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Note

Determination of optical purity by high-performance liquid chromatography on chiral stationary phases: pantothenic acid and related compounds

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In the last few years, numerous chiral stationary phases (CSPs) have been developed for optical resolution by high-performance liquid chromatography (HPLC). The CSPs include many types of columns[1], for instance, Pirkle[2], ligand-exchange[3], protein-conjugated[4,5], etc.

D-Pantothenic acid is a precursor of the biologically important coenzyme A. It is well known that D-(*R*)-pantothenic acid and related compounds are biologically active, but the L-isomers are inactive. A few chromatographic methods have been presented for the chiral separation of pantothenic acid and related substances by gas chromatography[6–9]. In these cases, it is necessary to prepare the chiral reagents or chiral stationary phases.

This paper describes two enantiospecific HPLC methods for the separation of pantothenic acid, panthenol and pantolactone enantiomers: (1) after conversion into pantoic acid or direct resolution on a ligand-exchange CSP, and (2) derivatization with the 3,5-dinitrobenzoyl reagents, followed by separation on chiral acrylic polymer CSP or "brush type"[1] CSPs.

EXPERIMENTAL

Chemicals and reagents

DL-Calcium pantothenate and DL-panthenol of reagent grade were obtained from Sigma (St. Louis, MO, U.S.A.), D-, L-calcium pantothenate, D-, L-pantolactone, D-panthenol of pharmaceutical grade from Daiichi Seiyaku (Tokyo, Japan), 3,5-dinitrobenzoyl chloride (DNBC) of reagent grade from Nakarai Chemical (Kyoto, Japan) and 3,5-dinitrophenyl isocyanate (DNPI) from Sumitomo Chemical (Osaka, Japan). All other reagents and solvents were of reagent grade.

High-performance liquid chromatography

An Hitachi Model L-6200 high-performance liquid chromatograph equipped with a spectrophotometric detector was operated at 254 nm.

Chromatographic separation of underivatized calcium pantothenate enantiomer and the related compound enantiomers, which were converted into pan-

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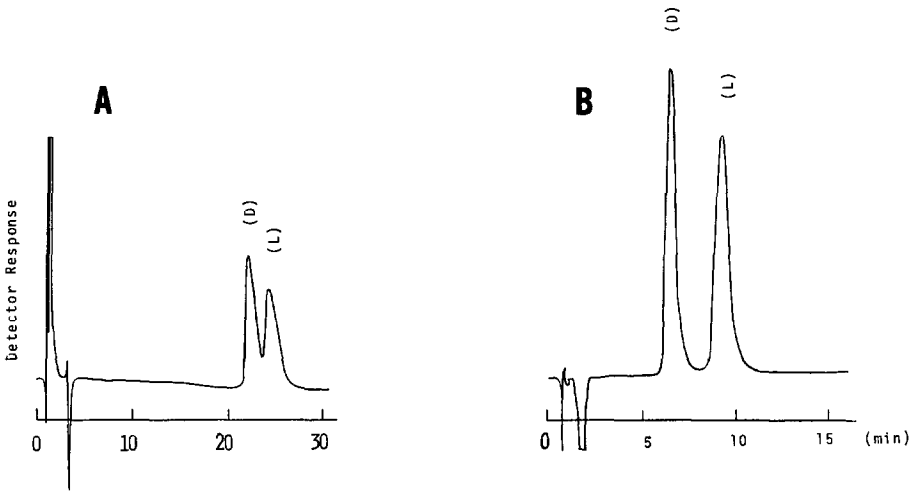


Fig. 1. Chromatograms of racemic calcium pantothenate (A) and racemic pantoic acid converted from panthenol (B). Chromatographic conditions: column; MCI gel CRS 10W 50 mm × 4.6 mm I.D. Mobile phases: 2 m*M* $CuSO_4$ (A), 2 m*M* $CuSO_4$ containing 10% acetonitrile (B). Flow-rates: 0.6 (A), 0.8 ml/min (B), Column temperatures: 25 (A), 35°C (B). Sample amount: *ca.* 50 μg as the racemic mixture.

toic acid, was achieved on MCI gel CRS 10W (50 mm × 4.6 mm I.D., Mitsubishi Kasei Kogyo, Tokyo, Japan), particle size 3 μm. Fig. 1 shows typical chromatograms of these enantiomers.

