

over the manometric method in regard to reliability of results and number of experimental observations necessary have been previously pointed out. This means that the new method can be applied to enzyme studies which were not feasible before because of the time required to obtain a fairly reliable measure of the enzyme.

Summary

1. A chronometric method is described for the measurement of the catecholase activity of tyrosinase using catechol as a substrate in the presence of ascorbic acid.

2. The enzyme activity has been shown to be a function of the time required by the enzyme to produce quinone just in excess of the quantity of ascorbic acid. The presence of quinone is detected by sampling the reaction mixture into a starch-hydriodic acid solution.

3. Curves correlating the chronometric method with the manometric method are given as well as expressions which allow the calculation of the enzyme activity.

4. Results with the chronometric method have been shown to be much more reliable than those obtained using the manometric method and can be obtained with considerably less effort.

5. The behaviors of high catecholase and high cresolase preparations have been compared using the new method. The importance of catechol concentration as an aid in characterizing the enzyme preparations has been pointed out.

6. The behavior of enzyme preparations whose properties resemble in varying degree those of the two main types has been observed using the chronometric method. They have been called intermediate preparations.

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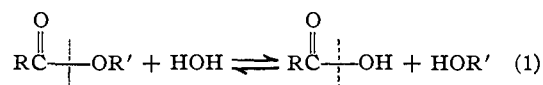
[CONTRIBUTION OF THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

Cleavage of the Alkyl-Oxygen Bond in the Hydrolysis of Esters. *t*-Butyl 2,4,6-Tri-methylbenzoate

BY SAUL G. COHEN AND A. SCHNEIDER

It is generally accepted that the hydrolysis of esters of carboxylic acids and the formation of these esters are reactions which involve no rupture of the carbon-oxygen bond of the alcohol.¹ The work of Reid, *et al.*,^{1a} on the esterification of thio-acids, the hydrolysis of thio-esters and the esterification of mercaptans gave indirect evidence for this. The sum of information which is available from the hydrolysis of carboxylic esters of optically active alcohols confirms this conclusion. There are many cases in which such esters have been hydrolyzed and there is no simple instance of inversion of configuration of the alcohol.² If the alkyl-oxygen bond were involved, it is likely that examples of inversion of configuration would be found. The base-catalyzed hydrolysis of amyl acetate in water enriched in the oxygen

isotope of mass 18,³ and the acid-catalyzed esterification of benzoic acid in methanol enriched in heavy oxygen⁴ gave direct evidence that these reactions involve no cleavage of the carbon-oxygen bond of the alcohol.



These conclusions have been based on a study of the reactions of primary and secondary alcohols and their carboxylic acid esters, and, apparently, are valid generalizations for these classes of compounds. There are a few exceptions in these classes: compounds which because of special structural features, show unusual reactivity. These include β -lactones,⁵ the hypothetical α -lactones⁶ and an ester of a secondary allylic alcohol.⁷

We have investigated the reactions of some es-

(1) After this manuscript had been prepared, a discussion of this problem appeared in the *Annual Reports on the Progress of Chemistry* for 1940, London, **38**, 1941, 229 ff. This article summarized the evidence which indicated that these reactions involve a rupture of the acyl-oxygen bond, and concluded that little if any contribution to ester hydrolysis is made by cleavage of the alkyl-oxygen bond.

(1a) Reid, *Am. Chem. J.*, **43**, 489 (1910); Pratt and Reid, *This Journal*, **37**, 1934 (1915); Sachs and Reid, *ibid.*, **38**, 2746 (1916).

(2) (a) Fischer, *Ann.*, **394**, 360 (1912); (b) Verkade, *et al.*, *ibid.*, **477**, 287, 297 (1930).

(3) Polanyi and Szabo, *Trans. Faraday Soc.*, **30**, 508 (1934).

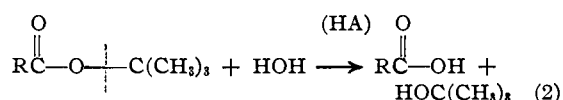
(4) Roberts and Urey, *This Journal*, **60**, 2391 (1938); **61**, 2584 (1939).

(5) Olson and Miller, *ibid.*, **60**, 2687 (1938).

(6) (a) Winstein, *ibid.*, **61**, 1635 (1939); (b) Bean, Kenyon and Phillips, *J. Chem. Soc.*, 303 (1936).

