TABLE I

ANALYTICAL DATA

Gas	Volume cc., S.T.P.	Volume Moles	KBr-KBrO$_3$ soln. Cc.	KBr-KBrO$_3$ soln. Moles Br_2	Double bonds
Propylene	86	0.00384	14.56	0.00390	1.01
Allene	105	.00469	34.60	.00926	1.97
From Reaction	129	.00578	24.70	.00661	1.14
From Reaction	146	.00652	27.00	.00722	1.11

Pseudo-nitrosites.—Two hundred cc. of propylene gas was confined for one to two days in a tube with 0.5 cc. of liquid nitrogen trioxide and 3–4 cc. of absolute ether. The propylene pseudo-nitrosite[16] which separated was crystallized from ethyl acetate; m. p. 120°; yield 0.2 g. The crystals did not decompose in a week. Allene pseudo-nitrosite,[17] m. p. 88°, was prepared similarly in about 0.1-g. yield. These crystals decomposed within twenty-four hours. The precipitate which formed from 200 cc. of a 3:2 mixture of propylene and allene was 0.1 g. of propylene pseudo-nitrosite, m. p. 120°. In another experiment 2 drops of diallyl was added to the mixture of ether and nitrogen trioxide before absorbing 160 cc. of propylene gas. The pseudo-nitrosite obtained in this case was a yellow, non-crystallizable oil, $n^{25}D$ 1.469.

Two 200-cc. samples of the gas from the reaction were treated similarly with the nitrogen trioxide reagent. Here, also, a yellow, non-crystallizable oil, $n^{25}D$ 1.460, was obtained.

(16) Demjanov, *J. Russ. Phys.-Chem. Soc.*, **33**, 275 (1901); *Chem. Zentr.*, **72**, II, 338 (1901).

(17) Demjanov and Ivanov, *Compt. rend. acad. sci.* (U. R. S. S.) [N. S.], **1**, 318 (1934); *C. A.*, **28**, 4374 (1934).

Summary

These new compounds have been prepared: 2,6-dipropyl-4-methylphenol, 2-allyl-6-propyl-4-methylphenol, allyl 2-propyl-4-methylphenyl ether, allyl 2,6-dipropyl-4-methylphenyl ether, 2,6-dipropyl-4-methylphenyl 3,5-dinitrobenzoate, 2-allyl-4-methylphenoxyacetic acid, and 2-propyl-4-methylphenoxyacetic acid.

During the rearrangement of allyl *p*-cresyl ether into 2-allyl-4-methylphenol, some 2,6-diallyl-4-methylphenol is formed concurrently.

Propylene, not allene, is the gas evolved during pyrolysis of allyl 2,6-dipropyl-4-methylphenyl ether.

EVANSTON, ILLINOIS RECEIVED JUNE 7, 1937

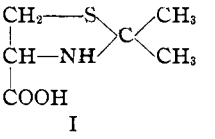
[CONTRIBUTION FROM THE BIOCHEMICAL RESEARCH FOUNDATION OF THE FRANKLIN INSTITUTE]

The Reaction of Cysteine with Acetone. A Note on the Titration of Cysteine by the Acetone–Hydrochloric Acid Method of Linderstrøm-Lang

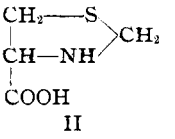
BY GLADYS E. WOODWARD AND E. F. SCHROEDER

During the course of experiments on the enzymatic hydrolysis of glutathione,[1] it was observed that amino acid titrations performed on the reaction mixtures by the Linderstrøm-Lang acetone–hydrochloric acid method[2] were generally much lower than those carried out by other methods.[3,4] The results obtained with the former indicated that only one of the peptide linkages of the glutathione molecule was being hydrolyzed, while the latter methods gave values agreeing closely with the theoretical for complete hydrolysis of both linkages.

