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Gas chromatographic–mass spectrometric studies of the isopropylation of amino acids

Our interest in the gas chromatography (GC) of drugs and their metabolites prompted a more detailed examination of peralkylation reactions utilizing bulky isopropyl groups. This technique was reported[1] to afford, in the case of simple amino acids, N,O-diisopropyl derivatives, though no information was available concerning yields, side products or failures of this derivatisation technique. However, it should afford, in successful cases, stable derivatives whose structures could be rigorously characterised.

By extending the original work we have been able to derivatise and separate, in a single programmed GC analysis, a mixture of twenty-three common amino acids (Fig. 1). Several amino acids gave no detectable derivatives, whilst others showed multiple peaks. Moreover significant amounts of side products were produced which could confuse the analysis. In this paper we wish to detail our experiences with the isopropylation technique.

Experimental

Gas chromatography. A Pye 104 dual-column instrument, operating as follows, was used throughout: Flame ionization detector (FID) (hydrogen flow 40 ml/min, air 600 ml/min); OFN* carrier gas flow 40 ml/min; glass column 5 ft. × ¼ in. O.D.; 5 % Carbowax 20M on Chromosorb G (AW DMCS); injection heater 70° in excess of oven; temperature program 50–240° at 2°/min.

Gas chromatography–mass spectrometry. A Pye 104 instrument, coupled to an AEI 902 mass spectrometer via a membrane separator[2], was operated as follows: helium carrier gas flow 40 ml/min (split 10:1, in favour of the mass spectrometer); temperatures of the components, capillary link 240°, membrane 180°, re-entrant 240°, ion source 250°; oven temperature program 50–240° at 4°/min.

The parallel FID/TIC** traces were almost identical. Mass spectra were obtained with 70 eV ionizing volts, 100 μA ionizing current and 8 kV accelerating potential.

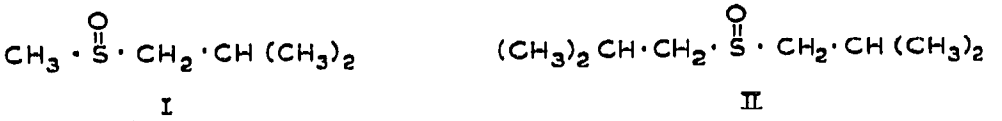
Derivatisation procedure. Essentially the method of PETTIT AND STOUFFER[1] was followed. All liquids were reagent grade and were dried over 4A molecular sieve prior to use. A BDH amino acid reference kit was used.

Preparation of N,O-triisopropyl tyrosine hydrobromide. Tyrosine (1 g) was derivatised and the product was obtained in 100 ml of dry ether. This solution was then saturated with dry hydrogen bromide gas. After cooling, the precipitate was collected and recrystallised twice from absolute ethanol to give white crystals (m.p. 186–190°). This material gave only one peak, identical with the normal tyrosine derivative, on gas chromatography–mass spectrometry (GC–MS) examination.

Results and discussion

Because of the vigorous nature of the derivatisation reaction, and because the derivatisation of a biological extract would usually involve an excess of alkylating

* OFN = oxygen-free nitrogen.
** TIC = total ion current.



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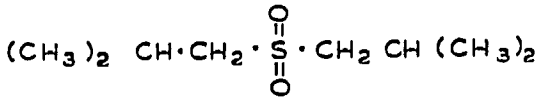

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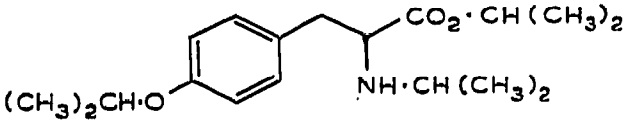

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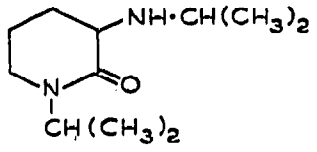

V

agent, we first examined a control reaction involving no amino acid. Examination of the basic and neutral products, by GC, revealed one major and two minor components, which could not be removed by washing with brine. MS identified these as compounds I, II and III, respectively. Possibly the sulphone (III) arose from our use of reagent-grade solvent. Attempts to moderate the reaction by the use of dimsyl sodium[3] and higher dilution only lead to diminished yields of alkyl amino acids and increased yields of side products.

A test mixture, containing equal parts, by weight, of twenty-three chromatographically-pure amino acids, was derivatised and examined by GC–MS (Fig. 1). Each peak was identified by GC–MS comparison with individually derivatised amino acids.

