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Note

Kinetics and yield of the esterification of amino acids with thionyl chloride in *n*-propanol

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The separation and quantitative measurement of amino acids by ion-exchange chromatography has been the standard procedure since its introduction by Moore and Stein[1]. The application of this principle[2] for suitable amino acid derivatives[3,4] to high-performance liquid chromatography has provided faster amino acid analyses with high sensitivity. In this respect gas chromatography offers the same advantages at a lower cost of equipment[4], but derivatization of the amino acids to suitable volatile compounds is essential. Various derivatives have been found to be of value[5], particularly the *n*-acyl esters of alkanols[6], which are usually obtained with an anhydrous hydrochloric acid–alkanol solution, at concentrations varying from 1.25 *M*[7] to saturation[8]. Increasing time, concentration and temperature are required as one goes from lower to higher alcohols, especially for amino acids such as the basics Cys and Ile[9].

The use of thionyl chloride in methanol[10] or thionyl chloride in hydrochloric acid–methanol mixtures[11] in place of hydrochloric acid have been used for methyl ester derivatives with reproducible yields. Based on these results, we have studied the reaction of amino acids with thionyl chloride in *n*-propanol in an effort to develop an alternative approach for the quantitative derivatization with alkanols. Kinetic results for neutral amino acids are reported.

EXPERIMENTAL

Reagents

L-Amino acids were purchased from Fluka (Buchs, Switzerland) or Merck (Darmstadt, G.F.R.). Thionyl chloride (technical grade) was refluxed with 10% linseed oil and distilled using a 50-cm Vigreaux column. *n*-Propanol was purified through the ternary azeotrope with benzene, and the fraction collected at 94–95°C (710 mmHg) was refluxed for 24 h with calcium oxide and redistilled.

Esterification reagent

Thionyl chloride was added dropwise to *n*-propanol at such a rate that the temperature of the mixture was kept at −5°C. After the addition was completed, the mixture was allowed to stand for 1 h at room temperature and kept at −5°C. The proportions of the reagents used were adjusted to give 1, 2, 3 and 4 *M* solutions.

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Esterification procedure

A 1–2-μmole amount of amino acid and 0.4 ml of reagent were placed in a screw-capped tube (60 × 11 mm) and heated from 30 to 100°C. The amino acids studied were Ala, Gly, Ile, Leu, Pro, Phe, Ser, Thr, Tyr, Val, Asp, Glu, Arg, His, Lys and Cys.

Kinetic measurements

Kinetic measurements were carried out with the neutral amino acids, except those with the third functional group, and were followed with a Beckmann Model 120-B amino acid analyser[12]. Esterification yields at levels above 95% were measured as follows: samples of 1, 3, 10, 20 and 30 nmole of amino acid were applied on a silica gel thin-layer chromatographic (TLC) plate (10 × 20 cm) and eluted with *n*-butanol–acetic acid–water (12:3:5). The plate was dried completely and the spots were detected by spraying with ninhydrin for 5 min at 100°C. The lower limit of detection (very light spot) was 3 nmole, no spot being detected at the 1-nmole level. A 20-μl volume (100 nmole) of the reaction mixture containing 2 μmole of amino acid in 0.4 ml of reagent was applied to a TLC plate, plus spots of 3 and 100 nmole of amino acid standard, and were detected by spraying with ninhydrin after elution. Levels greater than 95% were considered satisfactory when no ninhydrin-positive material was observed at the position corresponding to the free amino acid when compared with the 3-nmole spot.

RESULTS AND DISCUSSION

The order of reactivity established at 40°C with 2 *M* thionyl chloride by kinetic measurement of each individual amino acid is shown in Fig. 1 ($173 \cdot 10^{-3}$ min^{-1} > $k_{obs_{Leu}}$ > $3.0 \cdot 10^{-3}$ min^{-1}. The same order of reactivity was found under these conditions for the mixture. The dependence on thionyl chloride concentration and tem-