Further study of this anomalous situation has shown that cysteine, one of the constituent amino acids of the glutathione, reacts with acetone under the conditions of the titration to form the condensation product, 2,2-dimethylthiazolidine-4-carboxylic acid (I), which does not titrate in the Linderstrøm-Lang method. The structure of this compound, which could be isolated in good yield from cysteine–acetone reaction mixtures, is established by the following observations: (a) the empirical formula is $C_6H_{11}O_2NS$; (b) the amino group is absent, since the compound does not titrate with hydrochloric acid in the Linderstrøm-Lang method; (c) depression of the iodine uptake of the compound, as compared to cysteine, shows that the —SH group also is bound; (d) after hydrolysis of the compound in aqueous solution, acetone and cysteine (as insoluble cystine) can be recovered in nearly quantitative, equimolar amounts.



I



II

The reaction between cysteine and acetone is analogous to that recently described by Schubert[5] and later characterized by Ratner and Clarke,[6] in which cysteine reacts with formaldehyde to yield thiazolidine-4-carboxylic acid (II).

(1) Schroeder and Woodward, *J. Biol. Chem.*, in press.

(2) Linderstrøm-Lang, *Z. physiol. Chem.*, **173**, 32 (1928).

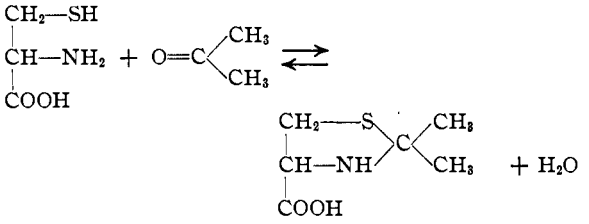
(3) Harris, *J Biol. Chem.*, **84**, 296 (1929).

(4) Linderstrøm-Lang, Weil and Holter, *Z. physiol. Chem.*, **233**, 174 (1935).

(5) Schubert, *J. Biol. Chem.*, **114**, 341 (1936).

(6) Ratner and Clarke, THIS JOURNAL, **59**, 200 (1937).

The new product (I), in contrast to that isolated by Ratner and Clarke (II), is extremely unstable in aqueous solution, being hydrolyzed into acetone and free cysteine. Like II, it has a high levorotation ($[\alpha]^{22}D$ in acetone, $-183°$). Since acetone is optically inactive and cysteine only slightly active, polarimetric measurements could be employed to advantage in investigating the conditions of formation and decomposition of the compound. These studies have shown definitely the existence of an equilibrium in cysteine–acetone–water solutions, probably according to the equation



In pure acetone, corresponding to the conditions under which the compound (I) was isolated, the equilibrium is shifted practically completely to the right. With increasing amounts of water, the equilibrium point is shifted toward the left, until in pure water complete dissociation of the compound occurs. The equilibrium point may be reached from either direction; it depends also on the *p*H of the acetone–water reaction mixtures, being shifted to the right by increasing the alkalinity. Ratner and Clarke have indicated the existence of a similar equilibrium in their cysteine–formaldehyde reaction, although in this case the equilibrium point lies far to the right under ordinary conditions, and can be displaced to the left only by an irreversible process, such as removal of formaldehyde from the sphere of reaction.

Experimental

Preparation of Condensation Product from Cysteine and Acetone: 2,2-Dimethylthiazolidine-4-carboxylic Acid.—One gram of free cysteine, prepared according to the method of Toennies and Bennett,[7] was refluxed in 250 cc. of acetone for one and one-quarter hours, at which time solution was almost complete. After cooling, a trace of undissolved material was removed by filtration. The filtrate was concentrated, by distillation, to 50 cc., or just to the point of hot saturation. After a second filtration, the solution was allowed to stand in the refrigerator overnight. Crystallization occurred slowly in the form of long rectangular prisms arranged as hard rosets, which had a tendency to stick to the bottom of the flask. The product was filtered off, washed with a minimum of cold acetone, and dried in air: yield, 840 mg., or 63%; on further concentration the yield was increased to 71%. For recrystallization, 1.33 g. was dissolved in 50 cc. of hot acetone, filtered, and allowed to stand in the refrigerator as above: yield, first crop, 840 mg., or 63%. The product was readily soluble in water and, in contrast to cysteine, in methyl alcohol, benzene, ligroin, chloroform, ethyl acetate, and ether.

