of new equilibria, but no further evidence is available on this point.

The rapid reaction of **2** with *n*-butyllithium was evident from observation of the nmr spectrum in the region downfield from tetramethylsilane. Shortly after addition of **2** a new singlet appeared upfield from that assigned to MPE which was attributed to the methoxyl protons of the metalated derivative. During the course of the reaction both singlets gradually moved upfield, indicating that these species were also involved in changing equilibria. Because the chemical shift of the methylene protons α to lithium also changed with time, it was necessary to adjust concentrations of *n*-butyllithium and **2** so as to maintain a constant reaction rate for all spectra and to record each at the same time into the run.

While the details are undoubtedly complex, it seems likely that the 14-fold increase in reactivity of **2** relative to **1** can be explained in part by the change in base–ether ratio from 1:1 in the case of **1** to 2:1 in the case of **2**.

Acknowledgments.—The authors are grateful to the Graduate School, Boston University, for support of the initial phase of this work. Funds from the Graduate School, University of Wisconsin, Madison, are gratefully acknowledged. We thank Christine Knapp (B. U.) and Larry Amich (U. W.) for technical assistance.

Registry No.—**1**, 100-66-3; **2**, 41532-81-4; **3**, 41894-71-7; **10**, 766-94-9; phenoxyacetic acid, 122-59-8; phenoxyacetic acid ethyl ester, 2555-49-9; 2-phenoxyethanol-*1,1-d*$_2$, 21273-38-1; 2-phenoxyethanol, 122-99-6; 2,3-benzofuran, 271-89-6; 2,3-dihydrobenzofuran, 496-16-2; (2-chloroethoxy)benzene, 622-86-6.

1-Butanol–Hydrogen Chloride. An Allegedly Anhydrous Esterification Reagent

JAMES P. HARDY, STEPHEN L. KERRIN, AND STANLEY L. MANATT*

Space Sciences Division, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91103

Received July 5, 1973

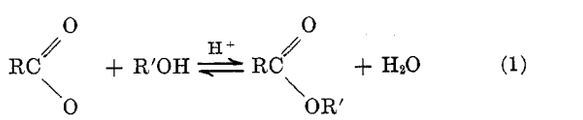
The stability of a common Fischer esterification reagent used extensively for preparation of amino acid and carboxylic acid esters, 2.1 *M* HCl–1-butanol, has been studied in detail. After 2 hr at 100 and 150°, the concentrations of 1-chlorobutane, di-1-butyl ether, and water in this reagent are 0.71, 0.04, and 0.75, and 2.36, 0.22, and 2.58 *M*, respectively. Approximate rate constants for formation of these products at 100 and 150° have been determined. It is concluded that esterifications with this reagent should be carried out below 100° to achieve best yields. An equilibrium constant of 0.15 ± 0.03 has been measured in the esterification of a typical aliphatic amino acid, leucine. The significance of the production of water in this esterification reagent is discussed especially in light of its use in amino acid esterification procedures where the carboxylic acid concentrations may be at the millimolar or lower concentration level.

The importance of amino acid chemistry and general lack of detailed quantitative studies of amino acid esterification reactions suggested to us that the latter problem deserves more extensive investigation. Herein we describe an investigation of some of the properties of the Fischer esterification reagent, 1-butanol–hydrogen chloride in our case, or in general an alcohol mixed with a strong acid, and its use for esterification of amino acids.

The Fischer esterification procedure for carboxylic acids was introduced in 1895[1] and synthetic procedures for esterification of amino acids by this method were published in 1901.[2] The mechanism of the reaction has been widely investigated and discussions of it may be found in most organic chemistry tests.[3] It is well known that, for equimolar quantities of reactant, the equilibrium in this reaction lies quite short of completion. To obviate this, an excess of alcohol or acid, product removal by distillation, or addition of a water scavenger is generally employed to shift the equilibrium in favor of the product.[3]

Recently, methods have been reported[4–8] utilizing Fischer esterification, usually with 1-butanol–HCl, and subsequent trifluoroacylation to render amino acids amenable to quantitative analysis by gas chromatography. Examination of the literature indicates that little systematic investigation has been directed toward the esterification reaction at concentration levels usually encountered in amino acid analysis. The quantitative yield of volatile amino acid derivatives is dependent upon the reproducibility and completeness of the derivatization reactions. Equilibrium constants, concomitant reactions of the esterification reagent, and the relationship of these to the total reaction become, therefore, important considerations in these analytical procedures and should be considered in any syntheses involving an expensive and/or small amount of carboxylic acid.

