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Note

Separation and determination of the enantiomers of pantolactone by gas–liquid chromatography

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Recently a number of papers have reported that optically active drugs showed different pharmacokinetic behaviour and/or pharmacological effects. It is therefore important to determine the optical purity of enantiomeric drugs in either chemical or biological applications.

D-Pantolactone is a constituent of D-pantothenic acid which is a precursor of the biologically important coenzyme A. D-Pantothenic acid and its analogues are biologically active, but the L-isomers are inactive.

A few gas–liquid chromatographic (GLC) methods have been presented for the separation of D- and L-pantolactone[1–4], most involving chiral stationary phases coated on capillary columns. Only one method succeeded in the separation of the enantiomers as their D-menthyl derivatives on a conventional column packed with QF-1[1]. These methods are useful for the separation of D- and L-pantolactone, but for routine work special columns may be required and the difficulty of D-menthyl derivatization remains a problem.

We devised a new GLC method for the separation and the determination of D- and L-pantolactone as the diastereoisomers derived from the reaction with (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPAC). This paper describes the method, and discusses its application to the determination of the optical purity of D-pantothenic acid and its analogues.

EXPERIMENTAL

Chemicals and reagents

D-, L- and DL-pantolactone of reagent grade were obtained from Tokyo-kasei Kogyo (Tokyo, Japan), D-Panthenol of reagent grade from Sigma (St. Louis, MO, U.S.A.), and calcium D-pantothenate and D-pantethine of pharmaceutical grade from Daiichi seiyaku (Tokyo, Japan). The reagent MTPAC was synthesized from (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid of reagent grade (Aldrich, Milwaukee, WI, U.S.A.) by the method of Gal[5]. MTPAC was dissolved in 1,2-dichloroethane to prepare a reagent solution of concentration 100 mg/ml, which was stored in a refrigerator. All other reagents and solvents were of reagent grade.

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Gas–liquid chromatography

A Shimadzu gas chromatograph GC-5A equipped with a flame ionization detector was used. A 2 m × 3 mm I.D. silanized glass column packed with 2% OV-17 coated on Gas Chrom Q (80–100 mesh) was used. The column and the injection port were maintained at 170 and 190°C, respectively. Nitrogen was used as a carrier gas at a flow-rate of 60 ml/min.

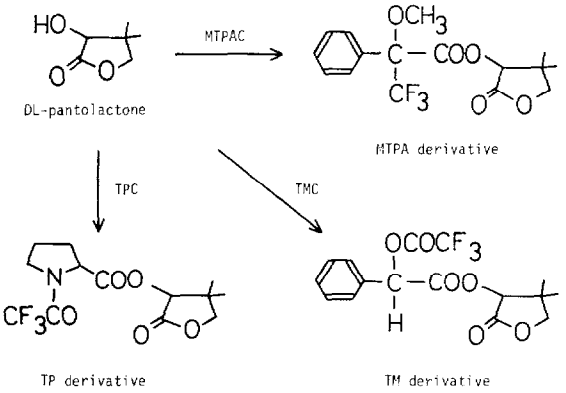
Derivatization

D-, L- and DL-pantolactone (1 mg) were dissolved in 1,2-dichloroethane (50 μl), treated with MTPAC solution (50 μl) and pyridine (20 μl) at 80°C for 30 min, and the reaction mixture was left overnight at room temperature.

RESULTS AND DISCUSSION

Choice of diastereoisomer

Three kinds of diastereoisomeric derivatives of DL-pantolactone were prepared as shown in Scheme 1, and a preliminary GLC separation of them was made using columns packed with 2% OV-17 (1 m) and 2% OV-1 (1 m). As a result, it was found that the MTPA derivatives were well separated compared with the (−)-trifluoroacetylmandelyl (TM) derivatives* and the N-trifluoroacetyl-L-prolyl (TP) derivatives[6]; also that the stationary phase OV-17 was preferable to OV-1.



Scheme 1. Diastereoisomers of DL-pantolactone.

The chemical structures of the MTPA derivatives were confirmed by chemical ionization mass spectrometry, as shown in Fig. 1.

GLC conditions

In order to determine the optimum separation conditions for the MTPA derivatives, further examinations were carried out on columns of 2% OV-17 (1 m and 2 m) and 7% OV-17 (2 m). Table I summarizes the experimental results, and Fig. 2

* (−)-Trifluoroacetylmandelyl chloride (TMC) was prepared from the reaction of (−)-mandelic acid with trifluoroacetyl anhydride and thionyl chloride.

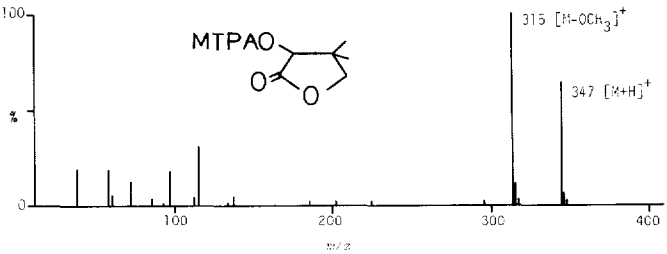


Fig. 1. Chemical ionization mass spectrum of the MTPA derivatives of D- or L-pantolactone.

shows the typical chromatograms of MTPA derivatives of DL-, D- and L-pantolactone. The L-pantolactone derivative was eluted before the D-isomer derivative. The separation factor, α, is almost independent of the kind of column and column temperature, whereas the resolution, R, is slightly affected by these parameters. As seen in Fig. 2b or c, a small peak was observed having the same retention time as that of the corresponding enantiomer. These phenomena are caused by contamination from a small amount of (+)-MTPAC in the derivatization reagent.

