

TABLE II
RATE OF REACTION OF RBr WITH 0.2 M NaSCN

R	Purity, %	No. of runs	$10^4 k$, l./mole-sec.
Ethyl	..	3	1.67
<i>n</i> -Propyl	99.5	2	1.15
<i>n</i> -Butyl	99.8	3	1.14
<i>n</i> -Amyl	99.6	3	1.20
<i>n</i> -Hexyl	99.8	2	1.22
<i>n</i> -Heptyl	99.4	2	1.24
<i>n</i> -Octyl	99.0	6	1.27
<i>n</i> -Decyl	98.6	2	1.23
Cetyl	98.3	3	1.20
Isobutyl	99.4	1	0.05
Isoamyl	..	1	0.73
Isohexyl	98.7	2	1.23

II. The average deviation from the mean k was less than 1% for each of the alkyl halides except *n*-amyl bromide, for which it was 1.3%, and isobutyl and isoamyl bromides, where only one run was made. There can be no doubt that *n*-amyl bromide is considerably more reactive than *n*-butyl bromide and that the reactivity increases with increasing chain length so that k for *n*-octyl bromide is 11% higher than for *n*-butyl bromide. The reactivity of the 10- and 16-carbon bromides appears to be slightly lower, though the lower degree of purity of these compounds causes some uncertainty in the value of k .

The data of Table II refer to an initial molarity of alkyl bromide of about 0.09. Because of the considerable differences in molecular weight, however, the percentage by weight of alkyl halide varied from 1.0 to 3.6. That this slight change in the medium was not entirely responsible for the variation in Table II was demonstrated by five experiments in which the molarity of *n*-octyl bromide ranged from 0.05 to 0.14. The extreme values of k differed by only 2% and moreover indicated the usual decrease in rate with increasing alkyl bromide concentration.⁴

It is difficult to formulate any simple effect of the alkyl group which would lead to greater reactivity with increasing chain length. An inductive effect should reduce the rate by increasing the electron density at the reaction center. Steric hindrance of the attacking reagent by the longer chain, as proposed by Brown, Taylor and Sujishi,² to explain the comparatively low basicity of *n*-propylamine in aqueous solution, would also decrease the rate here. The most satisfactory explanation, however, lies in the fact that the C-Br bond energy gradually decreases from 68.5 to 63.5 kcal./mole as the series is ascended from methyl to butyl bromide,⁵ and probably decreases somewhat further in the higher derivatives. Although the small differences in activation energy corresponding to these rate differences would be difficult to measure experimentally, the activation energies of several displacement reactions have been found to decrease slightly when the halide is changed from ethyl to *n*-propyl, though an entropy effect decreases the rate.⁴

(4) T. I. Crowell and L. P. Hammett, *THIS JOURNAL*, **70**, 3444 (1948); W. F. Johnson and I. M. Kolthoff, *ibid.*, **74**, 22 (1952).

(5) E. W. R. Steacie, "Atomic and Free Radical Reactions," Reinhold Publ. Corp., New York, N. Y., 1946, pp. 77-79.

Table II also shows that although isobutyl and isoamyl bromides react more slowly than their straight-chain isomers, the branching of the chain in isohexyl bromide is too far from the reaction center to affect the rate.

Experimental

Materials.—Commercially available alkyl bromides were shaken with H_2SO_4 , washed with water, dried over K_2CO_3 and fractionated. The purity given in Table II was estimated by Volhard titration for bromide after hydrolysis in a sealed tube. Isohexyl bromide (1-bromo-4-methylpentane) was prepared from formaldehyde and isoamyl alcohol by the Grignard reaction.⁶ Eimer and Amend C.P. sodium thiocyanate, dried at 130° for 24 hours, assay (Volhard) 99.8%, was used. The solvent was 95% ethanol (d_{25}^{25} 0.8004-0.8010).

Procedure.—The kinetic runs were started by weighing the alkyl bromide into a 100-ml. volumetric flask containing 25 ml. of solvent, adding 50 ml. of 0.4 M NaSCN (alcoholic) and diluting to the mark at 25.00°. Five-milliliter samples were withdrawn at one- or two-day intervals and added to 30 ml. of water. After adding 7 ml. of 0.5 M KCN and 50 ml. of 3 M HCl, the sample was titrated for thiocyanate with standard KIO_3 and starch indicator.⁷ The Volhard titer of the reaction mixture remained constant, and 99% of the theoretical quantity of thiocyanate ion was consumed after two months. This procedure parallels the standard preparation of alkyl thiocyanates.