The melting point in a closed or open capillary was 134–134.5° (corr.), with decomposition. On the hot stage of Kofler and Hilbck[8] the melting point behavior was different from that of the usual substances observed under the microscope. At 100° the crystals became smaller and a sublimate formed which changed at 130° to a fine needle-like structure. At 138–140° the "first melting point" occurred, being that of the larger bunches of crystals which had not sublimed. The sublimate underwent further changes in structure until at 200–210°, depending on the rate of heating, a "second melting point" occurred. Since free cysteine melts at about 220°, it appears probable from these observations that during the heating a portion of the condensation product decomposes into free cysteine, which sublimes and melts at the higher temperature. It is also probable that these hot stage melting points are not characteristic of the compound, but will depend greatly on conditions such as rate of heating and moisture content of the atmosphere.

Anal. Calcd. for $C_6H_{11}O_2NS$: C, 44.71; H, 6.88; N, 8.70; S, 19.91. Found: C, 44.90; H, 6.86; N, 8.62; S, 20.08. $[\alpha]^{22}D$ $-183°$, in acetone.

Polarimetric Data (Figs. 1–3).—These were obtained with solutions made by dissolving 50 mg. of free cysteine, or an equivalent amount (66.5 mg.) of 2,2-dimethylthiazolidine-4-carboxylic acid (I), in a total volume of 15 cc. of various acetone–water mixtures. In the indicated cases, a portion of the water was replaced by 0.2 *M* citrate–phosphate buffer. In the experiments with cysteine, the latter was first dissolved in the water or buffer, the required amount of acetone being added at zero time. The 2,2-dimethylthiazolidine-4-carboxylic acid was first dissolved in the acetone, water or buffer being added at zero time. All polarimetric readings were taken in 2-dm. tubes at room temperatures. Since free cysteine in water has only a slight levo-rotation (Fig. 1, curve I; $[\alpha]^{22}D$ $-12°$), no correction for this value has been made, only the observed readings being plotted.

Figure 1 demonstrates the effect of increasing the acetone concentration on the rate of reaction between cysteine and acetone. In preparing the reaction mixture having the highest acetone concentration (curve B, 14 cc. acetone to 1 cc. water), precipitation of cysteine occurred when the acetone was added to the aqueous cysteine solution. The acetone therefore had to be added in small portions with thorough shaking over a period of fifteen minutes. In this way a clear solution was obtained having a levo-rotation only very slightly below that of 2,2-dimethylthiazolidine-4-carboxylic acid in pure acetone (curve A). In the next lower acetone concentration (curve C, 12.5 cc. acetone to 2.5 cc. water) the reaction was so rapid that constant rotation had been practically

(7) Toennies and Bennett, *J. Biol. Chem.*, **112**, 497 (1935).

(8) Kofler and Hilbck, *Mikrochemie*, **8**, 38 (1931).

reached at the time of the first reading (three minutes). As the acetone concentration is decreased, the rate of rotational change also decreases, and the final constant value becomes successively lower. In the two lowest acetone concentrations (curves G and H) constancy had been reached at the nineteen-hour readings.

