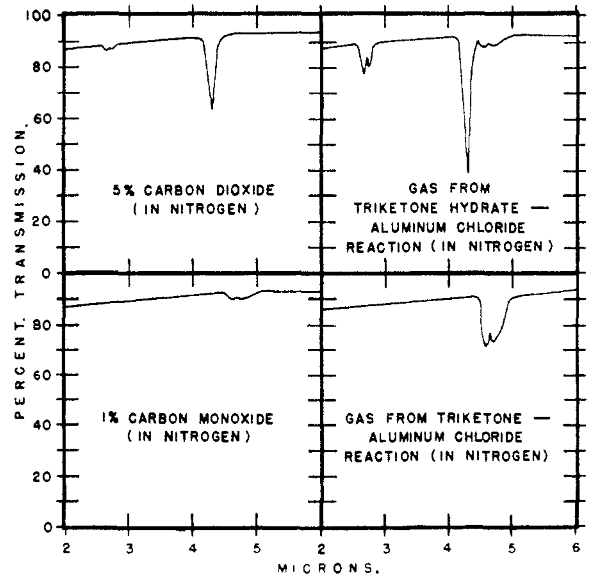


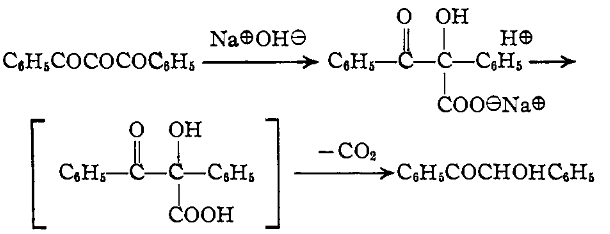
Fig. 2.—Infrared spectra.

ride gave radioactive benzil and essentially inactive carbon monoxide (Table I). A small-scale experiment with the C^{14}-labeled triketone hydrate gave comparably inactive carbon dioxide (Table I). These results like those obtained with cupric acetate indicate that the center carbonyl carbon is lost in the decarbonylation reaction.

Diphenyl Triketone with Sodium Hydroxide.—It has been reported by de Neufville and von Pechmann[4] that diphenyl triketone reacts with dilute alcoholic sodium hydroxide at room temperature to give, after acidification, benzoic acid, mandelic acid, benzoin and carbon dioxide.[14] Isotopic analyses of the products from C^{14}-labeled triketone (Ia) indicate that only the center carbonyl group of the triketone is converted to carbon dioxide in the alkaline decarbonylation (*cf.* Table I).

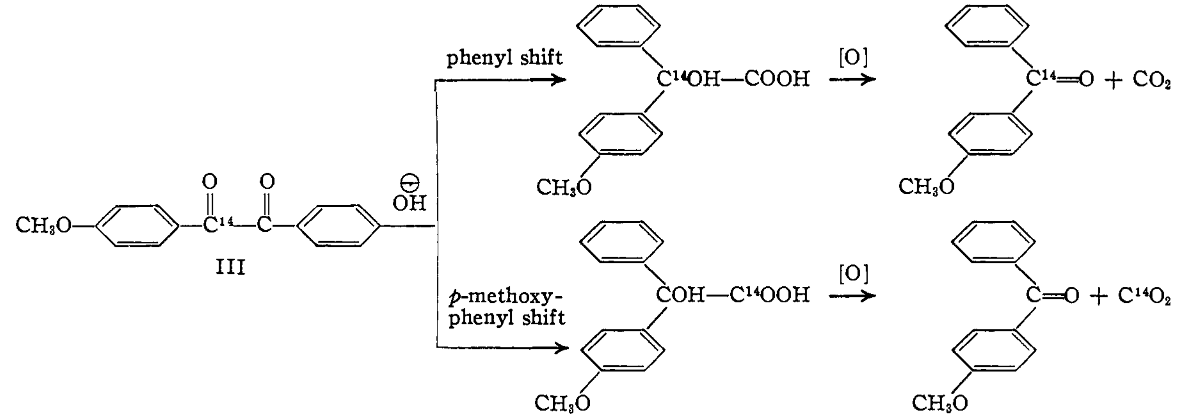
It seems unlikely that benzoin and carbon dioxide are formed directly in alkaline solution and most probably the initial reaction is a benzilic acid type rearrangement of the triketone with formation of the sodium salt of α-benzoylmandelic acid which, on acidification, is converted to benzoin and carbon dioxide.