(6) S. C. J. Olivier, *Rec. trav. chim.*, **55**, 1027 (1936).

(7) W. C. Oesper, "Newer Methods of Volumetric Analysis," D. Van Nostrand Co., Inc., New York, N. Y., 1938, p. 85.

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The Preparation of Acyl Trifluoroacetates from Trifluoroacetic Anhydride

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Recently the preparation of acyl trifluoroacetates from silver trifluoroacetate and acyl halides in ether has been reported.¹ It has now been established that acyl trifluoroacetates may be obtained in good yields from equimolar quantities of trifluoroacetic anhydride and carboxylic acids. Satisfactory procedures have been worked out for the preparation of benzoyl trifluoroacetate (55% yield), lauroyl trifluoroacetate (59% yield), furoyl trifluoroacetate (63% yield) and phenylacetyl trifluoroacetate (53% yield). Thus the hypothesis that the high order of reactivity of trifluoroacetic anhydride-carboxylic acid mixtures in acylation reactions is due to the *in situ* formation of the acyl trifluoroacetate is probably correct.²



We were originally interested in examination of the equilibrium between trifluoroacetic anhydride and carboxylic acids. The availability of pure samples of acyl trifluoroacetates made possible the development of an infrared procedure for following this reaction. Accordingly, the reaction between benzoic acid and trifluoroacetic anhydride was studied in *n*-butyl ether and in acetonitrile. Each of

(1) A. F. Ferris and W. D. Emmons, *THIS JOURNAL*, **75**, 232 (1953).
(2) E. J. Bourne, M. Stacey, J. C. Tatlow and J. M. Tedder, *J. Chem. Soc.*, 2876 (1949).

the four components of the equilibrium mixture was determined quantitatively. In both solvents it was found that equilibrium was established immediately and that within the accuracy of the analytical method (estimated at 5%) benzoyl trifluoroacetate was formed quantitatively. Furthermore, equimolar solutions of trifluoroacetic anhydride with lauric, furoic and phenylacetic acids had infrared spectra which indicated that only the acyl trifluoroacetate and trifluoroacetic acid were present, *i.e.*, the reaction was quantitative with these acids too. Therefore, the isolation of the acyl trifluoroacetates was undertaken and was easily effected by previously described techniques.¹

Experimental

Benzoyl Trifluoroacetate.—To 525 g. (2.5 moles) of trifluoroacetic anhydride was added 244 g. (2.0 moles) of benzoic acid. This mixture was then stirred and heated under reflux for 30 minutes. The trifluoroacetic anhydride and acid were distilled *in vacuo* and the benzoyl trifluoroacetate flash distilled at 100° (0.5 mm.), yield 240 g. (55%). The infrared spectrum of this material was identical to that prepared from silver trifluoroacetate and benzoyl chloride.¹

Lauroyl Trifluoroacetate.—To a solution of 20.0 g. (0.1 mole) of lauric acid in 100 ml. of dry methylene chloride was added 21.0 g. (0.1 mole) of trifluoroacetic anhydride. The methylene chloride was immediately distilled *in vacuo* and the lauroyl trifluoroacetate flash distilled at 160° (0.4 mm.), yield 17.1 g. (59%). The infrared spectrum of this preparation was identical with that of an authentic sample.

Reaction of Carboxylic Acids and Trifluoroacetic Anhydride.—Solutions of benzoic, furoic, lauric and phenylacetic acid were prepared in purified *n*-butyl ether.³ Aliquots of each acid and trifluoroacetic anhydride were made up to volume to give equimolar concentrations of each at the 0.1 *M* level. Infrared spectra were obtained on a Perkin-Elmer Model 21 spectrophotometer. The strong trifluoroacetic anhydride band at 1873 cm^{-1} was absent from the spectrum of each mixture. In its place were the following acyl trifluoroacetate carbonyl bands: benzoyl trifluoroacetate, 1835 cm^{-1} ; furoyl trifluoroacetate, 1835 cm^{-1} ; phenylacetyl trifluoroacetate, 1850 cm^{-1} ; lauroyl trifluoroacetate, 1850 cm^{-1} . These frequencies are in reasonable agreement with those previously reported for capillary layers of the mixed anhydrides.¹

