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Note

Enantiomeric purity determination of 3-aminoquinuclidine by diastereomeric derivatization and high-performance liquid chromatographic separation

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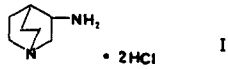
3-Aminoquinuclidine dihydrochloride (I) (1-azabicyclo[2,2,2]octan-3-amin) (CAS Reg. No. 6530-09-2) is an important intermediate in the synthesis of several pharmaceuticals[1]. For this reason the determination of its enantiomeric purity is of practical significance. No reference could be found in the literature on the optical resolution or optical purity determination of compound I.



I

Strong bases, such as 3-aminoquinuclidine are difficult to separate into enantiomers by direct chiral separation methods. Diastereomeric derivatives of compound I, on the other hand, were found to give relatively straightforward separations. In this work four different diastereomeric derivatization schemes have been used for high-performance liquid chromatographic (HPLC) separation of enantiomers of compound I.

EXPERIMENTAL

Materials and methods

TLC plates coated with silica gel 60 and RP-C_{18} (0.25 mm, F_{254}) were purchased from EM Science (Gibbstown, NJ, U.S.A.) and Whatman (Clifton, NJ, U.S.A.). Detection was by short-wavelength UV light. A Varian 5500 HPLC system equipped with a variable-wavelength UV detector, Model 200, a 6-port valve injector with a 10-μl loop, and a SP 4270 Spectra-Physics (Piscataway, NJ, U.S.A.) integrator was used. The columns used were 15 cm × 4.6 mm I.D. stainless steel packed with Zorbax C_8 and Zorbax Sil of 5 μm particle size from DuPont (Wilmington, DE, U.S.A.). ^{1}H NMR spectra were taken on a Varian 60 MHz EM 360 L spectrometer. Mass spectra were obtained on a Varian MAT Model 44 spectrometer. Infrared spectra were obtained in KBr pellets on a Nicolet Model 5 DX instrument. Optical rotation was measured using a Perkin-Elmer 241 polarimeter.

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The solvents used were spectroscopic grade. The dimethylformamide (DMF) and dioxane were further dried and purified by being passed through Sep-Pak Alumina B cartridge from Waters Assoc. (Milford, MA, U.S.A.). The non-chiral reagents were obtained from Aldrich (Milwaukee, WI, U.S.A.).

The following chiral reagents were used: *S*(−)-1-phenylethyl isocyanate (PEIC) from Fluka (Buchs, Switzerland); *R*(−)-1-naphthylethyl isocyanate (NEIC) (Aldrich); *R,R*-(+)O,O-dibenzoyltartaric acid (DBTA) (Aldrich); *S,S*-(−)O,O-dibenzoyltartaric acid (DBTA) (Aldrich); 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) (Polyscience, Warrington, PA, U.S.A.).

Anhydrides of *R,R*(+)- and *S,S*(−)-O,O-dibenzoyltartaric acid, although commercially available, were prepared by a modified literature procedure[2], as follows. The corresponding acid was dissolved in excess acetic anhydride and heated at reflux for 30 min. After cooling, the white solid was filtered and triturated with light petroleum (b.p. 35–60°C), then dried. Further purification by recrystallization from xylenes is optional. The *R,R*-(+)-O,O-dibenzoyltartaric acid anhydride (*R,R*-DBTAAN) had a melting point of 196°C and $[\alpha]_D^{20} = +152°$; the *S,S*-DBTAAN 191°C and −157°.

Derivatization procedures

The structures of the chiral reagents and the corresponding diastereomeric derivatives of compound I are listed in Table I, together with pertinent literature references.

All of the derivatizations required the free-base form of compound I, which was prepared by treating a methanolic solution of compound I with 2 moles of sodium methoxide. After a few minutes the methanol was removed under a stream of nitrogen and the residue dissolved in dry DMF (for the disubstituted urea and thiourea derivatives) or in dry dioxane (for the derivatization with DBTAAN). Filtration of the inorganic material was not necessary.

Disubstituted urea and thiourea derivatives

Samples of 1–2 mg in the free-base form in 1-ml dry DMF were treated with derivatization reagent added via syringe or as a solid (GITC) in one portion in a 10% molar excess. After Vortex stirring the reaction mixture was allowed to stand at room temperature for 30 min. Samples of the reaction mixtures were then diluted with the mobile phase and analyzed by HPLC.