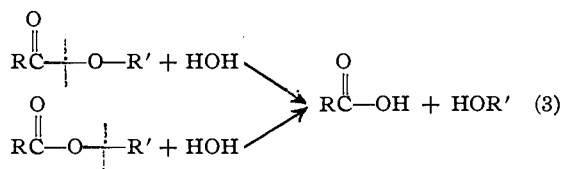
(7) (a) Kenyon, Partridge and Phillips, *ibid.*, 85 (1936); (b) Burton and Ingold, *ibid.*, 904 (1928).

ters of tertiary alcohols. Tertiary alcohols are compounds in which the carbon-oxygen bond is easily broken, and our results show that this property is also found in esters of these alcohols. We conclude that there is an important exception to the generalization that the hydrolysis of esters of carboxylic acids leaves the carbon-oxygen bond of the alcohol unbroken; the acid-catalyzed and uncatalyzed hydrolysis and alcoholysis of esters of tertiary alcohols involve a rupture of the molecule on the alcohol side of the ether linkage, a course which is not followed in the corresponding reactions of carboxylic acid esters of primary and secondary alcohols.

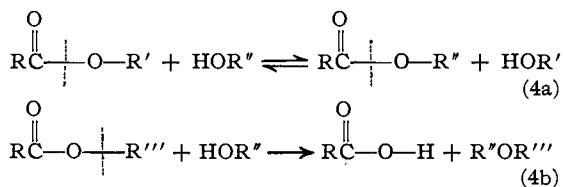


These acid-catalyzed reactions occur readily⁸ and it is practical to hydrolyze these compounds in an acidic rather than in a basic medium.

The hydrolysis of esters, because of the identity of the two atoms which are linked to oxygen in the water molecule, leads to the same products, whether the rupture occurs on the acid or the alkyl side of the ether linkage. In either case, an acid and an alcohol are formed



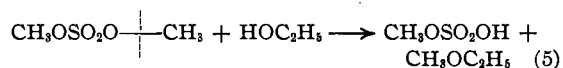
and the nature of the products does not tell where the cleavage of the ester molecule occurred. However, the alcoholysis reaction leads to different products, depending upon which bond of the ester is broken, and the position of the cleavage is revealed by a consideration of the nature of the products which are formed in this reaction



without recourse to the use of oxygen isotopes. Omitting considerations of the mechanisms of these reactions, the normal rupture of the acyl-oxygen bond (equation 4a) leads to ester interchange, while the rupture of the alkyl-oxygen

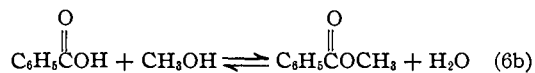
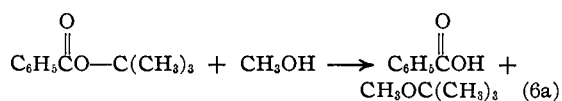
bond (equation 4b) will form an acid and an ether. The formation of these products, an acid and an ether, by the alcoholysis of an ester, must, in the absence of another mechanism for their production, be considered valid evidence for the cleavage of the ester at the alkyl-oxygen bond.

This mode of reaction, leading to these products, is shown by esters of the strong acids, both mineral and organic. The esters of sulfonic acids are hydrolyzed by cleavage of the alkyl-oxygen bond,⁹ and the alkylation of an alcohol by an alkyl sulfate proceeds by cleavage of this bond and leads to the corresponding acid and ether.



We have found that the alcoholysis of the weak carboxylic acid esters of tertiary alcohols similarly leads to the corresponding carboxylic acids and ethers, and with this as part of our evidence, we conclude that these reactions also proceed by rupture of the alkyl-oxygen bond (equation 4b). This reaction course, found in the strong acid esters of primary alcohols, does not seem to occur in the weak carboxylic acid esters until it is favored by the presence of the tertiary alkyl group.

When a solution of *t*-butyl benzoate in anhydrous methanol was boiled for four days, *t*-butyl methyl ether (60.7% yield), benzoic acid (22.6%) and methyl benzoate (61.9% yield) were recovered.



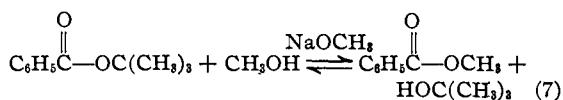
It was shown that the interaction of *t*-butyl alcohol and methanol in the presence of benzoic acid did not lead to *t*-butyl methyl ether. Tertiary-butyl alcohol is a product of the possible ester interchange reaction, and benzoic acid might arise from hydrolysis of the ester by traces of water. We believe that the benzoic acid and the *t*-butyl methyl ether arise according to equation 6a in an essentially irreversible reaction, and that most, if not all, of the methyl benzoate results from the subsequent esterification of the benzoic acid (6b). Since *t*-butyl alcohol would also be produced in an ester interchange, and we found no evidence of it, it is unlikely that more than traces of the methyl

(8) Norris and Rigby, *This Journal*, **54**, 2089 (1932).

