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Note

The gas chromatographic resolution of DL-isovaline

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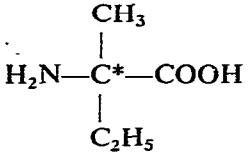
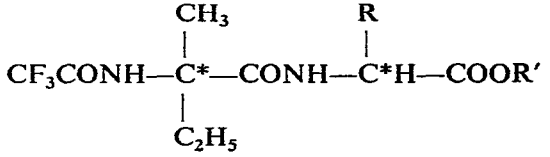
Isovaline (I) is of considerable interest cosmologically in that (1) it is one of the twelve non-protein amino acids which have been isolated from the Murchison meteorite[1] and (2) unlike the other chiral amino acids in this meteorite, it has no α-hydrogen at its asymmetric center and hence cannot racemize by the customary α-hydrogen dependent mechanisms which engender racemization in ordinary amino acids[2].



I



(DL) (L)

II

Accordingly, the enantiomeric composition of the Murchison isovaline sample should be that which prevailed at the time of its original synthesis in the meteorite[3] and could thus give a clue as to the primordial enantiomeric composition of the other protein and non-protein amino acids present in the meteorite[2]. Unfortunately, however, the gas chromatographic (GC) separation of the original mixture of amino acids (converted to diastereomeric N-trifluoroacetyl (N-TFA) (+)-2-butyl esters[4]) from the meteorite failed to resolve the isovaline component at all[1]. Pollock[5] later studied the GC resolution of five known non-protein amino acids as analogous derivatives containing other N-fluoroacyl and/or (+)-2-alkyl ester functions, and found that DL-isovaline could indeed be resolved to the extent of 78.8% as its N-TFA (+)-2-pentyl ester using a 150 ft. × 0.02 in. capillary column loaded with Dexsil 400 GC phase. Applying this same GC procedure to the isovaline isolated from the Murchison meteorite, Pollock *et al.*[2] achieved a 91% resolution and noted an enantiomeric composition of 52.6 ± 0.5% D(−) and 47.4% L(+). Since an approximately similar enantiomer ratio was noted for authentic DL-isovaline, the authors concluded that the Murchison isovaline must therefore be racemic[2].

Recently we have been interested in the precise stereochemical composition of the Murchison isovaline in another connection, and have accordingly undertaken

Since N-fluoroacyl (+)-2-alkyl esters had been quite thoroughly studied[5] and could not be resolved, we turned to another type of diastereomeric derivative which has been used for the GC estimation of enantiomeric composition, namely, N-TFA dipeptide esters[6,7]. Accordingly a series of N-TFA-DL-isovalyl-L-amino ester dipeptide derivatives of the type II was prepared, where the L-amino acid moiety included alanine, leucine and proline and the alkyl group of the ester function (R′) was methyl or isopropyl. Each of the derivatives was analyzed gas chromatographically using capillary or SCOT columns loaded with such conventional GC phases as Carbowax 20M, SE-30, NPGA, OV-17, Dexsil 300 and Ucon 75H. Resolution of the D,L-isovaline derivatives under these conditions proved inadequate, however, ranging from no separation to 90% or so. When the resolutions were attempted, however, using a 150 ft. × 0.02 in. capillary column loaded with the optically active GC phase, N-lauroyl-L-valyl-*tert.*-butylamide[8], the N-TFA-DL-isovalyl-L-leucine isopropyl ester derivative (II, R = $(CH_3)_2CHCH_2$–; R′ = $(CH_3)_2CH$–) proved to be resolvable with baseline separation of the two diastereomer peaks and with the two peaks appearing 8 min apart (90 min, 98 min, 140° isothermal). As seen in Fig. 1, a similar quantitative resolution of this derivative could be accomplished in a significantly shorter time using the more recently developed optically active phase, N-docosanoyl-L-valyl-*tert.*-butylamide[9], which can be employed at a higher temperature without column bleed. Electronic integration of the diastereomer peaks in five replicate analyses of the sort shown in Fig. 1 gave identical areas for each peak (1st: 49.95 ± 0.33%; 2nd: 50.05 ± 0.33%).

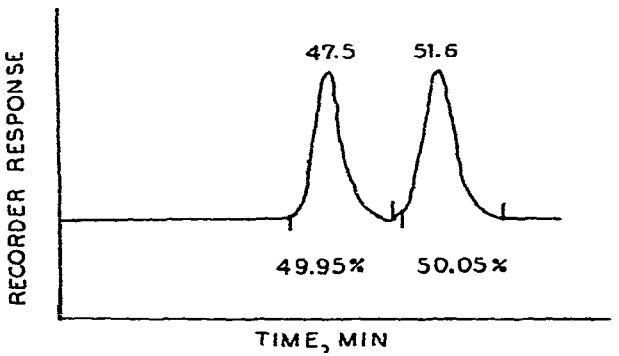


Fig. 1. Gas chromatographic analysis of N-TFA-DL-isovalyl-L-leucine isopropyl ester.

EXPERIMENTAL

N-TFA-DL-isovalyl-L-leucine isopropyl ester

The following procedure was adapted directly from that previously employed to synthesize N-TFA-L-prolyl-DL-leucine methyl ester[6]. N-TFA-DL-isovalyl chloride (III) was prepared by the action of trifluoroacetic anhydride on a suspension of DL-isovaline in dichloromethane (DCM), followed by solvent stripping and reaction of the crude product with thionyl chloride at room temperature. At the same time L-leucine isopropyl ester hydrochloride (IV) was prepared by refluxing for 3 h a solution of L-

leucine in 2-propanol saturated with hydrogen chloride, then stripping the solvents. A DCM solution of the acid chloride III was then added to the ester hydrochloride IV residue and the solution was chilled and treated dropwise under stirring with a solution of 2.2 equivalents of triethylamine in DCM. Processing and isolation of the N-TFA-dipeptide ester product followed the previous procedure[6].

Gas chromatography

One microlitre of a 10^{-2} *M* solution of the above dipeptide ester in nitromethane was injected into a 150 ft. × 0.02 in. capillary column coated with N-docosanoyl-L-valyl-*tert.*-butylaminde phase[9], installed in a Hewlett-Packard Model 5700A gas chromatograph coupled to a Hewlett-Packard Model 3380A digital electronic integrator–recorder. Operating conditions for analyses such as shown in Fig. 1 were: 150° isothermal, nitrogen flow-rate *ca.* 2.5 ml/min. Efflux times (min) and integrated peak area percents (average) are shown next to each diastereomer peak in Fig. 1.

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