Dibenzoyl tartaric acid monoamides

Samples of 1–2 mg in the free-base form in 1-ml dry dioxane were treated with a 10% molar excess of DBTAAN reagent as a dioxane solution added in one portion. After Vortex stirring the mixture was allowed to stand at room temperature for 30 min. A suspension was formed which was dissolved in the mobile phase. Samples of the reaction were analyzed by thin-layer chromatography (TLC) as shown in Table II. No detectable amounts of compound I were observed.

All derivatization products had ^{1}H NMR and chemical ionization mass spectra consistent with the structures inferred from synthesis. Statistical analysis of experimental data was performed with RS1 (BBN) software run on a DEC-VAX minicomputer.

TABLE I

CHIRAL DERIVATIZATION REAGENTS AND DERIVATIVES

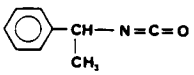
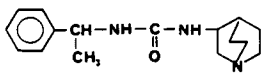
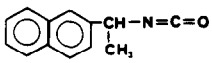
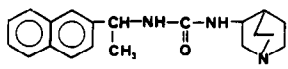
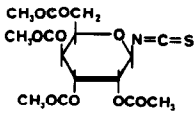
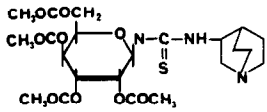
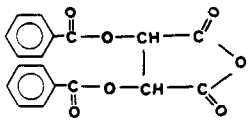
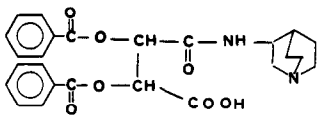
Chiral reagent	*Derivative*	*Reference*
 PEIC	 PUQ	3, 4
 NEIC	 NUQ	6
 GITC	 GTUQ	5, 7
 DBTAAN	 DBTAQ	6

TABLE II

TLC SEPARATION OF THE DIASTEREOMERIC DERIVATIVES OF COMPOUND I

Chiral reagents	*Diastereomers*	*TLC plate*	*Mobile phase*	*Detection method*	*R_F × 100 of diastereomers*	
					R	*S*
PEIC	PUQ	Silica gel	Ethylacetate–isopropanol–10% ammonium hydroxide (45:35:20)	UV (254 nm)	45	42
NEIC	NUQ	Silica gel	Ethylacetate–isopropanol–10% ammonium hydroxide (45:35:20)	UV (254 nm)	46	45
GITC	GTUQ	Silica gel	Ethylacetate–isopropanol 10% ammonium hydroxide (45:35:20)	UV (254 nm)	51	51
DBTAAN	DBTAQ	RP-C_{18}	Methanol–water (65:35)–0.1% ammonium acetate	UV (254 nm)	50	66

RESULTS AND DISCUSSION

TLC separation of the diastereomeric derivatives of compound I are listed in Table II. Normal-phase separations on silica gel were performed except in the case of DBTAQ where the separation was done on reversed-phase (C_{18}) plates. The HPLC separations are listed in Table III.

All diastereomeric derivatives were strong UV absorbers; the detection was made by monitoring absorbance at 254 nm. As indicated by the separation factors (α) listed in Table III, the separation of the diastereomeric derivatives of compound I is satisfactory in all instances. Any of the derivatization schemes can be used for the optical purity determination of compound I. However, the best separation is achieved using DBTAQ.

The DBTAAN reagents were prepared from both isomers of the O,O-dibenzoyltartaric acids, (*R*,*R*) and (*S*,*S*). This allows one to cause either isomer to elute first.

Mixtures of (*R*)-I and (*S*)-I in the range of enantiomeric ratios from 0.001 to 0.01 were analyzed by DBTAAN derivatization and separation under the conditions listed in Table III. Typical chromatograms of (*R*,*R*)-DBTAQ diastereomers are shown in Fig. 1. The minor peak is clearly observed at 0.1% minor enantiomer. Plots of enantiomeric ratios (ER) *vs.* enantiomeric peak area ratios were linear. The data were fit to eqn. 1 by linear regression and the statistics of this fit are shown in Table IV.

$$ER = k[A_1/A_2] \quad (1)$$

where $ER = C_1/C_2$, concentration ratio of the two derivatives; k is the slope; A_1 and A_2 are the peak areas of the two enantiomers derivatives.

The k values are respectively 0.801 and 1.234 and are reciprocals as they should be. The same k values were also obtained by analysis of racemic I.