(9) Ferns and Lapworth, *J. Chem. Soc.*, **101**, 273 (1912).

benzoate were due to an ester interchange occurring simultaneously with reaction 6a.

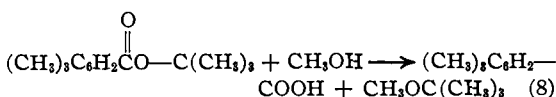
The course of this reaction was changed by alkali. When a solution of *t*-butyl benzoate (0.417 mole) in anhydrous methanol containing sodium methoxide (0.0416 mole) was boiled, the normal ester interchange took place. Methyl benzoate (71.6% yield) and *t*-butyl alcohol (81.7% yield) were obtained.



If *t*-butyl methyl ether was formed, there was too little for us to isolate.

To gain further information about the reactions of esters of tertiary alcohols, we prepared *t*-butyl 2,4,6-trimethylbenzoate and studied its reactions. The interaction of the silver salt of 2,4,6-trimethylbenzoic acid and *t*-butyl chloride, a standard method of esterifying this acid,¹⁰ regenerated the acid. 2,4,6-Trimethylbenzoyl chloride proved to be quite reactive and not subject to steric hindrance, and its reaction with *t*-butyl alcohol in pyridine solution produced the desired ester in 79% yield. Esters of 2,4,6-trimethylbenzoic acid normally show steric hindrance and resist hydrolysis.¹¹ The *t*-butyl ester proved to be stable to base, but was readily hydrolyzed in acid.

When a solution of *t*-butyl 2,4,6-trimethylbenzoate in anhydrous methanol was boiled for seven days, *t*-butyl methyl ether (12.5% yield), 2,4,6-trimethylbenzoic acid (6.1% yield) and the unchanged ester (82.5% yield) were isolated.



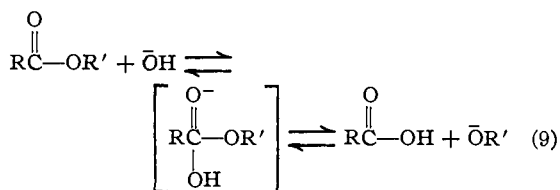
Our experiments showed that *t*-butyl methyl ether is not formed when *t*-butyl alcohol and methanol are boiled in the presence of 2,4,6-trimethylbenzoic acid.

As in the case of *t*-butyl benzoate, no evidence of this "abnormal" reaction was found in the presence of alkali. When a solution of *t*-butyl 2,4,6-trimethylbenzoate (0.35 mole) and sodium methoxide (0.0416 mole) in methanol was boiled for seven days, we recovered only the unchanged ester. We found no evidence either of ester interchange or of formation of the ether. The base-

(10) V. Meyer and Sudborough, *Ber.*, **27** 1586 (1894).

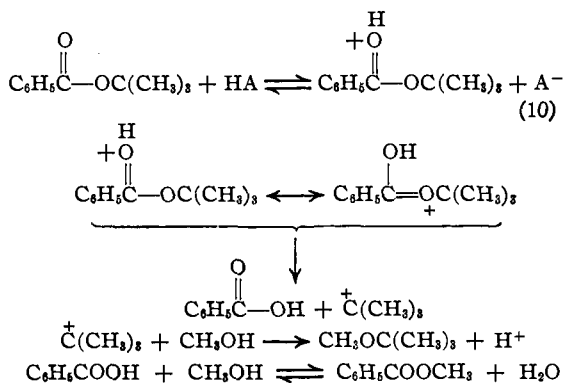
(11) Goldschmidt, "Stereochemie," Akademische Verlagsgesellschaft m. b. H., Leipzig, 1933, pp. 222 ff.

catalyzed ester interchange and hydrolysis reactions of esters probably proceed by a nucleophilic attack by an alkoxide or hydroxide ion on the carbonyl carbon atom of the ester.



Esters of 2,4,6-trimethylbenzoic acid show steric hindrance to reactions which proceed by this mechanism,¹¹ and we should expect the *t*-butyl ester to show such hindrance to a high degree. To this may be ascribed the resistance to the base-catalyzed ester interchange.