Chromatographic separation of the 3,5-dinitrobenzoyl (DNB) and 3,5-dinitrophenyl (DNP) derivatives was achieved on a YMC-packed A-KO3 column (250 mm × 4.6 mm I.D., Yamamura Chemical, Kyoto, Japan) which comprises a conjugated D-naphthylethylamine polymer on a silica gel surface, Sumipax OA-4000 (250 mm × 4.6 mm I.D.; Sumitomo Chemical, Osaka, Japan) and Enantio P1 (250 mm × 4.6 mm I.D.; TOSOH, Tokyo, Japan) which are brush-type columns.

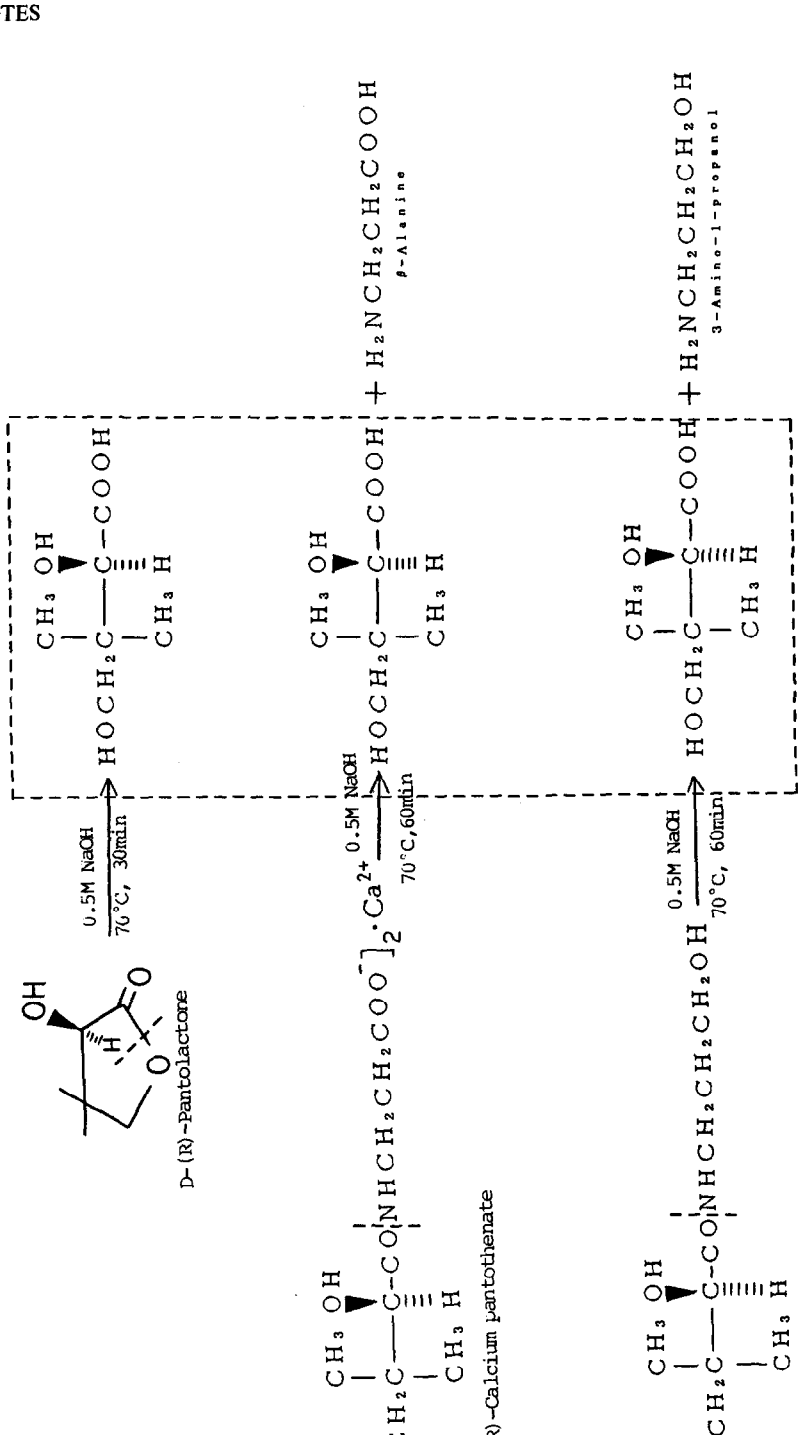
Conversion into pantoic acid (Scheme 1)

