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Note

Determination of optical purity by enantioselective capillary gas chromatography: panthenol and related compounds

WILFRIED A. KÖNIG*
Institut für Organische Chemie der Universität, D-2000 Hamburg 13 (F.R.G.)
and
UWE STURM
Schülke & Mayr GmbH, Forschung und Entwicklung, D-2000 Norderstedt (F.R.G.)
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D-Pantothenic acid [3-(2,4-dihydroxy-3,3-dimethylbutyramido)propionic acid] is a constituent of coenzyme A, which is of great importance for enzymastic acylations in living systems. Lack of D-pantothenic acid causes many kinds of deficiency disease in humans and animals: irritation of the skin, dermatitis, depigmentation of the hair and stunted growth. Only the *R*-configurated pantothenic acid acts as a vitamin, and it is a constituent of numerous pharmaceutical formulations[1]. Equally the reduction product of pantothenic acid, panthenol [2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutyramide], in its D-form (dexpanthenol), is used for treatment of many skin diseases, gastritis and inflammations of the respiratory system. It is oxidized enzymatically to pantothenic acid in the organism. Again, only the D-form is active and determination of optical purity in pharmaceutical preparations may be of interest.

D-Pantothenic acid and D-panthenol can be synthesized from *R*-pantoyl lactone, which may be obtained by asymmetric hydrogenation of 3-ketopantoyl lactone, as demonstrated by Ojima and co-workers[2,3]. In this case it is necessary to prove the stereoselectivity of the reaction.

We describe in this communication some simple methods to prove configurational purity of pantothenic acid, panthenol and pantoyl lactone by capillary gas chromatography (GC), using XE-60-L-valine-(*S*)- or (*R*)-α-phenylethylamide[4] as the chiral stationary phase.

EXPERIMENTAL

Gas chromatography

A Carlo-Erba Model 2101 AC gas chromatograph with split inlet and flame ionization detector was used.

Preparation of chiral capillary columns

The preparation of the chiral stationary phase and the method of coating of Pyrex glass capillary columns were described in refs. 4 and 5.

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Formation of volatile derivatives

Samples (1 mg) of DL and D-pantothetic acid were esterified with 1.5 *N* hydrochloric acid in dry methanol at 20°C for 1 h in a screw-cap vial with PTFE lining in the cap. After removal of the methanol in a stream of nitrogen, 200 µl of dichloromethane and 50 µl of trifluoroacetic anhydride were added, and the reaction mixture was kept at 20°C for 20 min. After removal of the excess reagent in a stream of nitrogen, the sample was dissolved in 1 ml of dichloromethane and used for GC investigation.

Samples (1 mg) of DL and D-panthenol were trifluoroacetylated for 15 min at 20°C as described above.

Pantoyl lactone (1 mg) was heated for 30 min at 100°C in a mixture of 100 µl of dichloromethane and 100 µl of isopropyl isocyanate (Fluka) in a thick-walled reacti-vial (Wheaton) or trifluoroacetylated at room temperature as described above.

RESULTS AND DISCUSSION

The chiral polysiloxane phases XE-60-L-Val-(*S*)- or (*R*)-α-phenylethylamide, coated on Pyrex glass or fused-silica capillary columns, have been used to separate a large number of chiral compounds[6]. The enantioselectivity of the stationary phases can be increased by preparing appropriate derivatives of a substrate that contains sites for molecular interaction with the chiral residues of the polysiloxane. As shown