The optimum GLC conditions presented in Experimental were determined from the analytical view point, that is, by consideration of the retention times of the eluates.

Derivatization conditions

The reaction of DL-pantolactone with MTPAC proceeded to completion under the conditions described in Experimental and no unchanged pantolactone was detected. The derivatization procedures are simple and the reaction is rapid.

Relationship between the theoretical and observed values for D-pantolactone

Mixtures (1 mg) of D- and L-pantolactone were prepared having various per-

TABLE I

CHROMATOGRAPHIC RESULTS FOR THE MTPA DERIVATIVES OF DL-PANTOLACTONE

Column	*Column temp. (°C)*	t_R *(min)*		α	R
		L-*Form*	D-*Form*		
2% OV-17 (1 m)	140	21.8	27.7	1.3	1.4
	150	13.5	16.8	1.2	1.4
	160	8.4	10.2	1.2	1.3
	170	5.7	6.9	1.2	1.1
2% OV-17 (2 m)	160	17.5	21.4	1.2	1.7
	170	11.5	13.9	1.2	1.9
	180	7.7	9.3	1.2	1.7
	190	5.3	6.3	1.2	1.2
7% OV-17 (2 m)	180	21.8	26.1	1.2	1.8
	190	15.1	17.8	1.2	1.6
	200	10.6	12.4	1.2	1.5
	210	7.5	8.7	1.2	1.4

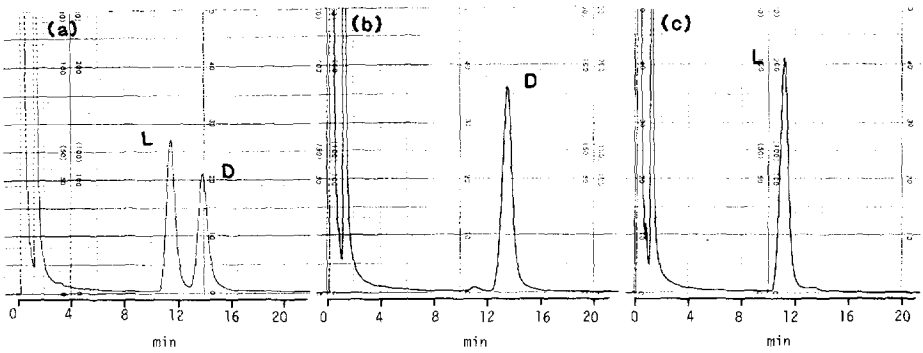


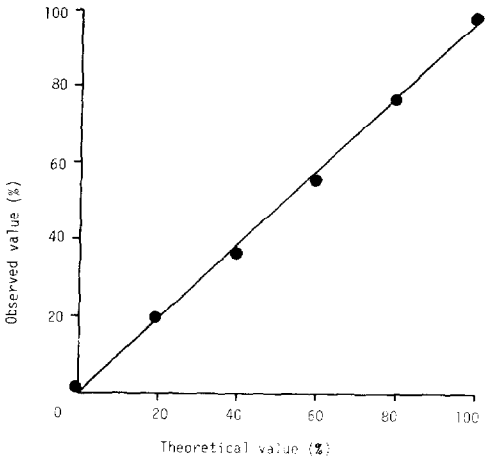
Fig. 2. Gas chromatograms of the MTPA derivatives of (a) DL-pantolactone, (b) D-pantolactone and (c) L-pantolactone.

centages of D-pantolactone, and were treated with MTPAC as described in Experimental. The MTPA derivatives were then subjected to GLC, and the ratio of D-pantolactone to the L-isomer was obtained by use of the peak-area method.

As seen in Fig. 3, the relationship between the theoretical value (%) and the observed value (%) of D-pantolactone showed a good linearity with a slope of 0.965. It is considered that the method devised would be acceptable for the determination of the optical purity of pantolactone enantiomers.

Applications

Prior to application of the method, it was confirmed that the racemization of D- and L-pantolactone did not occur in acidic or alkaline aqueous solutions. The method was applied to the determination of the optical purity of calcium D-pantothenate, D-panthenol and D-pantethine (Fig. 4). These compounds (5 mg) were hydro-



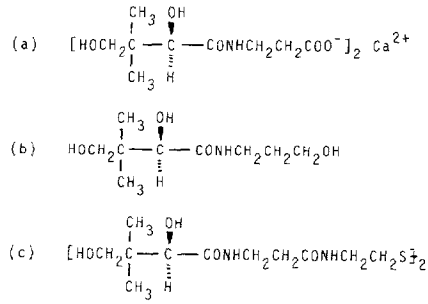


Fig. 3. Calibration curve for the theoretical value, x (%), of D-pantolactone *versus* the observed value, y (%). $y = 0.965x - 0.139$; $r = 0.9986$.

Fig. 4. Chemical structures of (a) calcium D-pantothenate, (b) D-panthenol and (c) D-pantethine.

lysed in 6 *M* hydrochloric acid at 80°C for 2 h to convert them into D-pantolactone. The latter was then extracted with dichloromethane, the organic layer was dried over anhydrous magnesium sulphate for 20 min and the solvent was removed under a stream of nitrogen. The residue was derivatized in the manner described, and then analysed by GLC.

It was found that the hydrolysis proceeded to completion and that racemization did not occur during the hydrolysis reaction. So, this method can be applied to the determination of the optical purity of calcium D-pantothenate, D-panthenol and D-pantethine.

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