D-, L-Pantolactone, D-, L-calcium pantothenate and D-, L-panthenol (10–20 mg) were incubated in 0.5 *M* NaOH (2 ml) solution in vials at 70°C for 30–60 min (see Scheme 1) in a water-bath. After the conversion into pantoic acid, this solution was injected into the chromatograph with a ligand-exchange CSP.

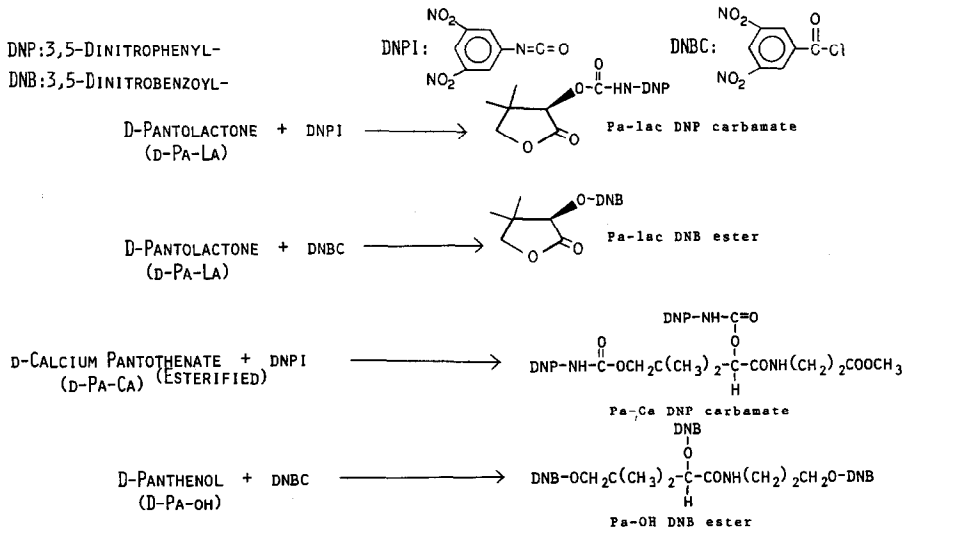
Derivatization with DNPI and DNBC (Scheme 2)

Samples (10 mg) of D-, L- and DL-pantothenic acid were esterified with 5 ml of 1.5 *M* hydrochloric acid in dry methanol at 50°C for 30 min in a screw-cap vial. After removal of the methanol under reduced pressure, 2 ml of dry toluene, 40–60 mg of DNPI and 50 μl of pyridine were added, and the reaction mixture was kept at 60°C for 30 min[10]. After removal of the excess of reagents, the sample was dissolved in 5 ml of chloroform, washed with 5 ml of 1 *M* HCl and water, dried with sodium sulphate (anhydrous) and used for HPLC investigation.