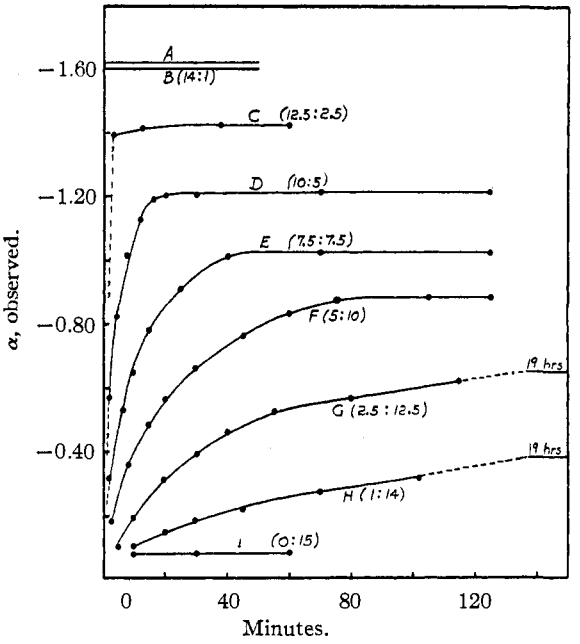


Fig. 1.—Reaction of cysteine with acetone in various concentrations: A, 2,2-dimethylthiazolidine-4-carboxylic acid in pure acetone; I, cysteine in pure water; B to H, cysteine in various acetone concentrations as indicated by the ratios, cc. acetone:cc. H_2O, in parentheses.

Figure 2 shows the rate of dissociation of 2,2-dimethylthiazolidine-4-carboxylic acid in water and in several concentrations of acetone. The dissociation is most rapid, and practically complete, in pure water; the rate and extent of dissociation decrease as the acetone concentration is increased. The rotation finally reaches a constant value, depending on the acetone concentration, the same final values being reached as are obtained from pure cysteine in corresponding acetone concentrations. The rotation of 2,2-dimethylthiazolidine-4-carboxylic acid in pure acetone remains perfectly constant; but when such a solution is diluted with an equal volume of water, the rotation falls and reaches the same final value as is obtained when the above product (I), or free cysteine, is dissolved in a solution of equal volumes of acetone and water (curves A and B). These results may be interpreted as indicative of the existence of a labile equilibrium between cysteine, acetone, water, and the condensation product (I), the point of equilibrium depending on the acetone concentration.

Figure 3 shows similar data obtained under controlled *p*H conditions, on solutions containing 5 cc. of acetone, 5 cc. of 0.2 *M* buffer, and 5 cc. of water. Again nearly the same final equilibrium rotations are obtained, starting either with cysteine or with the condensation product (I). When the acetone concentration is held constant, the rate and extent of the reaction of cysteine with acetone increases with increasing *p*H, as is true also in the reaction of Ratner and Clarke between cysteine and formaldehyde. The dissociation of the compound is also greater at *p*H 3.2 than at 5.2.

It will be observed (for example, Fig. 2, curves B, C, E, and G, dotted lines) that when the condensation product (I) is dissolved in the acetone–water mixtures, a very sharp drop in rotation apparently takes place before the first reading can be taken. This may mean that the compound itself has a lower rotation in these acetone–water mixtures than in pure acetone. A more likely explanation is that in the presence of water, one of the linkages, either S–C or N–C, is rapidly hydrolyzed to yield an intermediate product of lower rotation, and which is more slowly hydrolyzed. Because of the instability of the compound it was impossible to investigate this point by chemical means.