A possible course for the alcoholysis of these esters to form the corresponding carboxylic acids and ethers is the following. First, there may be a slow reaction, occurring in neutral solution and leading to a small amount of the products. We may consider this to be an uncatalyzed or solvolytic¹² reaction. The driving force for this reaction may be hydrogen bonding between an alcohol molecule and the carbonyl-oxygen atom of the ester, a weak electrophilic attack on oxygen, which is followed by ejection of the *t*-butyl group and the formation of the products. This is a slow reaction, accounting for a very small fraction of the products which are found. The major part of the products results from a similar, but acid-catalyzed, reaction. A small amount of acid is produced by the initial slow reaction, and this catalyzes the subsequent reaction.¹³



If the reaction were entirely solvolytic, result-

(12) Steigman and Hammett, *THIS JOURNAL*, **59**, 2536 (1937); Farinacci and Hammett, *ibid.*, **59**, 2542 (1937).

(13) Alternatively, the action of methanol as a weaker acid HA' in equation 10 may be considered the driving force for the initial reaction.

ing from an attack by alcohol molecules on the oxygen atoms of the ester, we should expect it to take place in the basic medium. The fact that the alcoholysis of *t*-butyl benzoate in the presence of sodium methoxide seems to give no evidence of rupture of the alkyl-oxygen bond and ether formation, need not be inconsistent with a solvolytic mechanism. If the base-catalyzed ester interchange were much faster than the ether forming reaction, the products of the latter reaction might not be detected. However, the reactions of *t*-butyl 2,4,6-trimethylbenzoate indicate that this is not the case. This compound, on treatment with methanol in the presence of alkali, failed to form detectable quantities of *t*-butyl methyl ether and 2,4,6-trimethylbenzoic acid, although ester interchange was not taking place. This indicates that, although this reaction starts in neutral solution, and may proceed very slowly in the presence of base, the major portion of the reaction depends on acid catalysis.

The ease with which the *t*-butyl group is ejected from these carboxylic esters is related to the special reactivity of the solvolytic or S_N1^{14} type shown by the analogous *t*-butyl halides. However, the alcoholysis of *t*-butyl chloride is solvolytic and its rate is independent of small concentrations of acid or base.^{15,16} It is possible that esters of highly arylated carbinols¹⁷ may show a rapid solvolytic reaction. We cannot say whether, in these reactions, the *t*-butyl group has any free existence as an ion, or reacts with the solvent without being completely liberated. More detailed information may be obtained from a study of the steric course of these reactions when made on esters of optically active alcohols which have very reactive hydroxyl groups.

A Stuart model of *t*-butyl 2,4,6-trimethylbenzoate shows that the oxygen atoms are not strongly hindered, although the carbonyl carbon atom is. The effect of acid catalysis in these reactions is shown by the following experiments. A solution of *t*-butyl 2,4,6-trimethylbenzoate in 39.5% hydrogen chloride in methanol was kept at 0° for 30 minutes and was then poured into ice and water. 2,4,6-Trimethylbenzoic acid was precipitated and was recovered in nearly quantitative yield. When the corresponding methyl ester

(14) Hughes, Ingold and Patel, *J. Chem. Soc.*, 526 (1933).

(15) Hughes, *ibid.*, 255 (1935).

(16) The hydrolysis of sulfuric and sulfonic esters is also solvolytic, the rates being independent of small concentrations of acid and base. Demény, *Rec. trav. chim.*, 50, 60 (1931).

(17) Gomberg and Davis, *Ber.*, 36, 3926 (1903).

was treated in the same way, no evidence of hydrolysis was found.¹⁸ The great difference in reactivity of the two compounds is consistent with a deep-seated change in mechanism in the reaction of the tertiary compound.

Similar evidence is obtained in hydrolysis experiments. When *t*-butyl 2,4,6-trimethylbenzoate was boiled for one hour in 18% hydrochloric acid, hydrolysis took place, and the acid was recovered in nearly quantitative yield. When the reaction was carried out in boiling 20% sodium hydroxide for one hour, no evidence of hydrolysis was found. This compound shows nearly prohibitive steric hindrance to the base-catalyzed mechanism, attack by a hydroxyl ion on the carbonyl carbon atom, followed by ejection of the *t*-butoxyl group (equation 9). That it shows little hindrance to the acid-catalyzed reaction is consistent with the alternative mechanism suggested here for esters of tertiary alcohols (equation 10), coordination of a proton with the carbonyl oxygen atom, followed by loss of the *t*-butylcarbonium ion. The steric hindrance is not an inherent property of the molecule, but is relative to the mechanism which is involved.