With few exceptions,[9,10] the esterification reagent has been generally considered anhydrous in that it contributes little or no water to the reaction. On this basis, it has been accepted that maximum water concentration at equilibrium would be equal to amino acid ester concentration according to eq 1. With a carboxylic acid



$$RC(=O)O + R'OH \overset{H^+}{\rightleftharpoons} RC(=O)OR' + H_2O \qquad (1)$$

(1) E. Fischer and A. Speier, *Ber.*, **28**, 3242 (1895).
(2) E. Fischer, *Ber.*, **34**, 433 (1901).
(3) See, for example, L. F. Feiser and M. Feiser, "Advanced Organic Chemistry," Reinhold, New York, N. Y., 1961, pp 371–376; C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1963, pp 752–781; V. M. Migrdichian, "Organic Synthesis," Vol. 1, Reinhold, New York, N. Y., 1957, pp 311–332; J. D. Roberts and M. C. Caserio, "Basic Principles of Organic Chemistry," W. A. Benjamin, New York, N. Y., 1965, pp 389–390, 519–521.
(4) C. W. Gehrke and D. L. Stalling, *Separ. Sci.*, **2** (1), 101 (1967).
(5) D. Roach and C. W. Gehrke, *J. Chromatogr.*, **43**, 303 (1969).
(6) C. W. Gehrke, R. W. Zumwalt, and L. L. Wall, *J. Chromatogr.*, **37**, 398 (1968).
(7) D. Roach and C. W. Gehrke, *J. Chromatogr.*, **44**, 269 (1969).
(8) C. W. Gehrke and K. Leimer, *J. Chromatogr.*, **53**, 195 (1970).
(9) W. M. Lamkin and C. W. Gehrke, *Anal. Chem.*, **37**, 383 (1965).
(10) W. Gerrard and H. R. Hudson, *J. Chem. Soc.*, 1059 (1963).

concentration at or below the millimolar level, a large excess of alcohol has been considered sufficient to drive the reaction to completion. However, the results reported here indicate that several orders of magnitude more water can be produced from the conversion of 1-butanol to 1-chlorobutane and di-1-butyl ether than is produced by the esterification reaction alone. The implications of the presence of large amounts of water in a quantitative esterification reaction are obvious. To assess the effect of these side reactions, we have examined and report here the degree and rate of production of 1-chlorobutane, di-1-butyl ether, and water from 1-butanol–HCl at times and temperatures most often used in amino acid esterification. We also report the equilibrium constant for the esterification of leucine, a typical aliphatic amino acid, at 60° in order to assay the magnitude of the effect of water formation on its esterification equilibrium.

Experimental Section

Materials.—Leucine was Calbiochem A Grade and used as received. 1-Butanol was Analabs pesticide grade, dried over and distilled from magnesium turnings in an all-glass system under dry nitrogen and subsequently acidified with Matheson anhydrous hydrogen chloride. The hydrogen chloride gas was added slowly and with cooling to reduce heat liberation and possible side reactions.

Instrumentation.—A Varian Model 1440 gas chromatograph with flame ionization detector and Hewlett-Packard 3370A digital integrator were used for all quantitative determinations. Combined gas chromatogaphy–mass spectrometry was performed on a Victoreen 4000 series gas chromatograph using a 10 ft × 0.050 in. Poropak Q column coupled through a single-stage Ryhage-type separator into a Hitachi Perkin-Elmer RMS-4 mass spectrometer.