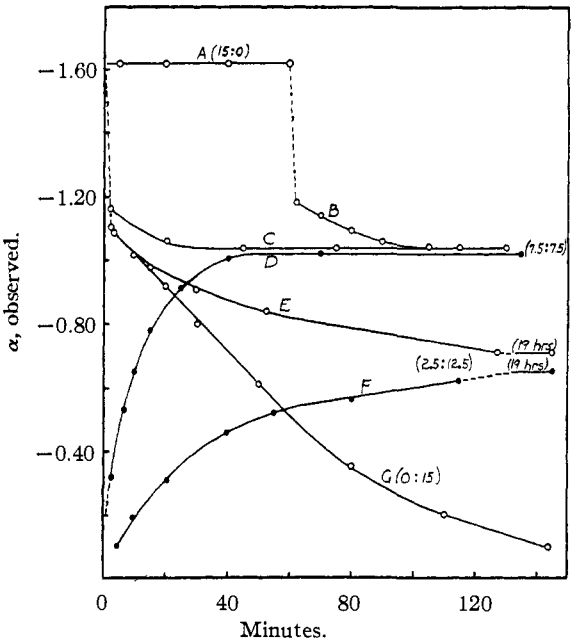


Fig. 2.—Formation and dissociation of 2,2-dimethylthiazolidine-4-carboxylic acid (I) in various acetone concentrations as indicated by the ratios, cc. acetone:cc. water, in parentheses: A, compound (I) in pure acetone, diluted at sixty minutes with an equal volume of water to give B (for curve B, the observed rotations were multiplied by two before plotting, to correct for the dilution); G, compound (I) in pure water; C and E, compound (I) in the indicated acetone–water concentrations; D and F, cysteine in the indicated acetone–water concentrations.

Identification of the Hydrolysis Products.—2,2-Dimethylthiazolidine-4-carboxylic acid (66.5 mg.) was dissolved in 15 cc. of water and treated at once with 27 cc. of a 0.5% solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid; no immediate precipitation occurred; when the mixture was permitted to stand at room temperature, the 2,4-dinitrophenylhydrazone of acetone slowly crystallized out in increasing amounts as acetone was formed by hydrolysis of the condensation product (I). After five hours the precipitate was filtered off, dried, and weighed: yield, 87 mg., or 87%. A similar aqueous

solution of the condensation product was allowed to stand at room temperature for four hours until completely hydrolyzed as shown by a drop in rotation to nearly zero. Addition of 2,4-dinitrophenylhydrazine solution caused an immediate heavy precipitation of the corresponding hydrazone of acetone. This was filtered off after fifteen minutes, dried and weighed; yield 70%. The melting point of the hydrazone in each case was 127–128°, identical with that of the 2,4-dinitrophenylhydrazone prepared from pure acetone; the mixed melting point was unchanged.

An aqueous solution of the condensation product gives a nitroprusside test, indicating the formation of free—SH groups almost immediately. A solution of the compound (66.5 mg.) in a mixture of 5 cc. of acetone, 5 cc. of water, and 5 cc. of citrate-phosphate buffer at *p*H 7 was allowed to stand for two weeks in a loosely stoppered flask. Hexagonal plates, identified as practically pure cystine by means of the Sullivan[9] test, separated out slowly. These had undoubtedly been formed by oxidation of the cysteine liberated on hydrolysis of the condensation product; yield 44 mg., or 89%.

Iodine Titration.—When the condensation product (I) was dissolved in glacial acetic acid, benzene, or acetone, it reacted slowly with iodine dissolved in the same solvents, cystine being precipitated. In no case was the iodine uptake in these solvents up to that required by theory for complete oxidation of the cysteine portion of the molecule to cystine. When the compound was dissolved in 2% sulfosalicylic acid and titrated immediately at 5° by the iodate procedure of Woodward and Fry,[10] the iodine uptake was about 20% of the theoretical; however, more iodine was slowly consumed, until the uptake reached a value slightly more than 100% of the theoretical required for complete oxidation to cystine. When the compound was first allowed to become completely hydrolyzed in 2% sulfosalicylic acid, as indicated by a drop in rotation to nearly zero, the theoretical amount of iodine was taken up at once. Iodate titration of cysteine in the presence of excess acetone was likewise depressed. Because of the extreme lability to the condensation product (I) these experiments are, quantitatively speaking, not wholly satisfactory; but they do show conclusively that the —SH group of the cysteine is involved in the condensation with acetone.