[14] The corresponding reactions with dimesityl triketone and mesityl phenyl triketone [Fuson, Matuszeski and Gray, THIS JOURNAL, **56**, 2099 (1934); Fuson, Weinstock and Ullyot, *ibid.*, **57**, 1803 (1935)] proceed in a somewhat different manner in that benzil (rather than benzoin) derivatives are formed. It is possible that these substances react by a mechanism fundamentally different from that which obtains with diphenyl triketone.



$$C_6H_5COCOCOC_6H_5 \xrightarrow{Na^{\oplus}OH^{\ominus}} C_6H_5{-}\overset{O}{\overset{\|}{C}}{-}\underset{COO^{\ominus}Na^{\oplus}}{\overset{OH}{C}}{-}C_6H_5 \xrightarrow{H^{\oplus}} \left[C_6H_5{-}\overset{O}{\overset{\|}{C}}{-}\underset{COOH}{\overset{OH}{C}}{-}C_6H_5 \right] \xrightarrow{-CO_2} C_6H_5COCHOHC_6H_5$$

Benzilic Acid Rearrangement of *p*-Methoxybenzil.—In order to gain information as to the relative ease of shifting phenyl and *p*-methoxyphenyl groups in reactions similar to the decarbonylation reaction a study was made of the benzilic acid rearrangement of a C^{14}-labeled *p*-methoxybenzil [1-(*p*-methoxyphenyl)-2-phenyl-1,2-ethanedione-1-C^{14}, III].[15] The rearrangement of III may proceed with shift of the phenyl or *p*-methoxyphenyl group. The identity of the product can be established by conversion to, and isotopic assay of, the corresponding *p*-methoxybenzophenone.



III was obtained from the cupric acetate induced decarbonylation of the C^{14}-labeled *p*-methoxyphenyl phenyl triketone (II) described earlier. The isotope-position assignment of III was verified by oxidation with alkaline hydrogen peroxide as employed for the corresponding oxidation of benzil.[16] Essentially all of the C^{14}-activity of III was accounted for in the resulting *p*-anisic acid. Proof that the C^{14} was in the carboxyl group of the *p*-anisic acid was obtained by the degradation of the acid through the Hofmann reaction to *p*-anisidine. The *p*-anisidine contained no significant amount of C^{14}. The activity data are given in Table I. The rearrangement of III was carried out in alkaline ethanol–water mixtures at 25° (four days), 70° (15 min.) and 100° (10 min.). The yields of *p*-methoxybenzilic acid were 86.2, 34.8 and 60.5%, respectively. The acid samples were oxidized with chromic acid to *p*-methoxybenzophenone which was analyzed for C^{14}. The ratio of the C^{14}-activity per labeled atom of the *p*-methoxybenzophenone to the difference in the activities of *p*-methoxybenzilic acid and *p*-methoxybenzophenone represents the ratio of phenyl to *p*-methoxyphenyl migration which for the three runs described above ranged from 1.72–2.17[17] (*cf.* the data of Table II).

TABLE II

RADIOACTIVITY ANALYSES OF BENZILIC ACID REARRANGEMENT PRODUCTS OF LABELED *p*-METHOXYBENZIL [1-(*p*-METHOXYPHENYL)-2-PHENYL-1,2-ETHANEDIONE-1-C^{14}]

Temp., °C.	Measured activity[a] of *p*-methoxybenzilic acid	Corrected activity[b] of *p*-methoxybenzilic acid	Measured activity[a] of *p*-methoxybenzophenone	Corrected activity[b] of *p*-methoxybenzophenone	Ratio of phenyl to *p*-methoxyphenyl migration
100	2197 ± 18	11380 ± 90	1543 ± 21	7459 ± 100	1.90
70	624 ± 2.5	3230 ± 13	422 ± 2.5	2040 ± 12	1.72
25	621 ± 4.2	3210 ± 22	456 ± 6.6	2198 ± 32	2.17

[a] See footnote (*a*) of Table I. [b] See footnote (*b*) of Table I.

Discussion

The decarbonylation of diphenyl triketone would be expected to proceed with loss of the center carbonyl group if the decarbonylation reaction were to involve a 1,2-shift of a benzoyl group. One of the side carbonyls would be lost if a 1,2-shift of a phenyl group were to take place. The experimental data indicate unequivocally that the benzoyl shift is highly favored for the aluminum chloride, cupric acetate and sodium hydroxide induced decarbonylations. This preferential shift of the benzoyl group would be expected if (a) the intrinsic migratory aptitude of the benzoyl group were greater than that of the phenyl group or (b) the function of the decarbonylation reagent were such as to effectively force the shift of the benzoyl group irrespective of its normal ease of migration relative to that of a phenyl group.

The relative migratory aptitudes of benzoyl and phenyl groups should be expected to depend on whether migration is being considered to a nucleophilic or an electrophilic center. The benzoyl group is certainly more strongly electron attract-

