High Sensitivity Optical Resolution of DL-Amino-acids by Gas Chromatography

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RECENT advances in synthetic peptide chemistry impel attention to optical purity of the amino-acids, which should have a steric impurity of less than 0·1% for use in peptide synthesis.[1] Optical rotation does not usually reveal this amount of contamination. Furthermore, there is no theoretical value for an optical rotation, but only the value on which most observers agree. Optically specific amino-acid oxidases and decarboxylases are more sensitive,[2] but are usually *not* chemically specific.

The advantages of gas–liquid chromatography (g.l.c.) for the separation of diastereoisomeric TFA-dipeptide esters have already been demonstrated by Weygand,[3] and we have recently

[1] J. P. Greenstein and M. Winitz, "Chemistry of the Amino-Acids," Wiley, New York, 1961, p. 946.
[2] A. Meister, L. Levintow, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, 1951, **192**, 535.
[3] F. Weygand, *Angew. Chem. Internat. Edn.*, 1963, **2**, 183.

applied this technique to show the asymmetry of some neutral amino-acids.[4] While this procedure gives excellent results with some neutral amino-acids, many TFA-dipeptide esters have such low volatility that g.l.c. analysis results in very long retention times and subsequently poor separation of the diastereoisomeric pairs.

In typical assays (Table 1), the amino-acid was esterified with thionyl chloride–methanol[6] and the excess of reagent and solvent removed. An excess of L-α-chloroisovaleryl chloride in an inert solvent was added to the residue and the suspension cooled and neutralized with triethylamine. After being washed (H_2O) and dried (Na_2SO_4), the solution was injected into the gas chromatograph.

The amino-acid may also be converted into its α-chloro-derivative and then resolved with L-valine methyl ester (Table 2).

Finally, α-halogeno-acids are of special interest in the studies concerned with Walden inversions in the amino-acid series. Reliable and sensitive methods of optical analysis therefore suggest a re-investigation of the work of Fischer and others.[7,8]

TABLE 1. *Gas-chromatographic separation of diastereoisomeric* N-α-*chloroisovalerylamino-acid methyl esters**

Amino-acid	Column†	Separation temp. (°C)	N_2 Flow (ml./min.)	R.t.‡ of diastereoisomer LD	R.t.‡ of diastereoisomer LL	Ratio of R.t.‡ LL/LD
Alanine	B	161	28	8·05	9·15	1·13
Valine	A	161	28	5·9	6·6	1·12
	B	161	28	8·1	9·9	1·22
Leucine	B	161	28	11·85	13·65	1·15
	C	175	56	11·1	12·7	1·14
Isoleucine	B	161	28	10·8	12·8	1·18
Proline	B	185	28	16·1	18·9	1·17
	C	200	56	18·0	21·2	1·17
Threonine	A	145	28	18·0	19·9	1·11
Phenylalanine	A	185	28	15·4	16·5	1·08

* G.l.c. analyses were carried out on a Wilkens 600C aerograph equipped with a flame ionization detector.
† A 5-foot steel column 5% SE 30 on Chromosorb W.
B 5-foot steel column 5% FFAP on Chromosorb W.
C 5-foot steel column 20% DEGS on Chromosorb W.
‡ R.t. = Retention time.

TABLE 2. *Gas-chromatographic separation of diastereoisomeric* N-*chloroacyl-valine methyl esters*

Chloro-acid from:	Column	Separation temp. (°C.)	N_2 Flow (ml./min.)	R.t. of diastereoisomer DL	R.t. of diastereoisomer LL	Ratio of R.t. LL/DL
Alanine	A	140	28	5·9	6·25	1·06
	B	161	28	5·75	6·5	1·13
Valine	A	140	28	11·85	13·5	1·14
	B	161	28	8·1	9·9	1·22
Isovaline	A	140	28	9·3	9·8	1·05
	B	161	28	4·6	4·95	1·08
Leucine	A	140	28	17·05	18·8	1·10
	B	161	28	10·15	12·0	1·18
Isoleucine	A	140	28	17·9	20·6	1·15
	B	161	28	10·35	12·9	1·25
Alloisoleucine	A	140	28	18·9	21·7	1·15
	B	161	28	10·5	12·85	1·22
Ornithine	A	175	28	10·4	11·4	1·10
	B	214	28	8·4	9·9	1·17
Lysine	A	175	28	15·7	17·5	1·10
	B	214	28	11·45	12·8	1·12

We have now found that α-halogeno-acyl chlorides are excellent reagents for quantitative g.l.c. analysis of amino-acids, both with respect to chemical structure and resolution of stereoisomers. In particular, L-α-chloroisovaleryl chloride can be used to analyze many DL-amino-acids and in most cases quantitative separation of the diastereoisomeric pairs is readily achieved on packed columns only 5 feet long. The reagent can be prepared from L-valine by Renard's procedure[5] followed by treatment with thionyl chloride; the product is 100% sterically pure.

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[5] M. Renard, *Bull. Soc. Chim. biol.*, 1946, **28**, 497.
[6] M. Brenner and W. Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.
[7] E. Fischer *et al.*, *Annalen*, 1905, **340**, 123; 1907, **357**, 1; 1911, **303**, 337; *Ber.*, 1908, **41**, 889, 2891; 1909, **42**, 1219.
[8] A. Neuberger, *Adv. Protein Chem.*, 1948, **4**, 297.