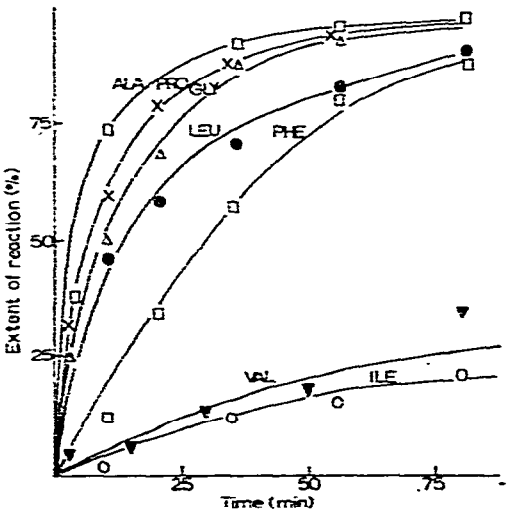

Fig. 1. Order of reactivity established for the neutral amino acids at 40°C using 2 *M* thionyl chloride in *n*-propanol.

TABLE I

DEPENDENCE OF THE RATE CONSTANT ON THE CONCENTRATION OF THIONYL CHLORIDE AND ON TEMPERATURE

Thionyl chloride concentration (M)	$k_{obs} \times 10^{-3}$ *(min)*				
	30°C	*40°C*	*50°C*	*60°C*	*70°C*
1		1.7			
2		3.0			
3		4.5	6.5	12.2	17.7
4	1.7	6.4		9.2	17.8

perature showed that whereas there is only a 3.7-fold increase in the rate constant on going from 1 to 4 *M* solutions, over the temperature range studied the increase was about 10-fold (Table I), so that on a comparative basis increasing the temperature has a greater effect on the reaction than increasing the thionyl chloride concentration (Fig. 2). Similar results have been obtained for the transesterification of methyl to *n*-butyl esters[13]. No significative differences in the rate constants were observed with 3 or 4 *M* thionyl chloride solution. Higher thionyl chloride concentrations were not studied because they are not physically stable (they boil on pipetting). Studies of esterification yields obtained with 3 *M* thionyl chloride solution between 70 and 110°C showed that most of the amino acids are esterified at levels greater than 95% at 80°C with variable times, 20 min for Pro up to 40 min for Val. His, Arg, Lys, Glu, Cys and Ile were only esterified at this level at temperatures of 100°C or higher, His and Arg being the most resistant.

Quantitative recoveries of the amino acids were obtained after alkaline hydrolysis at room temperature as measured by ion exchange. This result, plus the fact that

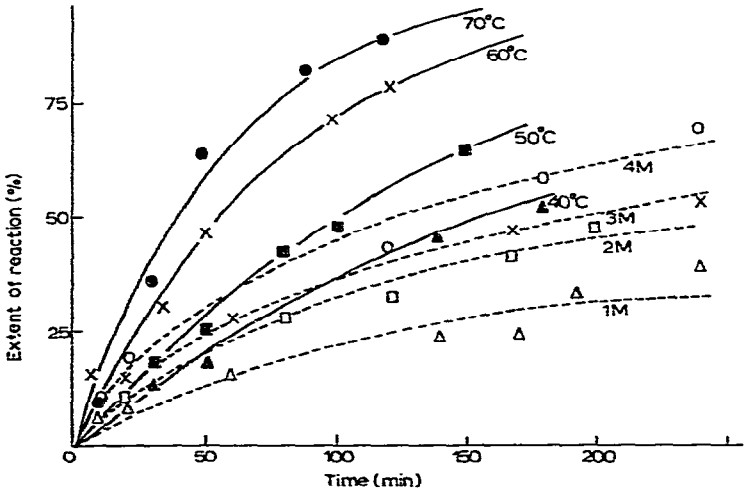

Fig. 2. Effect of temperature (solid lines) using 3 *M* thionyl chloride and of concentration (broken lines) at 40°C on the esterification kinetics of isoleucine.

only the spot corresponding to the ester is observed after complete esterification, suggests that no side-reaction has occurred as far as peptide formation is concerned. Study with Ile at 100°C and using 3 *M* thionyl chloride gave reproducible results with reagents kept for 3 months at −5°C. Incomplete reaction (< 95%) was observed when the reactions were carried out in reaction vessels double the size used here, especially those reactions which had been shown to be more resistant.

In conclusion, 16 amino acids have been esterified at levels greater than 95% using 3 *M* thionyl chloride in *n*-propanol at 100°C for 60 min, with reproducible results, thus offering an alternative approach for the synthesis of O-*n*-propyl esters of amino acids. Studies with lower thionyl chloride concentrations at higher temperatures are in progress.

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