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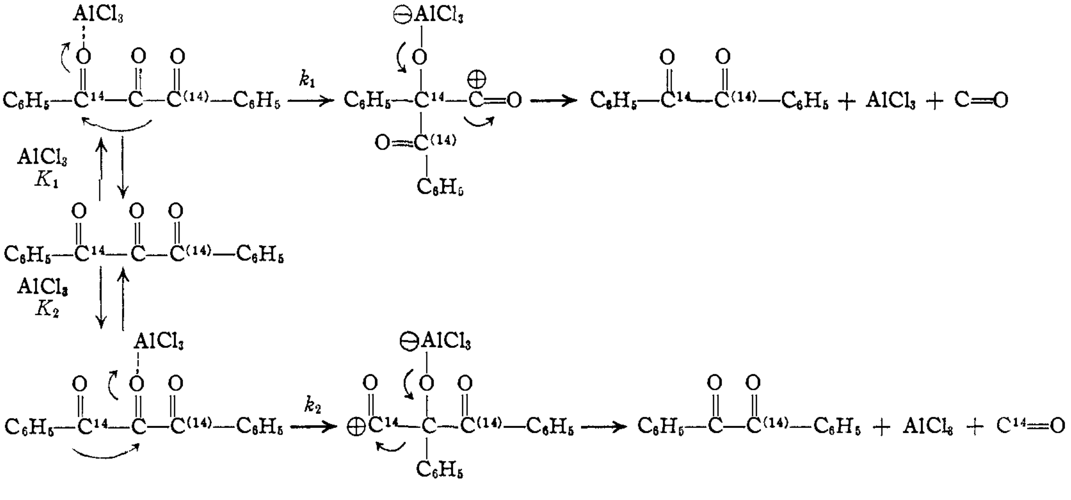
(15) The idea of investigating this type of reaction appears to have occurred to a number of people independently and has been discussed at length by Wheland, "Advanced Organic Chemistry," John Wiley and Sons, New York, N. Y., 1949, p. 510. An admirably critical study of the rearrangement of a variety of substituted α-diketones is being made by Dr. O. Kenton Neville of the Oak Ridge National Laboratory who reported a number of preliminary results at the Organic Chemistry Conference of the Brookhaven National Laboratory, January 20, 1950.

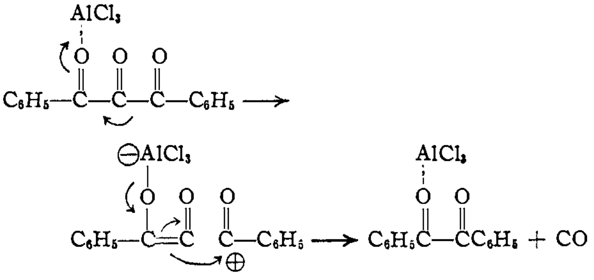
(16) Weitz and Scheffer, *Ber.*, **54**, 2327 (1921).

(17) The results of Stevens and Attree, *J. Chem. Phys.*, **18**, 574 (1950), indicate that an uncertainty of 10% or more may be introduced by neglect of the isotope effect in the benzilic acid rearrangement. This uncertainty is not, however, likely to influence our conclusion regarding the preferential migration of the phenyl group.

ing than the phenyl group (*cf.* the great acid strength of benzoylformic acid[18] relative to benzoic acid) and consequently the migration of the benzoyl group toward an electrophilic center would hardly be expected to be favored.[19] Nonetheless, the decarbonylating agents under discussion, except for sodium hydroxide, can be considered to be the type which normally induce organic reactions by tending to create electrophilic centers. Furthermore, if the sodium hydroxide-induced decarbonylation resembles its prototype, the benzilic acid rearrangement, it should likewise be characterized by the shift of a group to an electrophilic center.[20] The apparent ease of migration of the benzoyl group relative to a phenyl group in these reactions is unexpected and, if Wagner–Meerwein rearrangements are involved, seems only explicable by recourse to consideration of the role of the decarbonylating agents.

Aluminum chloride might reasonably be expected to function in the decarbonylation reaction by reversibly coördinating with the unshared electron pairs of the carbonyl groups of the triketone. Such coördination could occur at one of the side carbonyls or at a center carbonyl and would result in an increase in the electrophilicity of the carbon atom of the carbonyl at which coördination occurs. A 1,2-Wagner–Meerwein type rearrangement of a phenyl or benzoyl group with an electron pair to the electrophilic carbon and subsequent (or simultaneous) loss of carbon monoxide would give the final product. The carbonyl group which is lost preferentially would depend on the equilibrium constants, K_1 and K_2, of the coördination reactions, as well as on the rate constants, k_1 and k_2, for the shift of groups, and the ratio of the over-all rate of loss of center to side carbonyl is equal (as a first approximation) to k_1K_1/k_2K_2. For a Wagner–Meerwein type rearrangement, k_1 should be definitely smaller than k_2. However, K_1 should be greater than K_2 since the central carbonyl in the triketone is flanked by two strongly electron-attracting benzoyl groups while the side carbonyls have, on one side, relatively weakly electron-attracting phenyl groups. If k_1 is actually smaller than k_2, the experimental results indicate that K_1/K_2 must be several orders of magnitude greater than k_1/k_2 since within the errors of measurement only the center carbonyl is lost in the decarbonylation process. The results of the aluminum chloride induced decarbonylation of the labeled triketone hydrate may be explained by a somewhat similar reaction sequence.[21]





The cupric ion-induced decarbonylation of the triketone hydrate to give inactive carbon dioxide may be formulated in an analogous manner with the transfer of the benzoyl group being preceded by a reversible coördination of cupric ion with a side carbonyl. As explained earlier, the resulting benzoin should be oxidized by cupric ion to benzil. The extraordinary power of cupric ion in bringing about the reaction may be due to a facilitation of the formation of the rearrangement transition state by coördination of the metal ion at both carbonyl groups. The transition state could then be stabilized by resonance of the following sort. As pointed out previously, the substitution of *p*-methoxyphenyl for one of the phenyl groups seems to have no effect on the course of the reaction.