There are relevant kinetic data available for the hydrolysis of esters of the type CH_3COOR .^{19a,b} As the group R is varied from CH_3 to C_2H_5 , *i*- C_3H_7 and *t*- C_4H_9 , the rate of the base-catalyzed hydrolysis falls off, that of methyl acetate being 120 times that of *t*-butyl acetate. In this series the rate of the acid-catalyzed hydrolysis falls through a slight minimum and rises again, that of the *t*-butyl ester being about 15% faster than that of the methyl ester. These facts are consistent with the following interpretation: in the base catalyzed reactions, a single mechanism operates, the attack of the hydroxyl ion on the carbonyl carbon atom followed by ejection of the alkoxy group (equation 9). The rate of this reaction diminishes as the alkyl group is changed from primary to tertiary. In the acid catalyzed reaction, there is one mechanism available for esters of primary and secondary alcohols involving a rupture of the acyl-oxygen bond, and there is the alternative mechanism (equation 10) available

(18) The methyl ester may be hydrolyzed in concentrated sulfuric acid by rupture of the acyl-oxygen bond [Treffers and Hammett, *THIS JOURNAL*, 59, 1708 (1937)]. The conditions in our experiments were not drastic enough to bring this about, and the alternative mechanism is not available to the primary ester.

(19) (a) Skrabal and Hugetz, *Monatsh.*, 47, 17 (1926); (b) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 213.

for the tertiary alcohol ester, involving ejection of the alkyl group.²⁰

We thank Professor Paul D. Bartlett for valuable discussions and suggestions.

Experimental

Methanol was distilled from magnesium methoxide and redistilled through a 35-cm. column packed with glass beads: b. p. 64.7° (760 mm.), n_D^{20} 1.3263.

Pyridine.—Mallinckrodt analytical reagent pyridine was dried over solid potassium hydroxide.

***t*-Butyl alcohol** was dried roughly over potassium carbonate and was distilled over sodium. Material melting above 24° was satisfactory.

***t*-Butyl benzoate** was prepared by the interaction of *t*-butyl alcohol and benzoyl chloride in pyridine solution, the method of Norris and Rigby.⁸ The product was obtained in 80% yield, b. p. 112° (18 mm.), n_D^{25} 1.4896.

The Reaction of *t*-Butyl Benzoate with Methanol.—A solution of 74.3 g. (0.417 mole) of *t*-butyl benzoate in 300 cc. of anhydrous methanol was boiled under reflux for four days, and was then fractionated. Fractions boiling at 60–64° (69.5 g.) and at 64–66° (172.7 g.) were collected. No evidence was found for any product with a boiling point between that of methanol and that of the higher boiling material, methyl benzoate.

Water (50 cc.) was added to fraction 1, and fractionation was carried out through a 60-cm. Podbielniak column. The material boiling between 51.1 and 58° was collected (17.4 g.). Water (100 cc.) was added to the second fraction and the material boiling between 51.2 and 64° (16.9 g.) was collected. The combined lower boiling products were dried over sodium sulfate and distilled over 2 g. of sodium wire. *t*-Butyl methyl ether was collected, b. p. 54.3–55.1°, $n_D^{27.5}$ 1.3658, 22.8 g. (60.7%).

The residue in the reaction vessel, after removal of the *t*-butyl methyl ether and the methanol, was further distilled in vacuum. Methyl benzoate was collected, b. p. 70–71° (6 mm.), leaving a solid residue of benzoic acid. The methyl benzoate was redistilled, b. p. 60–62° (4 mm.), n_D^{24} 1.5133, 35.2 g., 61.9% yield. The benzoic acid residue was crystallized from benzene, 11.5 g., 22.6% yield, m. p. 121–122°, alone, and when mixed with an authentic sample.

***t*-Butyl methyl ether** was prepared by the method of Norris and Rigby,⁸ the reaction of *t*-butyl alcohol and methanol in 10% sulfuric acid. It was obtained in 60% yield, b. p. 54.9–55.2°, n_D^{20} 1.3665. An attempt to isolate it from a large amount of methanol, the conditions existing in the preceding experiment, led to 70% recovery. To a solution of 20 cc. of *t*-butyl methyl ether in 100 cc. of methanol, 40 cc. of water was added, and the solution was fractionated. The fraction boiling at 50.8–52° was collected, dried over sodium sulfate, boiled over sodium, and distilled. The ether was recovered (70%), b. p. 54.5–55.0° (748 mm.), n_D^{25} 1.3665.

The interaction of *t*-butyl alcohol and methanol in the presence of benzoic acid did not lead to *t*-butyl methyl

ether. A solution of 31 g. (0.417 mole) of *t*-butyl alcohol and 51 g. (0.417 mole) of benzoic acid in 300 cc. of anhydrous methanol was boiled for seven days. The characteristic odor of *t*-butyl methyl ether was not detected. Water (50 cc.) was added and the solution was distilled. The temperature rose immediately to 64.5°; we found no evidence of ether formation.