TABLE I

LINDERSTRØM-LANG AMINO ACID TITRATION DATA

Material titrated	Acetone added, cc.	HCl, cc. of 0.05 *N* required	theoretical
Cysteine	20	0.095	0.40
Cysteine	10	.10	.40
Cysteine + glycine	10	.41	.80
Cysteine + glutamic acid	10	.40	.80
Glycine	10	.39	.40
Glutamic acid	10	.39	.40
Glutathione	10	.38	.40
Cond. product (I)	20	.05	0
Cond. product	10	.06	0
Cond. product	10 (no H_2O)	.08	0

(9) Sullivan, *U. S. Pub. Health Service, Pub. Health Repts.*, **44**, 1421 (1929).

(10) Woodward and Fry, *J. Biol. Chem.*, **97**, 465 (1932).

Titration of Cysteine and the Condensate Product (I) by the Linderstrøm-Lang Acetone–Hydrochloric Acid Method.—As previously stated, the irregular results obtained with this titration method when applied to glutathione hydrolysis products gave the first indication of the occurrence of a reaction between cysteine and acetone. Table I presents some typical results obtained with the amino acids in question. To 1-cc. portions of 0.02 *M* aqueous amino acid solutions were added 10 or 20 volumes of acetone. Titration was then carried out with aqueous 0.05 *N* hydrochloric acid using naphthyl red as indicator. Titration of the condensation product (I) was carried out by dissolving 0.02 millimole (3.2 mg.) in 10 or 20 volumes of acetone, adding 1 cc. of water, then running in the acid.

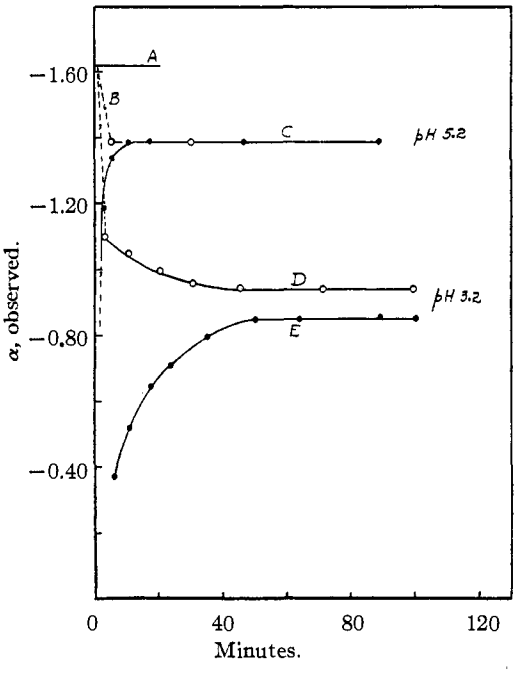


Fig. 3.—Effect of *p*H on the formation and dissociation of 2,2-dimethylthiazolidine-4-carboxylic acid (I) in an acetone–water solution (5 cc. acetone, 5 cc. water and 5 cc. buffer): A, rotation of compound (I) in pure acetone; B and D, compound (I) in acetone–water at *p*H 5.2 and 3.2, respectively; C and E, cysteine in acetone–water at *p*H 5.2 and 3.2, respectively.

Although glycine, glutamic acid, and glutathione titrate correctly in the above procedure, cysteine requires only about 25% of the theoretical hydrochloric acid. When glycine or glutamic acid is present with the cysteine, the hydrochloric acid uptake is even further depressed, only one equivalent instead of two being required. The condensation product (I) is highly acid in character, and it is probable that the glycine and glutamic acid act as buffers, keeping the *p*H of the mixtures high enough so that the cysteine–acetone reaction can proceed to completion. Rotational data indicate that the reaction is more rapid and complete at higher *p*H values. It has also been found that glycine increases the rate and extent of the change in levo-rotation in cysteine hydrochloride–acetone–water re-

action mixtures, again probably because of its effect in increasing the *p*H. The condensation product (I) takes up a slight amount of hydrochloric acid, probably because of partial dissociation under the conditions of titration, but the value is so low that it may be concluded that no free amino group is present in the compound.