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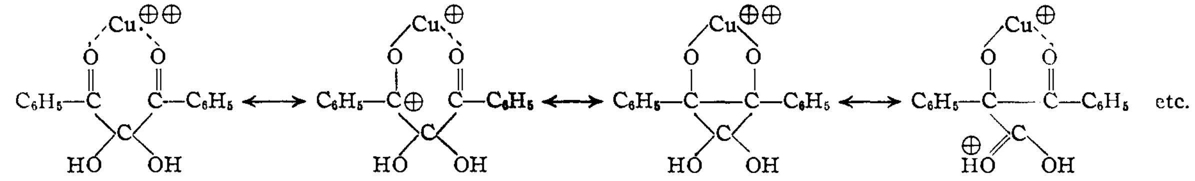
[18] Barre and Cornillot, *Ann. chim.*, [10] **8**, 329 (1927).

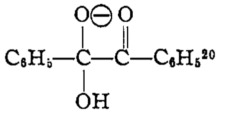
[19] Note that the intrinsic migratory aptitudes of various substituted phenyl groups in the symmetrical pinacol rearrangement as determined by Bachmann and Ferguson, THIS JOURNAL, **56**, 2081 (1934), closely parallel their electron-releasing effects as expressed by the σ-constants of the Hammett equation.

[20] *Cf.* Hammett, "Physical Organic Chemistry," McGraw-Hill Book Company, Inc., New York, N. Y., 1940, pp. 321-322.

[21] A Referee has suggested that a shift of a benzoyl group with a pair of electrons is unlikely and that the mechanism of the reaction with aluminum chloride might involve a benzoyl cation. The operation of such a mechanism is by no means excluded by the present experimental evidence for the aluminum chloride reaction. We prefer the Wagner–Meerwein type formulation chiefly because it enables all of the decarbonylation reactions to be explained on a common basis.

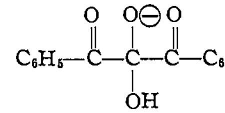


The function of hydroxide ion in the benzilic acid rearrangement is to produce the anion



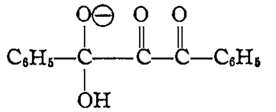
$$C_6H_5-\underset{OH}{\overset{O^{\ominus}}{C}}-\overset{O}{\overset{\|}{C}}-C_6H_5$$[20]

With diphenyl triketone and sodium hydroxide two different anions of this sort might be formed.



$$C_6H_5-\overset{O}{\overset{\|}{C}}-\underset{OH}{\overset{O^{\ominus}}{C}}-\overset{O}{\overset{\|}{C}}-C_6H_5$$

IV



$$C_6H_5-\underset{OH}{\overset{O^{\ominus}}{C}}-\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}-C_6H_5$$

V

Of these, IV should be most stable since it corresponds to the attack of hydroxide ion at the most electrophilic carbon in the molecule. Further indication of the stability of IV is afforded by the observation that the triketone hydrate is a sufficiently strong acid to dissolve in sodium carbonate solution. If we let K_1 and K_2 represent the respective equilibrium constants for the formation of IV and V and k_1 and k_2 the respective rearrangement rate constants, then it is clear from the experimental data that K_1/K_2 must be very large if k_1/k_2 is to be less than unity.

Dominance of equilibrium over rate factors is exhibited in the benzilic acid rearrangement of unsymmetrically substituted benzils and, in the rearrangement of 1-(*p*-methoxyphenyl)-2-phenyl-1,2-ethanedione-1-C^{14} (III), the over-all rate of phenyl migration was about twice that of the *p*-methoxyphenyl group. These results are quite comparable to those obtained in the unsymmetrical pinacol rearrangement[15] where the relative intrinsic migratory aptitudes of the groups are not the most important factor in determining the course of the reaction.

Acknowledgment.—We are indebted to Miss Winifred Bennett for most of the C^{14}-determinations.

Experimental

Acetophenone-carbonyl-C^{14} was prepared by the method of Brown and Neville[9] in 83% yield; b.p. 97.5–98° (10 mm.).