Synthesis.—Leucine 1-butyl ester hydrochloride was prepared by refluxing leucine in 1-butanol–2.7 *N* HCl for 15 min. The resultant mixture was taken to dryness on a glass–Teflon rotary evaporator. The solid (1-butyl)leucine 1-butyl ester hydrochloride was dissolved in 0.1 *N* KOH and sufficient base was added to ensure alkalinity. The free leucine 1-butyl ester was extracted into dichloromethane, washed twice with water, and reacidified with HCl gas. The dichloromethane was evaporated and the resultant leucine 1-butyl hydrochloride was recrystallized twice from hot diethyl ether, mp 94.5–95.5° (not previously reported). The chloride content was determined by potentiometric titration using silver nitrate. *Anal.* Calcd for $C_{10}H_{22}ClNO_2$: Cl, 15.87. Found: Cl, 15.7 ± 0.3.

Leucine–Leucine 1-Butyl Ester Equilibrium Standardization.—Accurately weighed amounts of leucine, leucine 1-butyl ester hydrochloride, and dodecane (internal standard) were added to 1 ml of 1-butanol–2.7 *N* HCl. This mixture was partitioned between 4 ml of petroleum ether (bp 20–40°) and 20 ml of 0.2 *N* KOH. The organic layer was chromatographed and molar responses relative to dodecane were determined. The precision of the relative molar response determinations was ±1.9% coefficient of variation. Chromatography was effected on a 6 ft × 2 mm glass column packed with 80/100 mesh GLC-110 coated with 0.2% OV-7, isothermal at 90° with a 20-ml/min helium flow.

Equilibration.—Accurately weighed amounts of leucine and dodecane were added to 1 ml of 2.7 *N* HCl–1-butanol and the mixture was sealed in ampoules. The ampoules were heated in an oil bath at 60° for 30, 60, and 90 min and the contents of each were subsequently extracted and analyzed exactly as for the standardization. Some experiments were done at 25° where the above mixture was sampled periodically, extracted, and analyzed.

Analysis of Products Found in 2.7 *N* HCl–1-Butanol. 1-Chlorobutane and Di-1-butyl Ether.—Accurately weighed amounts of 1-chlorobutane, di-1-butyl ether, and decane (internal standard) were dissolved in 1 ml of freshly prepared 1-butanol–2.7 *N* HCl and extracted as above, and relative molar responses were determined. Aliquots (1.5 ml) of 1-butanol–2.7 *N* HCl with a known amount of internal standard were sealed in ampoules and heated at 100 or 150° for 15, 30, 60, and 120 min. For analysis, 1-ml aliquots were removed and extracted. Chromatography was performed using a 20 × 0.125 in. stainless steel column packed with 80/100 mesh Poropak T programmed from 165 to 195° at 8°/min. Flow rate was 25 ml/min. To detect the possible product 1-butene, 1 ml of 2.7 *N* HCl–1-butanol was heated in a septum-capped vial for 3 hr at 100°. The head space vapor was sampled several times with a syringe and analyzed by combined gas chromatography–mass spectrometry. The limit of detectability was estimated to be about 0.1% for 1-butene and none was detected in these experiments.

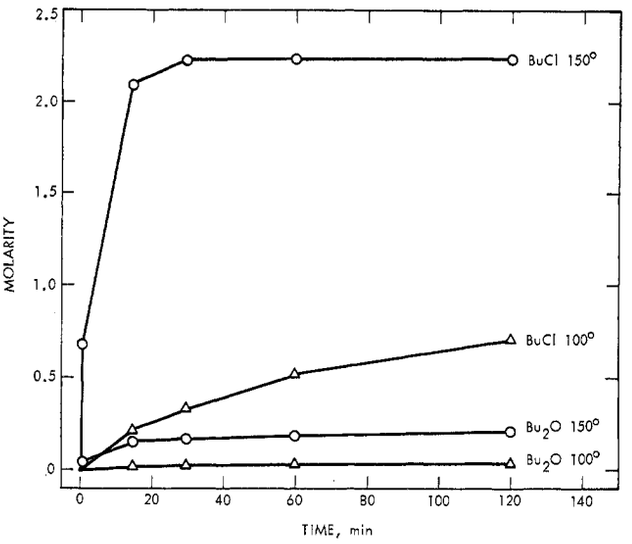


Figure 1.—Conversion of 2.7 *M* HCl–1-butanol to 1-chlorobutane and di-1-butyl ether at 100 and 150°.