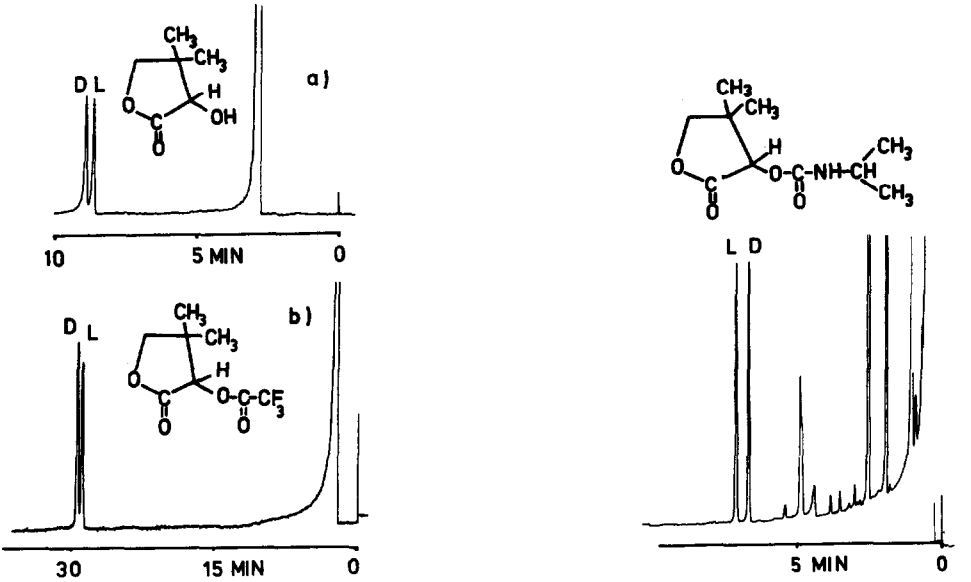

Fig. 1. (a) Separation of DL-pantoyl lactone on a 50-m fused-silica column coated with XE-60-L-Val-(*S*)-α-phenylethylamide. Column temperature, 160°C; carrier gas, 1.2 bar hydrogen. (b) Separation of DL-pantoyl lactone after trifluoroacetylation. Column, 40-m Pyrex glass capillary coated with XE-60-L-Val-(*S*)-α,α'-naphthylethylamide[12]; column temperature, 80°C; carrier gas, 1 bar hydrogen.

Fig. 2. Separation of isopropylurethane derivative of DL-pantoyl lactone. Column 15-m Pyrex glass capillary coated with XE-60-L-Val-(*R*)-α-phenylethylamide; column temperature, 145°C; temperature programme 2°C/min; carrier gas, 1 bar hydrogen.

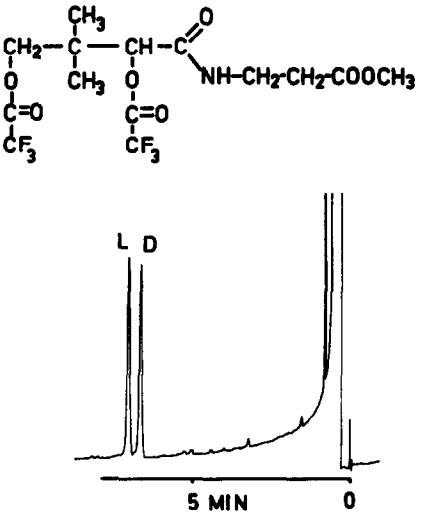

Fig. 3. Separation of DL-pantothenic acid after formation of methyl ester and trifluoroacetylation. Column, 15-m Pyrex glass capillary coated with XE-60-L-Val-(*R*)-α-phenylethylamide; column temperature 130°C; temperature programme 2°C/min; carrier gas, 1 bar hydrogen.

by Koppenhoefer *et al.*[7], pantoyl lactone can be seprated without derivatization on Chirasil-val. However, peak-tailing due to the polar hydroxy group may be encountered when the capillary column is not completely deactivated, or after prolonged use of a previously properly deactivated column, as shown in Fig. 1a. In earlier