1,3-Diphenyl-1,3-propanedione-1-C^{14} (C^{14}-Labeled Dibenzoylmethane).—From 35 g. of acetophenone-carbonyl-C^{14} there was obtained an over-all yield of 40.5 g. (62%) of 1,3-diphenyl-1,3-propanedione-1-C^{14} by the sequence of reactions: acetophenone → benzalacetophenone → benzalacetophenone dibromide → dibenzoylmethane.[10]

1,3-Diphenyl-2-acetoxy-1,3-propanedione-1-C^{14}.—A mixture of 40.5 g. (0.18 mole) of 1,3-diphenyl-1,3-propanedione-1-C^{14}, 72 g. (0.4 mole) of N-bromosuccinimide, a few mg. of benzoyl peroxide and 300 ml. of carbon tetrachloride was refluxed for 20 hours. During the reaction period, the mixture was irradiated by an ultraviolet lamp. The solid was removed by filtration and washed with hot carbon tetrachloride. The filtrate was combined with the washings, shaken with water and dried over magnesium sulfate. The solvent was evaporated and the residual crude bromide heated in 130 ml. of acetic acid with 33 g. of freshly fused sodium acetate for 1.5 hours. The mixture was cooled and mixed with water. The pasty solid was removed, dried and distilled at 5 microns. The distillate was crystallized from ligroin giving 26 g. (51%) of crude product, m.p. 86–89°. A small sample recrystallized from methanol had m.p. 95° (lit.,[11] 94°).

1,3-Diphenyl-1,2,3-propanetrione-1-C^{14} (Ia).—To a stirred cooled solution of 7 g. (0.025 mole) of 1,3-diphenyl-2-acetoxy-1,3-propanedione-1-C^{14} in 50 ml. of chloroform was added a solution of 1.5 ml. of bromine in 15 ml. of chloroform over a period of 30 minutes. The temperature during the addition was maintained at 4–7°. A stream of dry air was bubbled through the reaction mixture to remove the hydrogen bromide formed. After several hours at room temperature, the solvent was removed under reduced pressure. The viscous residue was then heated under reduced pressure with a soft bunsen flame for about 15 minutes. The evolution of acetyl bromide was then complete and the product was distilled in a von Braun flask at 15 microns. The distillate was recrystallized from anhydrous ligroin and yielded 5.0 g. (84%) of pure Ia, m.p. 68–69° (lit.,[11] m.p. 69–70°). The labeled triketone hydrate was obtained by recrystallization of the anhydrous material from dilute acetic acid and had m.p. 88–89° (lit.,[4] 90°).

1-(*p*-Methoxyphenyl)-3-phenyl-1,2,3-propanetrione-1-C^{14} (II) was prepared starting from sodium acetate-1-C^{14} by a series of reactions analogous to those used for Ia. The sodium acetate-1-C^{14} was converted to acetyl-1-C^{14} chloride[22] which was condensed with anisole in nitrobenzene solution using aluminum chloride as a catalyst. The resulting *p*-methoxyacetophenone–carbonyl-C^{14}, b.p. 151–153° (25 mm.) was condensed with benzaldehyde to give an 87% yield of benzal-*p*-methoxyacetophenone–carbonyl-C^{14}, m.p. 103–104.5° (lit.,[23] 104°) which with bromine in chloroform solution was converted in 73% yield to the corresponding dibromide, m.p. 161–162° (lit.,[24] 162°). The dibromide on treatment with sodium methoxide and subsequent acidification gave a 95.7% yield of 1-(*p*-methoxyphenyl)-3-phenyl-1,3-propanedione-1-C^{14}, m.p. 129–130° (lit.,[24,25] 131–132°).

Bromination of the labeled anisoylbenzoylmethane (17.5 g., 0.069 mole) was carried out in 60 ml. of chloroform with a solution of 4.0 ml. of bromine in 40 ml. of chloroform at 4–7° over a 2-hour period. The hydrogen bromide was removed by a stream of dry air and after 2 hours at room temperature, the chloroform was removed and the residue crystallized from benzene–hexane. The yield of 1-(*p*-methoxyphenyl)-2-bromo-3-phenyl-1,3-propanetrione-1-C^{14} was 18.5 g. (80.5%), m.p. 127–128° (lit.,[25] 128°). The product was refluxed with 8.0 g. of freshly fused potassium acetate in 70 ml. of acetic acid for 20 minutes. The mixture was then poured into water and extracted with ether. The extract was washed with sodium bicarbonate solution, dried over anhydrous magnesium sulfate and the ether removed under reduced pressure. The residual crude 1-(*p*-methoxyphenyl)-2-acetoxy-3-phenyl-1,3-propanetrione-1-C^{14} was dissolved in 80 ml. of chloroform and treated with a solution of 2.9 ml. of bromine in 40 ml. of chloroform at 5–10° over a period of 2 hours. The mixture was allowed to stand overnight and then the solvent was removed under reduced pressure. The residue was heated at 100° and 30 mm. until the evolution of acetyl bromide ceased (about 30 minutes). The product was distilled in a von Braun flask at 5 microns. The distillate was crystallized from ether and yielded 10.5 g. [71% from 1-(*p*-methoxyphenyl)-2-bromo-3-phenyl-1,3-propanetrione-1-C^{14}] of II, m.p. 65–66.5°, a second crystallization from anhydrous ether gave 9.6 g. of material, m.p. 66–67° (lit.,[12a] 65°).

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[22] Roberts, McMahon and Hine, THIS JOURNAL, **72**, 4237 (1950).