Samples (10 mg) of D-, L-panthenol or D-, L-pantolactone were converted into 3,5-dinitrophenyl carbamates in the same way as pantothenic acid, but without esterification.



Scheme 1. Conversion of pantothenic acid and related compounds into pantoic acid.



Scheme 2. Derivatization of pantothenic acid and related compounds with DNPI and DNBC.

Samples (10 mg) of D-, L-pantolactone were dissolved in 2 ml of tetrahydrofuran, and 40–60 mg of DNBC and 50 μl of pyridine were added. The mixture was heated at 60°C for 60 min[11]. After removal of the excess of reagent, the samples were dissolved in 5 ml of chloroform, washed with 5 ml of 1 *M* HCl, 5% $NaHCO_3$ and water, dried with sodium sulphate and used for HPLC investigation.

RESULTS AND DISCUSSION

MCI gel CRS 10W is a chiral stationary phase with ligand-exchange properties. Partial separation of the calcium pantothenate enantiomers was achieved directly with 2 m*M* copper sulphate solution. The complete resolution of pantoic acid enantiomers was obtained with 2 m*M* copper sulphate solution containing 10% acetonitrile. The peak area ratios of D-, L-pantoic acid are indications of the optical purity of the compounds. This method is applicable to the determination of the optical purity of D-pantolactone and D-calcium pantothenate. A good correlation between the theoretical value (D/D + L %) and the observed value was obtained for both pantolactone and calcium pantothenate (pantolactone: $y = 0.931x + 0.643$, $r = 0.992$; calcium pantothenate: $y = 0.971x + 0.801$, $r = 0.998$). In this application, it was confirmed that the racemization of D- and L-isomers did not occur in alkaline solution.

On the other hand, Pirkle *et al.*[12,13] and Ôi *et al.*[10,14] have reported the separation of many enantiomers as their 3,5-dinitrobenzoyl esters or their 3,5-dinitrophenyl carbamates. We, therefore, attempted to separate the enantiomers of pantothenic acid and related compounds as their 3,5-dinitrobenzoyl or 3,5-dinitrophenyl derivatives. The chromatographic results of these experiments are summarized in Table I and Fig. 2.