Results

Rates of Production of 1-Chlorobutane and Di-1-butyl Ether.—In a mixture of 1-butanol and hydrogen chloride, the 1-chlorobutane and di-1-butyl ether are formed according to eq 2 and 3. Each of these reactions

$$CH_3CH_2CH_2CH_2OH + HCl \rightleftharpoons CH_3CH_2CH_2CH_2Cl + H_2O \quad (2)$$

$$2CH_3CH_2CH_2CH_2OH + H^+ \rightleftharpoons (CH_3CH_2CH_2CH)_2O + H_2O \quad (3)$$

is acid catalyzed and each produces water. However, reaction 2 consumes acid, while reaction 3 does not. Each represents a reversible equilibrium, the reaction producing 1-chlorobutane being considerably more rapid than that producing di-1-butyl ether. Examination of Figure 1 and Table I reveals some of the details of these reactions. The 1-butanol is converted to 1-chlorobutane approximately ten times more rapidly than di-1-butyl ether. Starting with 2.7 *M* HCl in 1-butanol at 150°, an equilibrium amount of approximately 2.3 *M* 1-chlorobutane is produced along with an equivalent amount of water. In this process the HCl concentration is reduced from 2.7 to 0.4 *M*.

It appears that reaction 2 has reached equilibrium within 30 min at 150° while reaction 3 has not gone to completion within 2 hr. Di-1-butyl ether is formed quite rapidly in the first few minutes, as shown in Table I, but, as the acid is consumed by reaction 2, the rate drops rapidly. At 100° similar behavior is observed, but the rates are approximately ten times slower.

The high concentrations of reactants in this system cause the characteristics of the reaction medium to change markedly during the course of the reaction; consequently, only estimates can be made of the rate

TABLE I

CONVERSION OF 2.7 *M* HCl–1-BUTANOL TO BUTYL CHLORIDE AND DI-1-BUTYL ETHER

Temp	Time, min	[BuCl], *M*	[Bu_2O], *M*	Sum, *M*[a]
100°	0	0.010	0.008	0.018
	15	0.214	0.015	0.229
	30	0.330	0.027	0.357
	60	0.519	0.031	0.550
	120	0.709	0.042	0.751
150°	0	0.010	0.02	0.03
	1	0.68	0.05	0.73
	15	2.10	0.16	2.26
	30	2.23	0.17	2.40
	60	2.30	0.19	2.49
	120	2.36	0.22	2.58

[a] The sum of BuCl and Bu_2O concentrations represents the concentration of water formed.

TABLE II

ESTIMATION OF RATE CONSTANT FOR ACID-CATALYZED CONVERSION OF 1-BUTANOL TO DI-1-BUTYL ETHER AT 100°

Time, min	[Bu_2O], *M*	[HCl], *M*[a]	[BuOH], *M*[b]	10^3 k,[c] M^{-2} sec^{-1}
0	0.020	2.69	10.9	2.6
1	0.05	2.48	10.7	4.9
15	0.16	2.36	10.5	0.9
30	0.17	2.17	10.4	1.4
60	0.19	1.98	10.3	2.7
120	0.22	1.72	9.8	2.5 ± 1.1

[a] HCl concentration calculated from the initial concentration of acid minus the amount of butyl chloride formed. [b] Butanol concentration calculated from initial concentration and corrected for total products formed. [c] Rate constant calculated from the following expression: $d[BuOH]/dt = k[BuOH]^2[HCl]$.

constants. These constants for the two reactions at both temperatures are estimated from the data of Figure 1 and Tables I and II, and the calculations are based on eq 4 and 5.

$$d[BuCl]/dt = k[BuOH][HCl] \quad (4)$$

$$d[Bu_2O]/dt = k[BuOH]^2[HCl] \quad (5)$$

Table III summarizes these data at the two temperatures and includes the rate constants extrapolated to 60°. The rate of production of water at this lower temperature is important since the esterification equilibrium constant for conversion of leucine to leucine 1-butyl ester was measured at that temperature.