[23] Allen and Frame, *Can. J. Research*, **6**, 605 (1932).

[24] Barnes and Brandon, THIS JOURNAL, **65**, 1070 (1943).

[25] Pond and Shoffstall, *ibid.*, **22**, 658 (1900).

Decarbonylation Reactions. A. Diphenyl Triketone and Diphenyl Triketone Hydrate with Cupric Acetate.—The reaction vessel was a 200-ml. two-necked round-bottom flask. One neck of the flask was connected by a standard-taper joint to a curved tube which by rotation about the joint could be used to add solids to the flask. The other neck of the flask carried a gas inlet tube and a reflux condenser, the top of which was connected to a gas-handling system. A solution of 3.0 g. of cupric acetate monohydrate and 70 ml. of acetic acid was placed in the flask while the solid addition tube was charged with 1.5 g. of labeled triketone (Ia). The system was degassed and carbon dioxide-free nitrogen admitted. The triketone was added to the acetic acid solution and the mixture refluxed until the gas evolution ceased (90–100 ml. of gas was given off). The system was then flushed with nitrogen, the combined gases passed through sodium hydroxide solution and the resulting carbonate precipitated as barium carbonate. No carbon monoxide could be detected in the gaseous products by absorption in acidic cuprous chloride solution or infrared spectral analysis.

The contents of the reaction flask were mixed with an equal volume of water and the benzil which precipitated was collected and recrystallized from carbon tetrachloride. The yield of benzil, m.p. 93–95°, was 1.0 g. (75.5%). The results of the activity analyses are given in Table I.

A similar procedure gave comparable yields of carbon dioxide and benzil from non-isotopic triketone hydrate.

B. **Diphenyl Triketone and Diphenyl Triketone Hydrate with Aluminum Chloride.**—The reaction flask was charged with 5 g. of freshly sublimed aluminum chloride and the addition tube contained 1.5 g. of labeled triketone (Ia). The flask was evacuated and then filled with dry carbon dioxide-free nitrogen. The triketone was added to the aluminum chloride and the mixture heated to 130–140° for an hour. The evolved gas (shown by infrared spectrum in an inactive run to be only carbon monoxide) was collected as before, then mixed with oxygen and converted to carbon dioxide in a combustion train. The carbon dioxide was converted to barium carbonate for radioactivity determination. The decarbonylation reaction mixture was treated with dilute hydrochloric acid and extracted with ether. The ethereal extract yielded benzil, m.p. 93–95°, after crystallization from carbon tetrachloride. The infrared spectrum of the product (Fig. 1) indicated that no benzoin was formed in the reaction. The activity data are given in Table I.

A similar experiment using 1.5 g. of non-isotopic triketone hydrate and commercial aluminum chloride gave benzoin and carbon dioxide as the principal products although as shown by the infrared spectra (Figs. 1 and 2) some of the reaction gave benzil and carbon monoxide.

A small-scale experiment using 0.3 g. of labeled triketone hydrate and commercial aluminum chloride gave carbon dioxide and carbon monoxide as gaseous products. The carbon dioxide was absorbed in sodium hydroxide solution and then converted to barium carbonate. The residual carbon monoxide was burned in a combustion train and likewise converted to barium carbonate. The activity data are given in Table I.

C. **Labeled *p*-Methoxyphenyl Phenyl Triketone (II) with Cupric Acetate.**—A mixture of 4.0 g. of II, 8.0 g. of cupric acetate monohydrate and 190 ml. of acetic acid was refluxed in a flask equipped with gas inlet tube and reflux condenser for 2 hours during which time a stream of carbon dioxide-free nitrogen was passed through the flask. The carbon dioxide in the effluent gases was absorbed in 2 *N* sodium hydroxide solution and precipitated as barium carbonate (yield 2.66 g., 90.5%). The reaction mixture was diluted with water and extracted with ether. The extract was washed with sodium bicarbonate solution and dried over magnesium sulfate. The ether was evaporated and the residue crystallized from 95% ethanol. The yield of III was 3.1 g. (87%), m.p. 61–62° (lit.,[26] 62–63°). The results of the radiochemical assays are listed in Table I.

D. **Labeled Diphenyl Triketone (Ia) with Sodium Hydroxide.**—A solution of 1.3 g. of Ia in a few ml. of aqueous ethanol was mixed with 5 ml. of 15% carbonate-free sodium hydroxide solution and allowed to stand for 2 days at room temperature under a nitrogen atmosphere. The mixture was acidified with hydrochloric acid and the evolved carbon dioxide converted to barium carbonate for C^{14}-assay. The aqueous portion was extracted with ether and the extracts washed with alkali to remove any acidic cleavage products of the triketone. Evaporation of the ether gave, after crystallization from alcohol, 0.06 g. (5%) of benzoin, m.p. 129°. The activity analyses are given in Table I.