Reaction of Benzoic Acid and Trifluoroacetic Anhydride.—Mixtures were analyzed for each component in both acetonitrile and *n*-butyl ether for the system benzoic acid-trifluoroacetic anhydride. All measurements were made at 25–28°. Working curves were prepared from solutions of the pure components. Band intensities were measured for each solution at each analysis frequency to obtain corrections for mutual interference. Curvature of these optical density *vs.* concentration plots was too great to permit use of simultaneous equation solutions based on Beer's law. The method of successive graphical approximations was, therefore, used for determination of the concentrations of each of the components. The data obtained are summarized in Table I. The fact that an excess of trifluoroacetic acid over benzoyl trifluoroacetate was obtained is accounted for by some hydrolysis of trifluoroacetic anhydride by water intro-

duced into the system while the solutions were processed; accordingly, one-half of the excess trifluoroacetic acid found over benzoyl trifluoroacetate (Table I) represents the molar quantity of water introduced into the system. If this correction is taken into account, 0.109 mole of product was accounted for in acetonitrile out of 0.107 mole originally introduced. Similarly in *n*-butyl ether the analytical procedure accounted for 0.086 mole out of 0.084 mole introduced. It should also be mentioned that there was no evidence of disproportionation of benzoyl trifluoroacetate during any of these experiments.

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Further Studies on the Enzymatic Phosphorylation of Riboflavin¹

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In a previous communication,³ ADP⁴ was found to participate in the flavokinase reaction which catalyzes the phosphorylation of riboflavin to yield FMN. The inability to detect myokinase which catalyzes the reaction $2 \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$, in the enzyme preparations, plus the fact that the relative efficiencies of ATP and ADP in FMN synthesis are the same throughout purification, led to the conclusion that ADP participated directly in the synthesis of FMN. In view of the widespread acceptance that in most transphosphorylation reactions involving the adenylate system only the terminal phosphate of ATP can be directly transferred,⁵ the problem was reinvestigated, and myokinase activity was detected even in the purest flavokinase preparations. The finding of such a contaminant in the flavokinase preparations sug-

TABLE I

Each tube contained approximately 7.5 μM . of a preparation derived from Sigma Ba-ADP which consisted of 1.11 μM . AMP, 5.51 μM . ADP, and 0.66 μM . ATP; 1 mg. of flavokinase at an activity of 213, 375 μM . of tris-(hydroxymethyl)-aminomethane buffer at pH 7.41 and $1 \times 10^{-3} \text{ M}$ MgSO_4 or $6 \times 10^{-4} \text{ M}$ ZnSO_4 . The final volume of the reaction mixture was 5.0 ml. The tubes were incubated for 2 hr. at 33.5°, after which time they were immersed in a boiling water-bath for 5 min. prior to filtration through Whatman No. 2. A 3-ml. aliquot of each filtrate was diluted to 25 ml. with water and these solutions passed through Dowex-1 anion exchange columns according to the method of Cohn and Carter [THIS JOURNAL, 72, 4273 (1950)]. Results are recalculated on the basis of the 5.0-ml. final volume of the original reaction mixture.

TABLE I
REACTION OF BENZOIC ACID AND TRIFLUOROACETIC ANHYDRIDE

Compound	Analysis bands, cm^{-1}		Moles added		Moles found	
	Acetonitrile	Butyl ether	Acetonitrile	Butyl ether	Acetonitrile	Butyl ether
$\text{C}_6\text{H}_5\text{COOCOCF}_3$	1872	1873	0.035	0.057	None	0.028
$\text{C}_6\text{H}_5\text{COOH}$	714	708	0.066	0.025	0.036	None
$\text{C}_{12}\text{H}_{25}\text{COOCOCF}_3$	1832	1835	None	None	.030	.027
$\text{C}_6\text{H}_5\text{COOH}$	688	695	None	None	.043	.031
H_2O			0.006	0.002		
Total moles			0.107	0.084	.109	.086

(3) A. T. Blomquist and A. F. Ferris, THIS JOURNAL, 78, 7412 (1951).

	Mg ⁺⁺ and boiled enzyme, μM .	Mg ⁺⁺ and enzyme, μM .	Zn ⁺⁺ and enzyme, μM .
ΔAMP	+1.44	+1.65	+0.03
ΔADP	-2.96	-3.11	+ .14
ΔATP	+1.53	+1.58	+ .03

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(3) E. B. Kearney and S. Englund, J. Biol. Chem., 198, 821 (1951).

(4) The following abbreviations are used: AMP = adenosine monophosphate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; IMP = inosine monophosphate; FMN = flavin mononucleotide.

(5) S. P. Colowick, in Summer and Myrback, "The Enzymes," Vol. II, Part I, Academic Press, Inc., New York, N. Y., 1951, p. 114.