Equilibrium Constant for Esterification of Leucine.— The equilibrium constant for esterification of leucine hydrochloride in 2.7 *M* HCl–1-butanol was determined at 60°. Since under these conditions the conversion of the amino acid to ester is almost complete (97–98%), considerable attention was paid to the precision and accuracy of the analytical procedure. The difference between 98 and 99% represents approximately a factor of two in the equilibrium constant, while the difference between 99 and 99.9% represents a factor of ten. Also, owing to the inaccuracies inherent in chromatographic methods, 99% is indistinguishable from 99.9% and higher per cent conversions, and the equilibrium constant becomes essentially indeterminant. As shown by Table IV and the Experimental Section, the gas chromatographic analytical precision was on the order of 2%, coefficient of variation. In all cases, the per cent conversion of leucine to its ester was less than 98%. The error ranges in Table II represent standard

TABLE III

SUMMARY OF ESTIMATED RATE CONSTANTS AND ACTIVATION ENTHALPY AT 150 AND 100° EXTRAPOLATED TO 60° FOR CONVERSION OF 1-BUTANOL TO BUTYL CHLORIDE AND DI-1-BUTYL ETHER

Temp, °C	Reaction	*k*, M^{-1} sec^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}
150	$ROH + HCl \rightleftharpoons RCl + H_2O$	8×10^{-6} [a]	29.5
100		8×10^{-6} [a]	
60		(7×10^{-7})[b]	
	H_2	*k*, M^{-2} sec^{-1}	
150	$2\,ROH \rightleftharpoons R_2O + H_2O$	4×10^{-7} [c]	32.5
100		2.5×10^{-8} [d]	
60		(1×10^{-9})[b]	

[a] Rate constant estimated from initial slope of [BuCl] *vs.* time (Figure 1) and calculated from the expression $d[BuCl]/dt = k[BuOH][HCl]$. [b] Rate constants extrapolated from data at 100 and 150°. [c] Rate constant estimated from the average slope of [Bu_2O] *vs.* time (Figure 1) after 15-min reaction, calculated from the expression $d[Bu_2O]/dt = k[BuOH]^2[HCl]$. [d] Rate constant estimated from data in Table III.

TABLE IV

ESTERIFICATION OF LEUCINE HCl AT 60° WITH 2.7 *M* HCl–1-BUTANOL

Time, min[a]	Initial[b] leucine HCl, m*M*	(1-Butyl)leucine, m*M*[c]		Conversion, %	K_{eq}
30	37.02	35.5			
		36.6	36.2 ± 3.5	97.7 ± 1.3$_5$	0.146
		36.6			
		36.2			
60	53.7	52.1			
		51.6			
		54.3	52.4 ± 1.3	97.4 ± 2.3$_7$	0.178
		51.6			
120	45.7	43.7			
		43.1			
		44.1	44.3 ± 1.1	96.9 ± 2.4$_1$	0.128
		44.8			
		46.0			0.151 ± .026

[a] Esterification time at 60°. [b] Amount of leucine *HCl* weighed out. [c] Amount of (1-butyl)leucine extracted and quantitated by gas chromatography.

deviations, and the error in the average equilibrium constant is a reasonable estimate of the cumulative errors associated with its measurement. Thus, the precision and accuracy of the data are sufficiently high to establish that the per cent conversion of the amino acid to its butyl ester is less than and distinguishable from 100%.

