Communications to the Editor

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Separation of L- and D-Amino Acids as Diastereomeric Derivatives by High Performance Liquid Chromatography

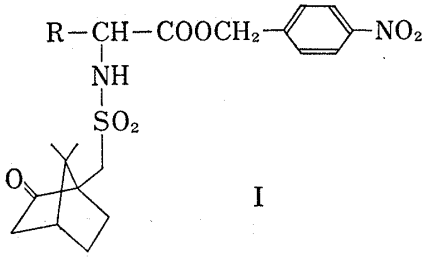
The optical resolution of racemic amino acids by high performance liquid chromatography (MicroPak Si-5, 1.5% iso-PrOH in iso octane) of corresponding diastereomeric *N*-*d*-10-camphorsulfonyl *p*-nitrobenzyl ester can be achieved.

The high performance liquid chromatography (HPLC) become used widely for the separation of complex mixtures because of its high sensitivity and versatility.

Recently, optical resolution of racemic amino acids has been carried out by gas chromatography.[1] But no convenient method for the HPLC resolution of amino acids has been described.

This communication is an approach to the optical resolution of amino acids as diastereomeric derivatives using HPLC.

The amino acids examined (alanine, leucine, isoleucine, and phenylalanine) were chromatographed in the form of N-*d*-10-camphorsulfonyl *p*-nitrobenzyl ester (I) of amino acids. The *d*-10-camphorsulfonyl moiety was used to introduce an additional asymmetric center and the *p*-nitrobenzyl moiety was introduced as the chromophor for detection.



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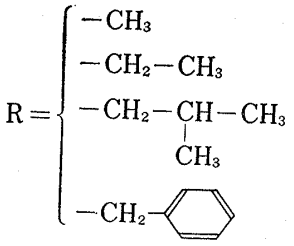

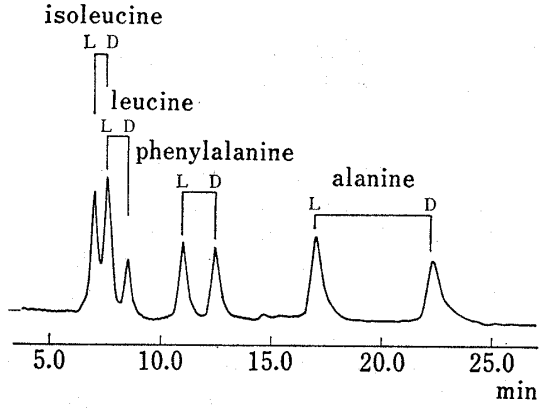

Fig. 1. Chromatogram of the Derivatives of Amino Acids

apparatus: FLC 350 equipped with a 254 nm UV-detector (JASCO); column: MicroPak Si-5 (2.0/250 mm) (Varian); solvent: 1.5% iso-PrOH in isooctane; flow-rate: 0.5 ml/min (pressure: 50—150 kg); column temperature: room temp

Table I. Retention Times of N-*d*-10-Camphorsulfonyl *p*-Nitrobenzyl Esters of Amino Acids

Amino acids	Retention times (min)	
	L-Enantiomer	D-Enantiomer
Isoleucine	7.0	7.6
Leucine	7.6	8.6
Phenylalanine	11.0	12.4
Alanine	17.0	22.3

1) E. Gil-Av, R. Charles and G. Fischer, *J. Chromatog.*, **17**, 408 (1965); S.V. Vitt, M.B. Saporowskaya, I.P. Gudkova, and V.M. Belikov, *Tetrahedron Letters*, **1965** 2575; B. Halpern, and J.W. Westley, *Tetrahedron Letters*, **1966** 2283; S. Nakaparksin, P. Birrell, E. Gil-Av, and J. Orò, *J. Chromatog. Sci.*, **8**, 177 (1970); W.A. Koenig, W. Parr, H.A. Lichtenstein, E. Bayer, and J. Orò, *J. Chromatog. Sci.*, **8**, 183 (1970); H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **22**, 8 (1974); H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **23**, 217 (1975).

The derivatives (I) were prepared by the reaction of amino acids (0.5 mmole) and *d*-10-camphorsulfonyl chloride[2] (0.5 mmole) in ether (10 ml) and 1N NaOH aqueous solution (10 ml) under vigorous stirring at room temperature for one hour, and then, the reaction mixture was acidified with 1N HCl and extracted with ether. The resulting sulfonylamide was treated with *p*-nitrobenzyl bromide (0.5 mmole) in chloroform solution under reflux for 30 min, and the chloroform solution was washed with H_2O, dried with anhyd. Na_2SO_4, and evaporated.

The experimental conditions of HPLC and the chromatogram of amino acids derivatives[3] were shown in Fig. 1.

In order to allocate the peaks, derivatives were prepared from optically active amino acids with the same reaction condition described above.[4]

The derivatives of D-amino acids had in all cases a longer retention times (Table I).

The application to the preparative resolution and the quantitative treatment are under investigation.

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2) P.D. Bartlett and L.H. Knox, "Organic Syntheses," Vol. 45, John Wiley & Sons, Inc., N. Y., 1965, p. 45.
3) The NMR spectra (100 MHz, in $CDCl_3$) of all compounds are in accordance with the structures.
4) For the derivatives of optically active amino acids have a single peak by HPLC, no racemization proceeds during preparation of the derivatives under the reaction condition employed.

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The Effect of AF2 [2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide] on Hepatic Microsomal Mixed Function Oxidase System in Rats

Male rats were fed for 16 days on the diet containing 0.2% of AF2. Hepatic microsomes were isolated and the activities of mixed function oxidase system were assayed. Cytochrome P-450 was significantly reduced in its content and, on the contrary, cytochrome b_5 was much elevated in the AF2-treated animals. AF2 treatment also depressed the activities of aminopyrine N-demethylase and aniline hydroxylase. The changes in the contents of hepatic constituents were also analyzed.

Furylfuramide [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, abbreviated to AF2] had been widely used as an antimicrobial preservative for foods in Japan for about ten years. But recently, the use of the compound as a food additive was prohibited by the Japanese Ministry of Health and Welfare because of its possible carcinogenic action when fed to experimental animals. The mutagenicity of AF2 has been also shown in the testing system in microorganisms. Apart from the carcinogenicity, few studies disclosed other important toxicities of AF2 on the experimental animals. Miyaki, *et al.*[1] observed the atrophy of testis in rats fed on AF2, but Miyaji[2] did not find any changes in testis by the administration of the compound. Liver hypertrophy is generally observed in animals treated with AF2 and Miyaji[2] deduced

1) K. Miyaki, M. Akao, K. Terao, and K. Kuroda, *Gann*, **103**, 167 (1969).
2) T. Miyaji, *Tohoku J. Exp. Med.*, **103**, 331 (1971).