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Note

High-performance liquid chromatographic determination of enantiomeric purity of 1-methyl-3-pyrrolidinol via derivatization with (*R,R*)-O,O-dibenzoyltartaric acid anhydride

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In spite of recent significant progress in chiral liquid chromatographic (LC) separation[1–3], oftentimes diastereomeric derivatization techniques are preferable.

The present work describes the enantiomeric purity determination of 1-methyl-3-pyrrolidinol (CAS Reg. No. 13220-33-2) using an extension of the method by Lindner *et al.*[4] of derivatization to (*R,R*)- or (*S,S*)-tartrate monoesters.

1-Methyl-3-pyrrolidinol is an intermediate in the synthesis of several drugs[5]. Having one asymmetric center it has one pair of enantiomeric forms. No reference was found in the literature concerning enantiomeric purity determination, chiral synthesis, or enantiomeric resolution of 1-methyl-3-pyrrolidinol, nor for the analogues 1-ethyl-, 1-butyl-, or 1-cyclohexyl-3-pyrrolidinol.

For the enantiomeric purity determination of 1-methyl-3-pyrrolidinol, derivatization with (*R,R*)- or (*S,S*)-dibenzoyltartaric acid anhydride is particularly advantageous. The dibenzoyl anhydride will generate UV absorbing monoesters, and consequently the detection of the derivatives is greatly facilitated. The second important advantage lies with the fact that only one pair of diastereoisomers is formed: (*R,R,R*) and (*R,R,S*), for example from (*R,R*)-anhydride. The two diastereomers have different non-chiral physical properties, and can be separated by conventional LC techniques, such as reversed-phase LC.

EXPERIMENTAL

Apparatus

The HPLC system used was a Hewlett-Packard Model 1087B liquid chromatograph equipped with Model 79875 variable-wavelength UV detector, two pumps, and automatic injector. Data and chromatograms were recorded and processed by the Model 79850 LC terminal.

Chemicals and reagents

(*R,R*)-Dihydro-3,4-dihydroxy-2,5-furandione dibenzoate ester, (O,O-dibenzoyltartaric acid anhydride, DBTAA) was prepared from (*R,R*)-tartaric acid (Fluka, Buchs, Switzerland) following a procedure described in the literature[6]. Pyrrolidinol

samples were: 1-methyl-3-pyrrolidinol, racemic (Lee Laboratories, Petersburg, VA, U.S.A.), 1-methyl-3-pyrrolidinol, enantiomers (Preparation Laboratory, A. H. Robins Co., Richmond, VA, U.S.A.). All pyrrolidinol samples were examined by capillary gas chromatography prior to use and found to be at least 99.5% pure (chemical purity). Tetrahydrofuran (Burdick & Jackson) was used freshly filtered through basic alumina. Trichloroacetic acid (J. T. Baker), triethylamine p.a. (J. T. Baker), high-performance liquid chromatography (HPLC)-grade methanol (E. M. Science) used as received. The water used was triple distilled.

Chromatographic procedure

The column used was Zorbax C_8 5-μm, 15 cm × 4.6 mm I.D. from DuPont. The mobile phase was a mixture of methanol–0.1% aqueous triethylamine solution (50:50), adjusted to pH 4.2 with glacial acetic acid. A flow-rate of 2 ml/min was used with detection at 254 nm.

Derivatization

Approximately 10 mg 1-methyl-3-pyrrolidinol was dissolved in 1 ml tetrahydrofuran. Amounts of 35 mg trichloroacetic acid and 80 mg DBTAA are added.

The mixture was heated to 50°C for 4 h in a sealed vial. The reaction mixture was diluted 1:10 with HPLC-grade methanol, and 3 μl of this solution was injected for HPLC analysis.

RESULTS AND DISCUSSION

The chromatograms of *R*, *S* and the racemic mixture of 1-methyl-3-pyrrolidinol DBTA monoesters are presented in Fig. 1. The separation factor (α) of the two enantiomers is 1.6.

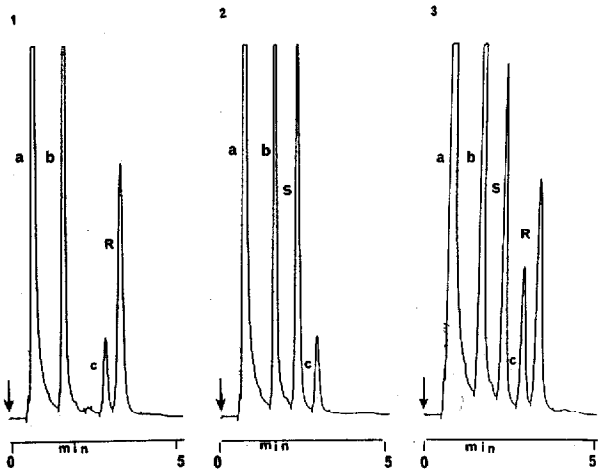

Fig. 1. Chromatograms of 1, *R* isomer; 2, *S* isomer; 3, racemic mixture. Peaks: a = dibenzoyltartaric acid; b = unknown; S = *S* isomer derivative; c = DBTAA; R = *R* isomer derivative. Absorbance scale, 0.128 a.u.f.s.d.

Linearity of detector response was checked by diluting a standard preparation of the racemic in steps down to 12:1. Linearity was maintained. The response factors of the diastereomers are slightly different at 254 nm.

A correction factor (k) was determined from six runs using racemic 1-methyl-3-pyrrolidinol; it is the mean ratio of the (R,R,S) to (R,R,R) diastereomer areas:

$$k = \frac{A^{rac}_{(R,R,S)}}{A^{rac}_{(R,R,R)}} = 1.14 \pm 0.03 \text{ (standard deviation, } N = 6)$$

The enantiomeric purity is calculated from the ratio of the integrated areas (A) of the chromatographic peaks multiplied by this correction factor as follows:

$$\%(R) = \frac{A_{(R,R,R)} \cdot k}{A_{(R,R,R)} \cdot k + A_{(R,R,S)}} \cdot 100$$

where $A_{(R,R,R)}$, $A_{(R,R,S)}$ are the integrated areas of the diastereomer peaks.

The results of enantiomeric purity determination of several samples are listed in Table I.

TABLE I

ENANTIOMERIC PURITY OF 1-METHYL-3-PYRROLIDINOL SAMPLES AS % (w/w) *R*

Sample	*Mean*	*S.D.**	*N***
1	92.4	0.3	5
2	95.7	0.1	4
3	92.3	0.3	8
4	95.6	0.0	2
4 + 1% (w/w) *S*	94.5	0.3	2
4 + 2% (w/w) *S*	93.4	0.1	2
4 + 5% (w/w) *S*	90.0	0.1	2

* Standard deviation, or 1/2 (range) for duplicates.

** Number of replications.

To a sample (No. 4, Table I) of high % (w/w) *R*, 1%, 2%, and 5% pure *S* isomer was added; the expected decrease of enantiomeric purity was observed.

From these results it appears that the enantiomeric purity of 1-methyl-3-pyrrolidinol can be determined via diastereomeric derivatization with (*R,R*)-O,O-dibenzoyltartaric acid anhydride followed by reversed-phase HPLC separation with UV detection.

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