To minimize the production of water by reactions 2 and 3, the equilibrium constant for the esterification of leucine was measured at a temperature (60°) significantly lower than the 100–150° at which amino acid esterifications are carried out in several currently used analytical procedures.[4–8] Because the effect of temperature on this equilibrium is unknown, in the following discussions of the effect of water it will be assumed that the equilibrium constant is essentially invariant over the temperature range discussed here.

Figure 2 shows some calculations of the per cent amino acid ester which would be present at equilibrium in 1-butanol–HCl for a number of different initial concentrations of water and for three equilibrium constants. The dotted lines below and associated with each curve represent the per cent ester formed as a

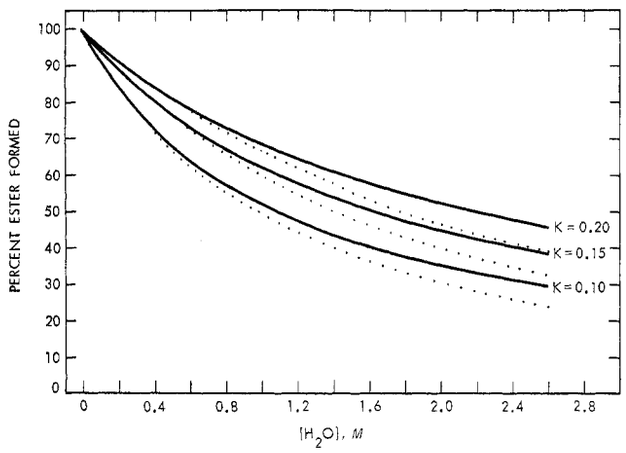


Figure 2.—Calculated per cent amino acid ester present at equilibrium for a number of different initial water concentrations. Dotted line below and associated with each curve represent the per cent ester formed if the initial water present were produced by the 1-butanol (see Results).

function of initial water concentration if the water were produced from the 1-butanol. That is, the initial 1-butanol concentration (10.9 *M*) is reduced by the amount of water "formed." Calculations for initial amino acid concentrations ranging from 10^{-6} to 10^{-2} *M* gave curves which were essentially the same. The initial 1-butanol concentration of 10.9 *M* was calculated from the density of 1-butanol at 25°. No correction was made for density changes with temperature.

Rate of Esterification.—As indicated by Table IV, leucine esterification is essentially complete in less than 30 min at 60°. Additional qualitative evidence suggests that esterification is quite rapid even at 25° in 2.7 *M* HCl–BuOH. In fact the rate of esterification of solid leucine hydrochloride may be limited by the rate of solution of the solid amino acid hydrochloride in 1-butanol–HCl. In one experiment, leucine hydrochloride was placed in a Teflon capped glass vial and 2.7 *M* HCl–1-butanol was added. The tube was agitated, at room temperature, with a Vortex mixer until the visible traces of solid amino acid hydrochloride were just gone. This took approximately 15 min. The excess 1-butanol–HCl was evaporated at room temperature under a stream of dry nitrogen. Analysis of the residue indicated that approximately 30–40% of the amino acid had been converted to the 1-butyl ester under these conditions.

Discussion

Under conditions normally employed for direct esterification of amino acids, the esterifying reagent, HCl–1-butanol, reacts to produce 1-chlorobutane, di-1-butyl ether, and water.[11] The water produced in this reaction interferes in the quantitative conversion of the amino acids to their esters. The maximum magnitude of the problem is related to how much water is produced at equilibrium (equilibrium between 1-butanol–HCl and 1-chlorobutane, di-1-butyl ether and water), and the equilibrium constant for esterification of the amino acid. Thus, the importance of the rate of esterification

(11) Water could also be produced by dehydration of 1-butanol to form 1-butene, although this reaction is unlikely for a primary alcohol under the conditions discussed here, and none was detected in this work as mentioned in the Experimental Section.