Proof of Isotope Position Assignment of Labeled *p*-Methoxybenzil [1-(*p*-Methoxyphenyl)-2-phenyl-1,2-ethanedione-1-C^{14}, III].—A solution of 1.0 g. of III in 30 ml. of methanol was mixed with 15 ml. of 15% hydrogen peroxide solution. Ten ml. of 2 *N* NaOH was added and the mixture shaken vigorously for several minutes. Twenty ml. of 6 *N* HCl was added and the crude *p*-anisic acid which amounted to 0.44 g. (66.3%), m.p. 177–179° was collected, washed with water and dried. After one recrystallization from water, the m.p. was 184°. The filtrate from the acidified reaction mixture was extracted with ether and from the extract, after several fractional crystallizations from water, was obtained 0.01 g. of benzoic acid, m.p. 121°. The radioactivity analyses (Table I) of the products indicate that at least 98.5% of the C^{14} of the labeled *p*-methoxybenzil (III) could be accounted for in the anisic acid. Actually, the slight activity of the benzoic acid may have been due to contamination by anisic acid since the activity of III and the *p*-anisic acid agree well.

The labeled *p*-anisic acid was mixed with some of the inactive acid and converted to the corresponding amide, m.p. 164–165°, in 82% yield by successive treatments with thionyl chloride and dry ammonia in ether solution. The amide (1.00 g.) was heated on a steam-bath for one-half hour with a solution of sodium hypochlorite[27] prepared by dissolving 180 ml. of chlorine gas in 45 ml. of 10% sodium hydroxide.

The reaction mixture was extracted with ether, the ether was removed and the residue crystallized from an ether-pentane mixture. The yield of *p*-anisidine, m.p. 57°, was 0.47 g. (53.4%). The C^{14} analysis of this material (Table I) indicates that essentially all of the C^{14} activity of the *p*-anisamide resided in the carbonyl group.

Benzilic Acid Rearrangement of Labeled *p*-Methoxybenzil (III).—Three runs were carried out under different conditions.

A.—III (2.0 g.) was mixed with a solution of 2.0 g. of potassium hydroxide in 8 g. of 50% aqueous ethanol. The mixture was heated on a steam-bath under reflux for 10 minutes. The dark blue solution was diluted with 30 ml. of water and extracted with ether. The aqueous residue was acidified with concentrated hydrochloric acid and on cooling *p*-methoxybenzilic acid precipitated. The crude acid was collected, washed with water and dried. The yield was 1.30 g. (60.5%), m.p. 143–145°. After two recrystallizations from benzene, 1.05 g. of material was obtained with m.p. 148–149° (lit.,[28] 148–149°).

B.—For the later runs, III was mixed with some *p*-methoxybenzil prepared in 71% yield by the oxidation of benzanisoin[29] with iodine and sodium methoxide in boiling methanol.[30] The diluted III (2.0 g.) was heated with a solution of 2.0 g. of potassium hydroxide in 8 g. of 50% aqueous ethanol at 70° for 15 minutes. The blue solution was worked up as before. The yield of *p*-methoxybenzilic acid was 34.8%.

C.[31]—A mixture of 1.08 g. of III and a solution of 9 g. of sodium hydroxide in 15 ml. of 95% ethanol and 30 ml. of water was stirred at room temperature for 4 days. The resulting yellow solution was worked up as before. The yield of *p*-methoxybenzilic acid was 1.00 g. (86.2%).

Oxidation of *p*-Methoxybenzilic Acid to *p*-Methoxybenzophenone.—Two procedures were used. For the rearrangement product from A (above), 1.00 g. of the acid was oxidized by heating on a steam-bath with a solution of 2.0 g. of potassium dichromate in 3 g. of water and 3 g. of concentrated sulfuric acid. The *p*-methoxybenzophenone was isolated, after dilution of the reaction mixture with water, by ether extraction, reduced pressure distillation and crystallization from a methanol-water solution. The yield was 0.47 g. (57.2%), m.p. 61–62°.

The rearrangement products from B and C (0.60 g. and 0.80 g., respectively) were oxidized with 1.0 g. of chromic

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[26] Arnold and Fuson, THIS JOURNAL, **58**, 1295 (1936).

[27] Graebe and Rostowzev, *Ber.*, **35**, 2747 (1902).

[28] Christie, McKenzie and Ritchie, *J. Chem. Soc.*, 153 (1935).

[29] McKenzie, Luis, Tiffeneau and Weill, *Bull. soc. chim.*, **45**, 414 (1929).

[30] Corson and McAllister, THIS JOURNAL, **51**, 2822 (1929).

[31] The rearrangement conditions in this experiment are those used by Neville (ref. 15 and private communication).

anhydride in 10 ml. of acetic acid and 5 ml. of water on the steam-bath for 20 minutes. The products (64–70% yields) were isolated as before.