The Reaction of *t*-Butyl Benzoate with Methanol in the Presence of Sodium Methoxide.—A solution of 74.3 g. (0.417 mole) of *t*-butyl benzoate in 300 cc. of methanol containing 2.25 g. (0.0416 mole) of sodium methoxide was boiled under reflux for four days and was then fractionated.

Fraction 1, methanol, b. p. 64.4–65.0° (157.4 g.), was discarded. No evidence of *t*-butyl methyl ether was found. Fraction 2, a mixture of methanol and *t*-butyl alcohol, b. p. 65.0–72.0° (51.5 g.), was collected. At this point, a gelatinous precipitate interfered with further distillation. The residue was washed with water and dried over sodium sulfate; the water was extracted with ether, and the ether extracts were added to fraction 2. The aqueous layer was distilled, the fraction boiling at 76–93° was collected and added to fraction 2. The residue was acidified; no evidence of benzoic acid was found. The combined fraction 2 was then distilled through a 60-cm. Podbielniak column, and the fraction boiling at 71–78.4° was collected (28.1 g.). This was boiled over 3 g. of sodium wire for two hours and distilled, yielding *t*-butyl alcohol, b. p. 81.8–82.6°, $n_D^{28.5}$ 1.3850, m. p. 21–22°, 25.2 g., 81.7% yield.

The higher boiling reaction residue, which had been washed with water and dried, was twice distilled in vacuum, yielding methyl benzoate, b. p. 71° (7 mm.), n_D^{27} 1.5140, 40.7 g., 71.6% yield. No *t*-butyl benzoate was recovered.

Bromomesitylene was prepared by the bromination of mesitylene.²¹

2,4,6-Trimethylbenzoic acid was prepared by carbonation of mesitylenemagnesium bromide,²² m. p. 151–152°.

***t*-Butyl 2,4,6-Trimethylbenzoate**.—A suspension of 1 g. of silver 2,4,6-trimethylbenzoate in 20 cc. of *t*-butyl chloride was agitated for one-half hour and allowed to stand at room temperature for fifteen hours. The solid which formed was collected and was apparently silver chloride. The filtrate was evaporated to dryness on the water-bath. The residue was crystallized from benzene, and was identical with 2,4,6-trimethylbenzoic acid.

A solution of 50 g. of 2,4,6-trimethylbenzoic acid in 100 g. of thionyl chloride was boiled under reflux for two hours, and was fractionated. The acyl chloride was obtained in 94% yield, b. p. 124° (26 mm.).

To a solution of 15.8 g. of *t*-butyl alcohol in 23.9 g. of pyridine, 26.6 g. of 2,4,6-trimethylbenzoyl chloride was added with stirring. The temperature rose rapidly to 65° and pyridine hydrochloride precipitated. Stirring was continued for two hours, and the mixture was allowed to stand overnight. It was poured into water, extracted with ether, washed with sodium carbonate, dried and distilled. *t*-Butyl 2,4,6-trimethylbenzoate was obtained in 79% yield, b. p. 114–115° (3 mm.), 142° (13 mm.), d_4^{25} 0.9654, n_D^{25} 1.4920.

(21) Smith, *Org. Syn.*, 11, 24 (1931).

(22) Smith and Byrkit, *This Journal*, 55, 4307 (1933).

(20) In experiments, which we are not reporting at this time, we have found, as confirmation for this, that the reaction of *t*-butyl acetate with methanol, both in the presence and absence of added weak carboxylic acids, produces *t*-butyl methyl ether. Cf. Tronov and Seibgatullen, *Ber.*, 62B, 2850 (1929).

Anal. Calcd. for $C_{14}H_{20}O_2$: C, 76.32; H, 9.15.
Found: C, 76.15, 76.12; H, 9.08, 9.06.

The Reaction of *t*-Butyl 2,4,6-Trimethylbenzoate with Methanol.—A solution of 92.5 g. of the ester in 300 cc. of methanol was boiled under reflux for seven days. The material boiling at 62–64° was collected. To it 60 cc. of water was added, and the part of this which boiled below 58° was collected. This was washed, dried over sodium sulfate, boiled over sodium, and distilled. *t*-Butyl methyl ether was obtained, b. p. 54.7–55.0°, n_D^{27} 1.3661, 4.6 g., 12.5% yield.

The methanol was then distilled from the reaction mixture. No evidence of *t*-butyl alcohol was found. The residue was further distilled in vacuum, yielding 76.4 g. (82.5% recovery) of *t*-butyl 2,4,6-trimethylbenzoate, b. p. 114–116° (3 mm.), n_D^{25} 1.4920. The white solid residue was dissolved in 10% sodium carbonate, precipitated by acidification and crystallized from ligroin, yielding 5.8 g. of 2,4,6-trimethylbenzoic acid, m. p. 151–152°, a 48% yield, based on the amount of ester consumed.