Remarks on the Use of the Linderstrøm-Lang Method in the Presence of Cysteine.—In a private communication Dr. Linderstrøm-Lang of the Carlsberg Laboratories, Copenhagen, informed us that he was able to confirm, qualitatively, these results on the titration of cysteine provided that our procedure was followed (addition of all the acetone first, then titration with hydrochloric acid). However he pointed out that the original directions for the method called for the addition of most of the required hydrochloric acid before adding the acetone, then completing the titration in acetone. When this is done, cysteine, both in the presence and absence of glycine, gives very nearly the theoretical values. Apparently when most of the acid is added first, the *p*H of the mixture is depressed to a point where the reaction between cysteine and acetone occurs only very slowly. The reason assigned, in the original description of the method, for adding the hydrochloric acid first was to avoid precipitation of any of the amino acids by the acetone before titration has been completed; the hydrochlorides as a rule are more soluble than the free amino acids in the acetone–water mixtures. Since in our work no such precipitation was ever observed, we used the procedure, frequently employed by others, of adding the entire amount of acetone before titration. From the present work a second reason is apparent for following the original instructions as to order of additions, namely, the obviation of low titrations which might result due to the condensation with the acetone of any cysteine present.

Acknowledgments.—The authors wish to thank Dr. H. L. Fisher of the Air Reduction Company, Stamford, Conn., for valuable advice during the course of this work; also Drs. H. K. Alber and J. Harand of this Laboratory for carrying out the micro analyses and melting point determinations reported.

Summary

Cysteine reacts with acetone with loss of water to form 2,2-dimethylthiazolidine-4-carboxylic acid (I), which is unstable in aqueous solution, decomposing into the original constituents. Polarimetric data indicate the existence of an equilibrium, cysteine + acetone $\leftrightharpoons$ (I) + H_2O, the point of equilibrium depending on the acetone concentration and the *p*H. Because of the occurrence of this reaction, it is essential, in applying the Linderstrøm-Lang amino acid titration method in the presence of cysteine, to follow exactly the original directions with respect to order of addition of the reagents.

PHILADELPHIA, PENNA. RECEIVED JULY 2, 1937

[A COMMUNICATION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Replacement Series of Alkyl Groups as Determined by Alcoholysis of Esters. II

BY GEORGE BATES HATCH AND HOMER ADKINS

A comparison of the relative replacing power of alkyl groups in the type reaction

$$AcOR + R'OH \longleftrightarrow AcOR' + ROH$$

has been extended using the methods previously reported.[1] This involves allowing an equimolecular mixture of an alcohol and an acetate, of relatively widely different volatilities, to react in a steel vessel at 200° for seventy hours under hydrogen in the presence of a few drops of water. The more volatile alcohol and ester are then removed by distillation, and the amount of the less volatile ester determined by saponification. In most cases the volume of the reactants was approximately 70 ml., although a smaller reaction vessel[2] containing only 20 ml. gave equally good results in the cases in which it was used. The comparisons reported for the first time in this paper were all made by reaction of: (a) the alcohol with ethyl acetate, and (b) ethanol with the acetate of the alcohol, equilibrium being established from both directions.

A summary of the relative replacing power of twenty-seven alcohols is given in Table I. The figures in the column headed "Replacement Values" represent the moles of the alkyl acetate per mole of methyl acetate which would be found at equilibrium in a system which before reaction contained equimolecular amounts of the alcohol and methyl acetate. For the purpose of tabulation and comparison the results are given as though the comparisons were made with methanol. If the comparisons were actually made against ethanol, then the replacement value so calculated (AcOR/AcOEt) was multiplied by 0.81, the value for ethanol in terms of methanol.

(1) Fehlandt and Adkins, THIS JOURNAL, **57**, 193 (1935).
(2) Adkins, *ibid.*, **55**, 4272 (1933).