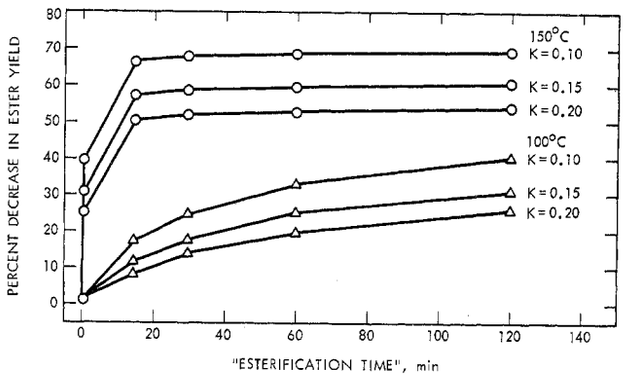


Figure 3.—Per cent decreases in ester yield as a function of time. Time is a measure of the amount of water formed by the HCl–1-butanol reactions.

relative to the rate of water production needs to be realized.

Some assessment of the interaction of these two rate and equilibrium effects on the final ester yield is given by Figure 3. This figure is a plot of calculations of the per cent decrease in ester yield as a function of "esterification time" using the kinetic data obtained in this work for reactions 2 and 3. The decrease in ester yield is the difference between amino acid ester which would be formed if there were no water present in the HCl–1-butanol reagent (aside from the water formed as a result of the esterification reaction) and the amount of ester which would be formed at equilibrium if there were as much water in the esterifying reagent as would be formed if the reagent had been held at the given temperature, 100 or 150°, for the various times. This plot might be thought of as the equilibrium ester concentrations as a function of the kinetic water concentrations. The ester concentrations were calculated for three equilibrium constants at the two temperatures indicated.

As we noted earlier, the esterification reaction is probably quite rapid once the amino acid hydrochloride is in solution, but the rate of solution is, under some conditions, slow. The rate of esterification is even more complicated, since, not only is the final ester yield influenced by the water produced by the HCl-butanol reaction, but the esterification reaction is acid catalyzed. The conversion of 1-butanol to 1-chlorobutane consumes acid and the acid-catalyzed rate consequently drops. This slower rate then demands an even longer esterification time for equilibration, hence more time for an additional amount of water to be produced and an even lower yield of ester.

Some of the previous argument is based on data obtained for the amino acid leucine. We expect that esterification equilibrium data for most of the other amino acids should be similar, although we were surprised to find no other quantitative data in the literature of a similar nature for other amino acids. Steric effects undoubtedly play a role in the amino acid esterification as they do in aliphatic carboxylic acid systems.[12] However, most of the amino acids are already α,β-substituted acids and subsequently the effect of changing the size of the β substituent will be much less pronounced than if the amino acids were not already

(12) M. S. Newman, *J. Amer. Chem. Soc.*, **72**, 4783 (1950); K. L. Loening, A. B. Garrett, and M. S. Newman, *ibid.*, **74**, 3929 (1952).

so highly substituted. The effect of the α-amino group should be essentially the same for most amino acids except perhaps for tryptophane or proline. In any event, the variation in esterification rates for the amino acids will act mainly to demand increased esterification time to reach equilibrium. The importance of the amino acids would seem to warrant some studies along these lines.

It has been our experience that an important difference in behavior between the amino acids is the rates of solution of their hydrochloride salts.[13] The magnitude of this feature has not as yet been investigated in a systematic manner. In any case it is clear from the preceding data that increasing the temperature of the esterification reagent to speed the rate of solution of amino acid hydrochlorides may not be a wise approach. With the thought in mind that a salt with an anion larger than chloride would dissolve more rapidly, we observed the rate of solution of a mixture of amino acid hydrobromides and found no dramatic increase in solubility rate. Sulfate salts do dissolve rapidly[14] but the acid, H_2SO_4, is not volatile and would interfere with steps subsequent to esterification. Gehrke[9] has suggested sonic energy to aid in dissolving the amino acid hydrochlorides.