A solution of 76 g. (0.35 mole) of *t*-butyl 2,4,6-trimethylbenzoate in 250 cc. of methanol containing 2.25 g. (0.0416 mole) of sodium methoxide was boiled for seven days, and was then fractionated. The methanol was recovered; no *t*-butyl methyl ether was found, nor was *t*-butyl alcohol. The higher boiling residue was washed with water, dilute acid and water, and was distilled. The ester was recovered, 61 g. (81%), n_D^{25} 1.4920, b. p. 115° (3 mm.). The alkaline wash waters were acidified, but no 2,4,6-trimethylbenzoic acid was precipitated.

Acid Hydrolysis of *t*-Butyl 2,4,6-Trimethylbenzoate.—A suspension of 0.288 g. of the ester in 5 cc. of 1:1 (18%) hydrochloric acid was boiled for one hour, during which time the 2,4,6-trimethylbenzoic acid precipitated. The mixture was cooled and the solid was collected, 0.210 g., 97% yield, m. p. 150–151°, alone and when mixed with an authentic sample.

Treatment of *t*-Butyl 2,4,6-Trimethylbenzoate with Alkali.—A suspension of 0.483 g. of the ester in 5 g. of 20% sodium hydroxide was boiled for one hour. The cooled mixture was extracted with 6 cc. of ether, and the ether was washed with a little water. The aqueous portions were combined, boiled to remove dissolved ether and acidified with 9 cc. of concentrated hydrochloric acid. No 2,4,6-trimethylbenzoic acid was precipitated. Blank tests showed that not more than 18 mg. of the acid would be lost in these manipulations, indicating that the reaction had proceeded certainly less than 5%, and probably less than 3%.

Treatment of the Esters of 2,4,6-Trimethylbenzoic Acid with Alcoholic Hydrogen Chloride.—Hydrogen chloride, dried through sulfuric acid and phosphorus pentoxide was dissolved at 0° in anhydrous methanol. The solution was 39.5% in hydrogen chloride.

(1) A solution of 1.90 g. of *t*-butyl 2,4,6-trimethylbenzoate in 20 cc. of 39.5% hydrogen chloride in methanol was allowed to stand for thirty minutes at 0° in a paraffin-sealed glass-stoppered flask. The solution was poured into 100 cc. of ice water; a copious white solid was precipitated. The mixture was extracted with 100 cc. of ether, and the ethereal solution was extracted with 100 cc. of 10% sodium carbonate. The carbonate solution was boiled to remove ether, and acidified with 40 cc. of concentrated

hydrochloric acid. The solid was collected and washed with water, 1.347 g., 96.5% yield, m. p. 150–151° alone, and when mixed with 2,4,6-trimethylbenzoic acid.

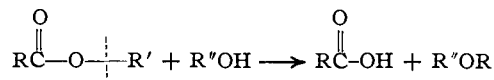
(2) In an earlier experiment, a 10% solution of *t*-butyl 2,4,6-trimethylbenzoate in fuming hydrogen chloride in absolute methanol was prepared under somewhat less strictly anhydrous conditions. It was allowed to stand at room temperature for three hours, and then the methanol and hydrogen chloride were removed in vacuum under nitrogen. A solid residue, 2,4,6-trimethylbenzoic acid, was left in practically quantitative yield.

Methyl 2,4,6-trimethylbenzoate¹⁰ was prepared by the action of methyl iodide on silver 2,4,6-trimethylbenzoate, b. p. 156° (72 mm.), n_D^{27} 1.5054.

(3) A solution of 2 cc. of methyl 2,4,6-trimethylbenzoate in 20 cc. of 39.5% alcoholic hydrogen chloride, prepared in the same way as in experiment 1, above, was allowed to stand for thirty minutes at 0° in a paraffin-sealed glass-stoppered flask. The solution was poured into 100 cc. of ice water; oil droplets formed, but no solid appeared. The mixture was extracted with 100 cc. of ether, and the ether was extracted with 100 cc. of 10% sodium carbonate. The carbonate solution was boiled, cooled and acidified. No precipitate was found.

Summary

Although the hydrolysis of carboxylic esters leads to the same products, an acid and an alcohol, whether the rupture occurs on the acid or the alkyl side of the ether linkage, in the alcoholysis reaction, the nature of the products does depend on the position of the cleavage. The formation of the corresponding acid and ether in an alcoholysis, instead of the normal ester-interchange products, is evidence for cleavage of the ester at the alkyl-oxygen bond.