The oxidation products did not depress the m.p. of authentic *p*-methoxybenzophenone and had the same infrared spectra. In each experiment, the carbon dioxide formed in the oxidations was swept with nitrogen into carbonate-free sodium hydroxide solution and converted to barium carbonate. Although the barium carbonate samples were radioactive, the yields were substantially higher than theoretical and indicated that some over-oxidation occurred. Consequently, the activities of the barium carbonate samples were not useful in determining the ratio of phenyl to *p*-methoxyphenyl migration in the rearrangement. The activities of the samples of III, the methoxybenzilic acids and *p*-methoxybenzophenones are given in Table II.

Summary

The aluminum chloride, cupric acetate and sodium hydroxide induced decarbonylations of diphenyl triketone have been shown by C^{14}-tracer studies to result with loss of the center carbonyl group.

The cupric acetate-induced decarbonylation of *p*-methoxyphenyl phenyl triketone was found to proceed in a similar manner.

The benzilic acid-type rearrangement of *p*-methoxybenzil has been found to involve preferential migration of the phenyl group.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

The Molecular Weight of Lysozyme after Reduction and Alkylation of the Disulfide Bonds

BY H. FRAENKEL-CONRAT, A. MOHAMMAD, E. D. DUCAY AND D. K. MECHAM

Most proteins containing disulfide bonds are rendered insoluble when treated with mercaptans. This phenomenon is probably correlated with the known tendency of reduced proteins to form molecular aggregates, possibly through new hydrogen bonds involving their –SH groups.[2] This difficulty has at times been circumvented by performing the reduction in the presence of denaturants which serve the dual purpose of rendering the disulfide bonds more reactive, and keeping the reduced protein in solution.[3,4] There remains the further difficulty that protein –SH groups are often readily autoxidizable. Unless extensive precautions are observed, a reduced protein cannot be assumed to contain the same number of –SH groups, after it is isolated and freed from reagent mercaptan, as it had in the reaction mixture. This difficulty has been removed in the present study by alkylation of the –SH groups prior to isolation of the protein.

When lysozyme is treated with thioglycol (mercaptoethanol) in saturated urea solution at *p*H 5, it remains dissolved and its activity disappears rapidly, quite in contrast to the slow insolubilization and inactivation occurring in the absence of urea. Subsequent addition of iodoacetamide to the reaction mixture, followed by sodium hydroxide until a constant *p*H of 8.5–9 is obtained, causes alkylation of the thio groups of both the excess thioglycol and the reduced protein and thus terminates the reaction and stabilizes the product. Further, this technique facilitates the analytical evaluation of the extent of reduction, since one amide group is stably introduced into the protein for each –SH group alkylated. The loss of cystine + cysteine is an additional criterion of the extent of reduction–alkylation.[5]

In the specific case of lysozyme the alkylated product differs from the unreacted protein in its low solubility in water above *p*H 7. This affords a simple technique of separation. Depending upon the concentration of urea, and on the reaction time, more or less of the insoluble inactive product is obtained, while the residual amount of protein appears to be only slightly affected in composition as well as in enzymatic activity. Thus the reduction of lysozyme in urea, in contrast to most protein modifications, is largely an all-or-none phenomenon.

While the *amount* of insoluble inactive product formed in saturated urea depends upon reaction time, thioglycol concentration, etc., the *extent* of its reduction appears almost the same in all samples (Table I). The amount of amide nitrogen introduced is approximately equivalent to the cystine originally present, and less than 5% of the original cystine remains. In contrast, the soluble fraction, which after 20–30 minute reaction periods comprises about 50%, retains most of the original activity and shows amide-N and residual cystine values indicating that no more than one-fifth of the –S–S– bonds had been reduced and alkylated. Thus reduction of the first or second disulfide bond appears to be the rate-limiting reaction, followed by a rapid reductive cleavage of all other disulfide bonds.

Disulfide bonds have been shown to supply the crosslinks between peptide chains of proteins. Thus the sub-unit of insulin (mol. wt. 12,000) appears to consist of four peptide chains held together by 6 disulfide bonds. If lysozyme were similarly constituted, reduction–alkylation should yield a mixture of the individual peptide chains. On the basis of microbiological and chemical analyses it was recently concluded that lysozyme contained only one or two peptide chains.[6] The cleavage of

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

[2] H. S. Olcott and H. Fraenkel-Conrat, *Chem. Revs.*, **41**, 162 (1947).

[3] H. Fraenkel-Conrat, *J. Biol. Chem.*, **142**, 119 (1942).

[4] G. L. Miller and K. J. I. Andersson, *ibid.*, **144**, 465, 475 (1942).

[5] Principally the same technique may be, and has been, applied to the determination of –SH groups of proteins. In the absence of small-molecular mercaptans, the alkylation of protein –SH groups can be determined potentiometrically by adjusting both the protein-urea and the iodoacetamide solutions to *p*H 8.5, then mixing them and titrating the HI liberated until the solution shows a constant *p*H of 8.5 for at least 30 minutes. The product can then be isolated by dialysis and the titration results confirmed by amide-N, and possibly also cystine analyses. Egg albumin was found to contain 1.0 –SH groups per 10^4 g., in agreement with the literature.

[6] J. C. Lewis, N. Snell, D. J. Hirschmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **186**, 23 (1950).