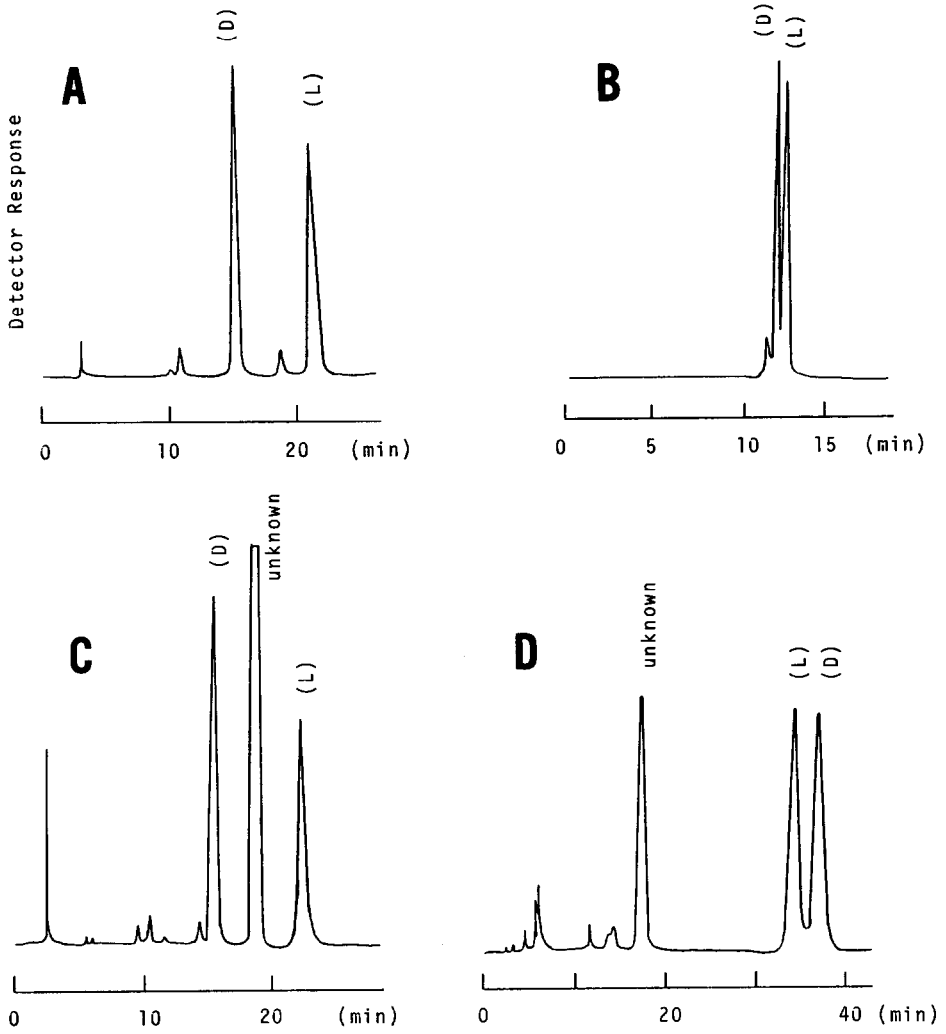


Fig. 2. Typical chromatograms of enantiomeric 3,5-dinitrophenyl (-benzoyl) carbamates (esters) on CSP (YMC-KO3): (A) Pa-lac DNP carbamate; (B) Pa-lac DNB ester; (C) Pa-Ca DNP carbamate; (D) Pa-OH DNB ester. Chromatographic conditions as in table I.

Retention volumes of D-isomer derivatives were smaller than those of L-isomer derivatives except in the case of panthenol. As a result, it was found that the 3,5-dinitrophenyl carbamate derivatives were better separated than the 3,5-dinitrobenzoyl ester derivatives. It can be assumed that the 3,5-dinitrobenzoyl esters lack the –NH– function for hydrogen bonding interaction with the CSPs which is important for chiral recognition.

In conclusion, the methods described here are expected to be useful for determination of the optical purity of pantothenic acid and related compounds by HPLC.

TABLE I

ENANTIOMER SEPARATION OF CALCIUM PANTOTHENATE (Pa-Ca), PANTOTHENYL ALCOHOL (Pa-OH) AND PANTOYL LACTONE (Pa-lac) AS 3,5-DINITROPHENYL (-BENZOYL) DERIVATIVES WITH CHIRAL STATIONARY PHASES (CSPs)

Racemate	*CSP I*			*CSP II*			*CSP III*		
	α^a	k'^b	*Mobile phase*[c]	α^a	k'^b	*Mobile phase*[c]	α^a	k'^b	*Mobile phase*[c]
Pa-Pc DNP carbamate	1.52	4.90	A	1.55	1.46	C	1.37	2.89	C
Pa-lac DNP carbamate	1.51	4.79	A	1.54	1.45	C	1.37	2.67	C
Pa-lac DNB ester	1.09	0.88	A	1.00	0.67	D	1.00	0.67	C
Pa-OH DNB ester	1.08	12.05	B	1.00	10.58	D	1.00	3.18	C

[a] The separation factor of the enantiomers, α, is the ratio of the capacity factors of the enantiomers.

[b] k' is the capacity factor for the first enantiomer eluted (D-isomer except in the case of Pa-OH).

[c] Mobile phases: hexane–dichloromethane–ethanol, 70:30:8 (A), 70:30:10 (B), 70:30:5 (C), 70:30:1 (D). CSP: YMC A-KO3 (I), Sumipax OA-4000 (II), TSKgel Enantio Pl (III). Flow-rates of 0.8 ml/min were typically used at 35°C.

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