

Note

Paper chromatography of the ethyl esters of aspartic acid

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(Received April 4th, 1975)

Treatment of aspartic acid with ethanolic hydrogen chloride gives a solution containing all the possible ethyl esters of this amino acid. The data given in Table I, though limited, are sufficient for unequivocal identification of the esters. This is important partly because these esters are useful synthetic intermediates^{1,2} and partly because their chromatographic characteristics have been incorrectly reported in the literature^{3,4}.

The ester having an R_F value of 0.45 (I) was definitely β -ethyl aspartate because, on reaction with ethylamine, it gave a compound which co-chromatographed with a sample of N^4 -ethylasparagine which had been synthesized by an independent route⁵. Similarly, the component having R_F 0.54 was identified as α -ethyl aspartate by heating it with anhydrous ammonia in ethanol and showing that the product was inseparable from authentic isoasparagine. Both comparisons were made by paper chromatography using *n*-butanol-acetic acid-water (90:10:29, v/v), *n*-butanol-ethyl methyl ketone-ammonia (sp. gr. 0.880)-water (4:4:1:1, v/v), *tert.*-butanol-methanol-water (4:5:1, v/v), *tert.*-amyl alcohol-ethyl methyl ketone-water (6:2:2, v/v), phenol-water (3:1, w/v) and phenol-formic acid-water (100:10:29, v/v).

The identity of spot II having R_F 0.79 was also rigorously established by comparison with a sample of α,β -diethyl aspartate, synthesized by the method of Fischer⁶ and characterized by nuclear magnetic resonance. However, Koch and Hanson³ have previously identified spot II as the free base form of β -ethyl aspartate and suggested that I was only given by β -ethyl aspartate hydrochloride. We have shown that samples of the β -monoester, initially adjusted to pH values of 0.8, 3.6, 8.0 and 12.5, all chromatograph as a single spot having an R_F value of 0.45 in *n*-butanol-acetic acid-water (90:10:29, v/v). Thus the initial ionic state of β -ethyl aspartate does not affect its mobility and Koch and Hanson's identification of II must be in error. DeVay *et al.*⁴ have also identified spot II incorrectly: they were misled because a sample of II, purified by preparative chromatography and eluted from paper with 0.01 *N* HCl, gave C, H and ethoxy analyses which suggested that it was a monoester. In fact, chromatography shows that α,β -diethyl aspartate decomposes to a mixture con-

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TABLE I
CHROMATOGRAPHIC PROPERTIES OF THE ETHYL ASPARTATES

Solvent, *n*-butanol-acetic acid-water (90:10:29, v/v). Descending chromatograms, run on Whatman No. 3MM paper, were dipped through 0.2% ninhydrin in industrial ethanol and heated at 100° for 10 min. Each R_f value, the mean of at least four observations, is given with its standard deviation. The data published by Koch and Hanson³ are shown in parentheses for comparison; the solvent was *n*-butanol-acetic acid-water of unspecified composition.

<i>Ester</i>	R_f	<i>Spot</i>	<i>Ninhydrin colour</i>
β -Ethyl aspartate	0.45 \pm 0.04 (0.44)	I	Brown-yellow-green (grey-green)
α -Ethyl aspartate	0.54 \pm 0.03		Mauve
α,β -Diethylaspartate	0.79 \pm 0.02 (0.73)	II	Grey-purple-brown (red-brown-green)

taining aspartic acid and both monoesters when eluted from paper chromatograms with aqueous solvents.

ACKNOWLEDGEMENTS

Thanks are due to Dr. R. Walter and Professor L. Fowden who kindly supplied authentic samples of N⁴-ethyl asparagine and isoasparagine respectively. This work was supported by a studentship awarded by the Science Research Council of Great Britain.

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