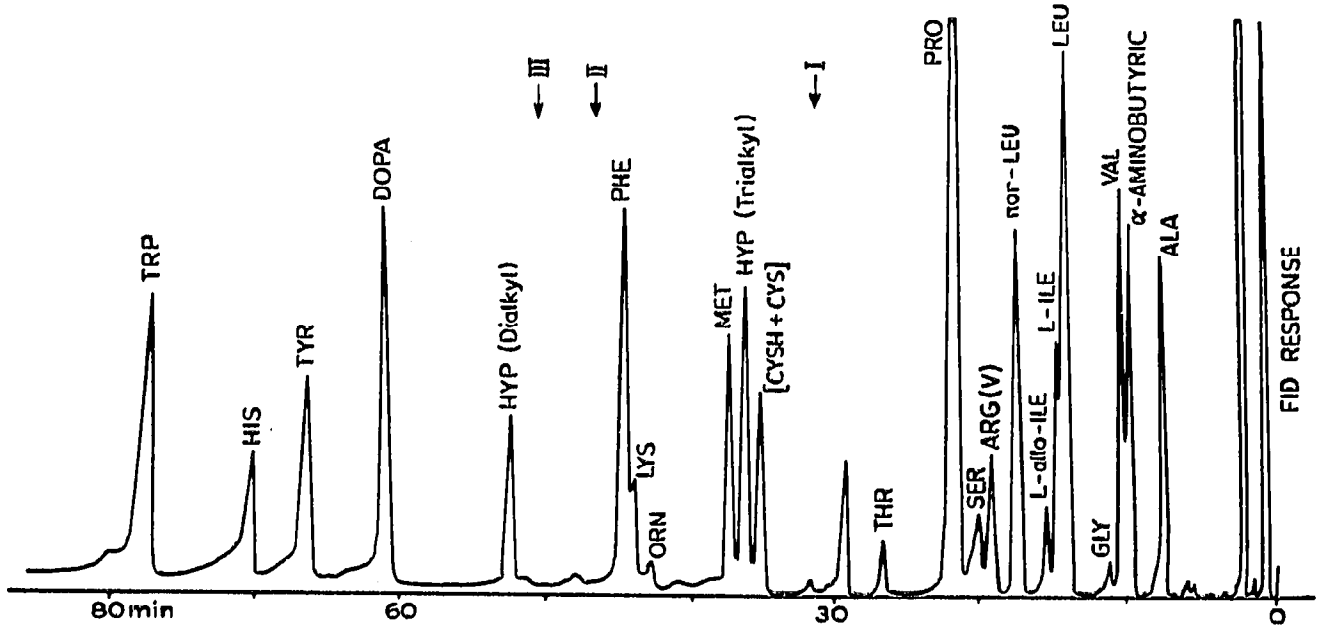

Fig. 1. Chromatogram obtained on GC analysis of a mixture of amino acids as their isopropyl derivatives.

It was clear, on the basis of a peak-height analysis, that yields varied considerably depending upon the amino acid chosen. Tyrosine was selected as a standard and its N,O-triisopropyl derivative (IV) was obtained as a crystalline hydrobromide. From this material standard solutions of both the free base and the hydrobromide were prepared. The GC responses of these two solutions were almost indistinguishable. Comparison with several derivatisations of tyrosine gave an average yield of 75 % for this amino acid.

Based upon a peak-height analysis the responses, relative to tyrosine, of the other amino acids in the test mixture were evaluated. Those amino acids containing alkyl or aryl side chains were derivatised in good yield, whilst cystein, cystine (which was presumably reduced during the reaction to cystein) and methionine gave satisfactory yields. The hydroxy and basic amino acids gave low yields but taurine, glutamic, aspartic acids and the amides asparagine and glutamine gave no detectable derivatives.

In the case of hydroxyproline we observed significant amounts of a second derivative whose mass spectrum corresponded to the triisopropyl compound. Two peaks were also found with 'L-isoleucine', both with identical mass spectra, neither of which corresponded to leucine nor norleucine. The sample of 'L-isoleucine' used was a mixture of L-isoleucine and L-*allo*-isoleucine so presumably the two peaks correspond to the two diastereoisomers.

Arginine produced a single peak whose mass spectrum was almost identical with that of the ornithine derivative, though its retention time was quite different. We believe this derivative corresponds to the lactam (V) because no ester absorption (1715 cm^{-1}) appears in its IR spectrum.

The electron impact induced fragmentation of the majority of these derivatives is consistent with bond rupture directed by the α-nitrogen. Only in the cases of lysine and ornithine are fragmentations involving ring formation required to rationalise their mass spectra and these have been encountered in fragmentation studies of the ethyl esters of lysine[4] and ornithine[5].

Tabulated low-resolution MS of all of the compounds included in this work have been submitted to the Mass Spectrometry Data Centre, AWRE, Aldermaston, Great Britain.

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