

per litre, was allowed to drain and dried at 100° C. The benzoates when chromatographed on this paper were revealed in ultra-violet light as dark spots on a yellow fluorescent background, a spot containing 10⁻⁸ gm. mole being just visible. Spots containing more than 10⁻⁶ gm. mole tend to overload the paper.

The normal technique of descending irrigation was slightly modified in order to maintain a saturated atmosphere in the chamber with the volatile solvents used. The chamber lid, fitted with a rubber gasket and a thumbscrew tightening device, was completely airtight. Inside were arranged three troughs side by side. From the outer ones were hung large sheets of filter paper.

The benzoates in benzene solution were applied in the normal way to a prepared strip of paper. This was hung from the centre trough at evening and the outer troughs were filled, one with methanol saturated with heptane, the other with heptane saturated with methanol. Next morning the centre trough was filled with the heptane phase through a hole (normally closed) in the chamber lid. The solvent front moves 25 cm. in about one and a half hours.

For the dinitrobenzoates of the following alcohols, R_F values (± 0.02) were obtained for a run of about 25 cm. at 20° C. :

Methanol	0.24	2-Butanol	0.61
Ethanol	0.39	2-Methyl-1-propanol	0.55
1-Propanol	0.46	1-Pentanol	0.66
2-Propanol	0.51	3-Methyl-1-butanol	0.65
1-Butanol	0.57	1-Hexanol	0.72

If the values for the homologous series of derivatives of normal alcohols are used for calculating the corresponding R_M values¹ and these are plotted against the number of methylene groups in the alcohols, the points lie approximately on a straight line.

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¹ Rice, R. G., Keller, G. J., and Kirchner, J. G., *Anal. Chem.*, **23**, 194 (1951).

² Kariyone, T., Hashimoto, V., and Kimura, M., *Nature*, **168**, 511 (1951).

³ White, J. W., and Dryden, E. C., *Anal. Chem.*, **20**, 853 (1948).

⁴ Bate-Smith, E. C., and Westall, R. G., *Biochim. et Biophys. Acta* **4**, 427 (1950).

Feasibility of Quantitative Separation of the Threo- and Erythro-Forms of Amino-Acids by Column Chromatography

THE methods that are at present available for the separation of the diastereoisomeric forms of amino-acids are subject to several limitations. In the usual fractionation procedures only one of the isomers, at most, is obtained pure, the other remaining contaminated. A high degree of purity is realized only at the expense of yield. The difficulties inherent in isotopic synthesis make the above operations increasingly wasteful, thus further limiting the practicability of these methods.

We wish to report that the application of column chromatography, using an ion-exchange resin ('Dowex-50', 250/500 mesh), led to the clean-cut and quantitative separation of *dl*-threonine and *dl*-allothreonine. On the scale employed (3 mM), the chromatographic separation was complete, whereas chemical separation, although yielding microbiologically pure *dl*-threonine, resulted in a *dl*-allothreonine fraction containing much of the threo-form. The carbon-14 labelled products of both chemical and chromatographic separation have been tested for purity by microbiological assay with *L. faecales*.

The quantitative recovery, the completeness of the separation and the economy of effort resulting from the use of the chromatographic procedure offer great advantages. The investigation is being extended to other β -substituted serines and isoleucine. Details of this work will be published elsewhere.

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Counter-Current Distribution of Nisins.

It has been suggested¹ that the phrase "100 extractions" quoted from my work by Hirsch² is liable to misinterpretation. Amplification is therefore needed.

"Extraction" was used by Bush and Densen³ to describe the process occurring in each single unit (for example, separating funnel) at each stage of the counter-current distribution. With their technique, n^2 extractions are made in a chain of n units, giving n top layers and n bottom layers. "Extraction" must therefore be distinguished from "transfer".

The technique of Bush and Densen was applied to various samples of nisin, *A* and *L*, mentioned by Hirsch, giving the distribution shown in Fig. 1. Batch 138 is included as an example of a preparation with two active components. "Commercial B", different from *B* of batch 138, was distributed earlier by Craig's method⁴ in twenty test-tubes (actual number of extractions 210, not 200) and appeared to give two major components, both differing from *A* and *L*; but the measured partition

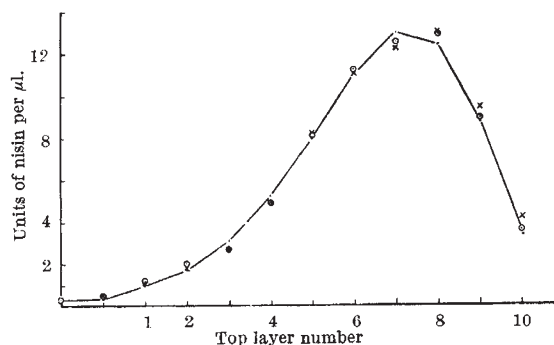


Fig. 1. Distribution of nisins *A* and *L*. Theoretical curve for a single substance; O, experimental points for *L*; X, experimental points for *A*.