Clearly, then, esterification of amino acid hydrochlorides at 100° is marginal with respect to reproducible quantitative conversion to esters. If the acids are esterified within less than 5–10 min the water produced at 100° can cause at most a 2–10% decrease in the yield of ester formed depending on esterification equilibrium constant. A rapidly dissolving and esterifying amino acid would be little influenced by the water from the 1-butanol–HCl.

(13) Unpublished work of J. P. Hardy and S. L. Kerrin.
(14) G. E. Pollock, private communication.

Conclusions

It has been shown that, above 100°, 2.7 *M* HCl–1-butanol Fischer esterification reagent produces considerable water in a time comparable to the times required for esterification of the amino acids and probably other carboxylic acids. Thus, to realize good yields or satisfactory quantitative results in esterification reactions with Fischer-type reagents, the reactions should be carried out at temperatures below 100° and for times long enough to ensure complete solution of the amino acid hydrochlorides. To ensure reproducible quantitation of amino acids by gc analysis of their volatile derivatives, such as the *N*-trifluoroacetyl-*O*-butyl esters,[4–8] *N*-trimethylsilyl-*O*-1-butyl esters,[15] and other ester derivatives, the initial esterification step with a Fischer-type reagent should be carried out at temperatures below 100°.

Acknowledgment.—The authors wish to thank Dr. E. A. Cohen for assistance in putting together the Hewlett-Packard 2116 computer program to perform the equilibrium calculations. This paper presents the results of one phase of research carried out at the Jet Propulsion Laboratory, California Institute of Technology, under Contract No. NAS 7-100, sponsored by the National Aeronautics and Space Administration.

Registry No.—1-Butanol–HCl, 42031-19-6; leucine 1-butyl ester hydrochloride, 42031-13-0; 1-chlorobutane, 109-69-3; di-1-butyl ether, 142-96-1; 1-butanol, 71-36-3; leucine HCl, 760-84-9.

(15) J. P. Hardy and S. L. Kerrin, *Anal. Chem.*, **44**, 1497 (1972).

Notes

O-Carbamoyloximes[1]

DAVID R. DALTON* AND H. GRANT FOLEY

Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122

Received June 25, 1973

Shortly after the turn of the century, a general azoxirane synthesis was reported by Conduché.[2] The method for realizing these compounds (**1**) involved careful treatment of an aqueous suspension of aldehyde with hydroxyurea (presumably generated *in situ* from hydroxylamine hydrochloride and potassium cyanate) or, alternatively, of the hydrogen chloride salt of the oxime of the aldehyde with potassium cyanate.

The same products were reportedly obtained by Bellavita and Cagnoli[3] utilizing the first method of Conduché, although the structural class was modified to that of the nitrones (**2**). The new structures were preferred largely because the compounds appeared more stable than would be expected were their structures **1** and, so it was reported, treatment of the compounds **2** with cyanide ion in aqueous solution resulted in formation of the ureides **3**.

The alternative structure **4**, *i.e.*, an *O*-carbamoyloxime, for the original azoxirane was considered possible by Grammaticakis,[4] but clearly this would not satisfy the ureide formation of Bellavita and Cagnoli[3] and he concluded (largely on the basis of ultraviolet spectral comparisons with oximes) that **4** could be rejected.

Finally, however, the elegant interpretation of the available spectral and chemical data by Exner[5] sug-

(1) A communication dealing with a portion of the material contained herein has appeared: D. R. Dalton, H. G. Foley, K. N. Trueblood, and M. R. Murphy, *Tetrahedron Lett.*, 779 (1973).
(2) A. Conduché, *Bull Soc. Chim. Fr.*, [3] **35**, 418 (1906); *Ann. Chim. Phys.*, [8] **12**, 533 (1970); [8] **13**, 5 (1908).
(3) V. Bellavita and N. Cagnoli, *Gazz. Chim. Ital.*, **69**, 583, 602 (1939).
(4) P. Grammaticakis, *Bull. Soc. Chim. Fr.*, **8**, 101 (1941).
(5) O. Exner and M. Horack, *Collect. Czech. Chem. Commun.*, **24**, 2992 (1959), and earlier papers.