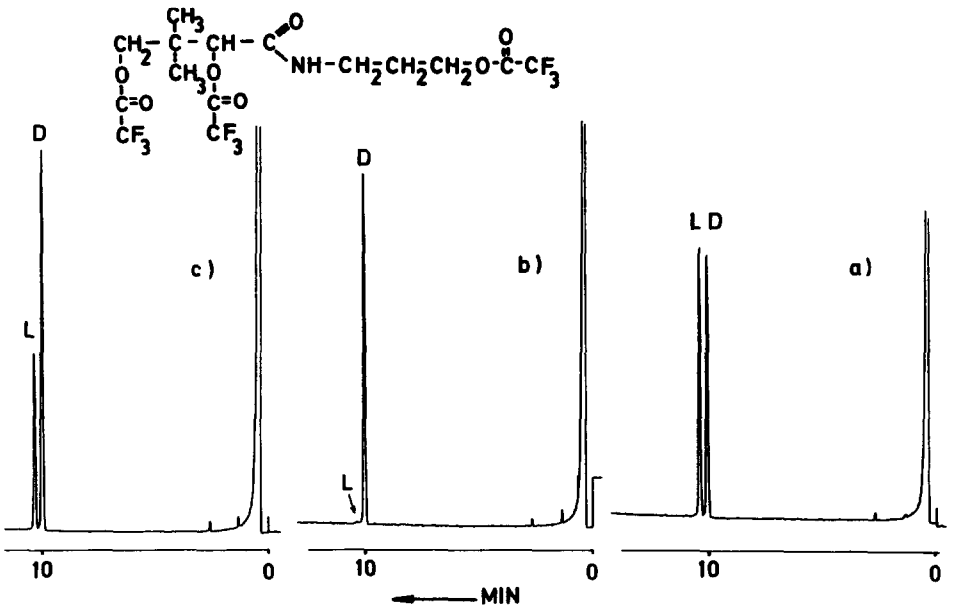

Fig. 4. Separation of (a) racemic panthenol, (b) "pure" D-enantiomer, and (c) a mixture of (a) and (b) after trifluoroacetylation. Column, 15-m fused-silica capillary coated with XE-60-L-Val-(*S*)-α-phenylethylamide; column temperature, 130°C; temperature programme, 1.5°C/min.

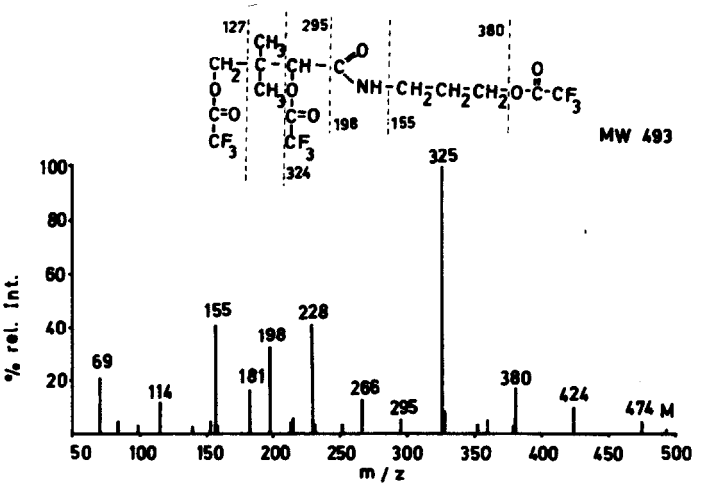

Fig. 5. Electron impact (70 eV) mass spectrum of O-trifluoroacetylated panthenol. Hewlett-Packard 5985 A quadrupole mass spectrometer (with Hewlett-Packard 5840 gas chromatograph); column 25-m fused-silica capillary column, coated with OV 1; column temperature, 150°C; temperature programme, 5°C/min.

communications, Ôi *et al.* described the separation of pantoyl lactone on a triazine derivative with L-lysine as a chiral ligand[8], and on N-(1*R*, 3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)-ethylamide[9]. Chiral alcohols can be separated more generally after conversion into urethanes by reaction with isopropyl isocyanate[10,11]. This reaction proceeds without racemization, and the urethane derivative of pantoyl lactone is separated very well on short capillary columns with XE-60-L-Val-(*S*)- or (*R*)-α-phenylethylamide, as shown in Fig. 2. Pantoyl lactone may also be separated after trifluoroacetylation; however, much longer columns are necessary for complete separation (Fig. 1b).

The enantiomers of pantothenic acid can easily be separated after formation of the methyl ester and trifluoroacetylation (Fig. 3). Again, racemization during derivatization could be excluded by investigating a pure D-enantiomer.

In the case of panthenol, the reduction product of pantothenic acid, a time-consuming and rather inaccurate microbiological test has been so far used for establishing the optical purity[13,14]. Trifluoroacetylation under mild conditions yields a volatile O-trifluoroacetyl derivative, which is separated on both XE-60-L-Val-(*S*)- and (*R*)-α-phenylethylamide (Fig. 4). The derivative was investigated by GC–MS (Fig. 5) to make sure that acylation of the NH group is avoided, which may occur after prolonged reaction with trifluoroacetic anhydride even at room temperature. The N-acylated derivative, which lacks the NH function for hydrogen bonding interaction with the chiral stationary phase, is not separated.

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