The *t*-butyl esters of benzoic, 2,4,6-trimethylbenzoic and acetic acids, on treatment with methanol, form the corresponding acids and *t*-butyl methyl ether. This reaction is inhibited by alkali. It is suggested that the uncatalyzed and acid-catalyzed alcoholysis and hydrolysis of esters of tertiary alcohols (as examples of alcohols with reactive hydroxyl groups) proceed by rupture of the ester at the alkyl-oxygen bond. The rapidity of the acid-catalyzed hydrolysis of *t*-butyl 2,4,6-trimethylbenzoate, one of a class of esters which normally resists hydrolysis because of steric hindrance, is also evidence for a change in mechanism in this reaction. The data which are available for the rates of the base- and acid-catalyzed hydrolysis of esters of the type CH_3COOR , where R is successively CH_3 , C_2H_5 , *i*- C_3H_7 , and *t*- C_4H_9 ,

are also consistent with the hypothesis that the mechanism of the acid-catalyzed hydrolysis of esters of tertiary alcohols is different from that of

other ester hydrolyses, and involves a rupture of the alkyl-oxygen bond.

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Anomalous Dispersion of Dipolar Ions

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Low frequency measurements of the dielectric constant of amino acids, peptides and other similar dipolar ions in solution in polar liquids indicate polarizations which are the same as those calculated statistically on the assumption of free rotation about the various single bonds of the chain between the charges.¹ These low frequency measurements cannot, as shown by Kuhn,² distinguish between polarizations resulting from orientation of the molecule as a rigid body and polarizations resulting from distortion under the influence of the field. The latter possibility is a likely one if the rotation about the bonds is unhindered. If, on the other hand, the rotation is subject to steric hindrance no rotation will occur in the short period of high frequency oscillations. The molecule will then orientate as a rigid body. To distinguish between these two alternatives, we must make observations at frequencies comparable with those corresponding to the relaxation times of the molecules regarded as rigid bodies. If the dipolar ions contribute to the polarization by orientation as rigid bodies a region of anomalous dispersion will be observed at frequencies corresponding to this relaxation time. If, however, free rotation still occurs at these high frequencies, orientation of the dipoles with the electric field may be expected to occur by a deformation of the loose jointed chain. The region of anomalous dispersion would then occur at much higher frequencies.

The two alternatives are illustrated by solutions of the proteins and of decanoic acid polymers.³ Both molecules have large electric moments, the proteins by virtue of their dipolar structure and the decanoic acid polymers because of the large number of ester linkages. In the case of the proteins relaxation times have been observed at radio frequencies of the order of those calculated by

Stokes' law. The solutions of decanoic acid polymers, however, which, as spheres, would be expected to have a relaxation time corresponding to ordinary radio frequencies on the basis of Stokes' law show no dispersion at frequencies as high as 100 megacycles. Moreover, the measured polarizations are the same as those calculated for independent orientation of the polar groups. The present problem is to distinguish between these two alternatives in the case of solutions of amino acids and peptides.

Unfortunately, even for the largest available dipolar ions other than proteins, the relaxation times predicted by Stokes' formula, on the assumption that the ions are rigid spheres, correspond to frequencies of the order of 10^8 cycles. Relaxation times may be calculated from the variation of either the observed dielectric constant or the power absorption, or both, over a frequency range. Measurements at these ultra high frequencies are, however, difficult in view of the importance of distributed inductance and capacitance and the resulting complication of the electrical circuit analysis. A further restriction is the large a. c. conductivity associated with the very power absorption effect we wish to measure.^{3a} For the case of concentrated solutions of highly polar solutes, this may be sufficient to make the measurements inaccurate or even impossible. Consideration must also be given to limiting the amount of substance required for an experiment in view of the small amounts of large dipolar ions available.

Very little previous experimental work has been done on this problem of determining the relaxation times of amino acids and peptides. Fricke and Parts⁴ made some observations at 4.6 meters (65.6 megacycles) on various amino acids. The

(1) J. Wyman, Jr., *J. Phys. Chem.*, **43**, 143 (1939).

(2) W. Kuhn, *Z. physik. Chem.*, **A175**, 1 (1935).

(3) J. Wyman, Jr., *THIS JOURNAL*, **60**, 328 (1938).

(3a) Theoretically the ratio of resistance to reactance at the frequency for which this is a maximum increases without limit with the static value of the dielectric constant.

(4) H. Fricke and A. Parts, *J. phys. Chem.*, **42**, 1171 (1938).