TABLE III

HPLC SEPARATION OF DIASTEREOMERIC DERIVATIVES OF COMPOUND I

Chiral reagent	*Diastereomers*	*Mobile phase and flow-rate*	*Column*	*k'(R)*	*k'(S)*	*α*[a]
PEIC	PUQ	Ethyl acetate–methanol–conc. ammonium hydroxide (85:10:5), 2 ml/min	Silica gel	2.04	1.77	1.15
NEIC	NUQ	Ethyl acetate–methanol–conc. ammonium hydroxide (85:10:5), 2 ml/min	Silica gel	1.98	1.60	1.23
GITC	GTUQ	Methanol–acetonitrile–water (25:15:60)–(0.1% triethanolamine, 0.15% acetic acid), 2 ml/min	RP-C_8	10.65	9.24	1.15
DBTAAN	DBTAQ	Methanol–acetonitrile–water (25:15:60)–(0.1% triethanolamine, 0.15% acetic acid), 2 ml/min	RP-C_8	2.50	1.60	1.56

[a] $\alpha = k'(R)/k'(S)$, where k' is the capacity factor.

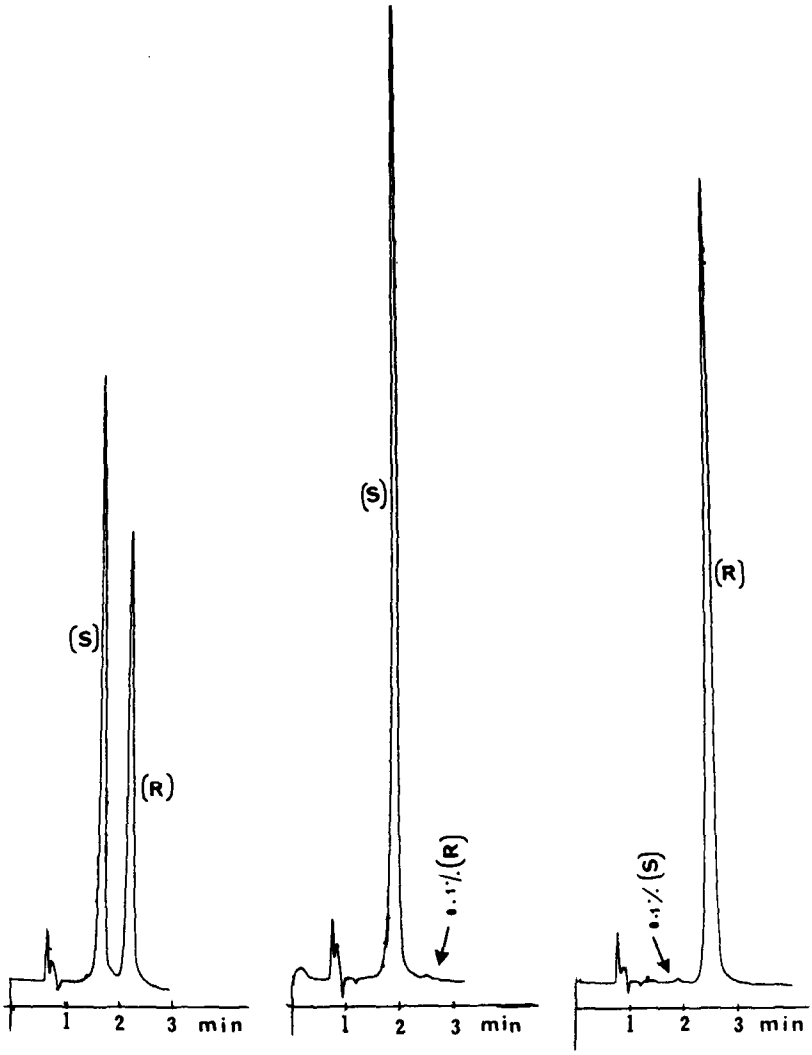


Fig. 1. HPLC separation of (*R*)-I and (*S*)-I mixture derivatized with (*R,R*)-O,O-dibenzoyltartaric acid anhydride. Column: 150 × 4.6 mm, I.D., Zorbax C_8; mobile phase: 0.15% acetic acid in water (adjusted with triethylamine to pH 4.2)–methanol–acetonitrile (60:25:15); flow-rate: 2 ml/min; detection: UV 254 nm.

TABLE IV

LINEAR REGRESSION STATISTICS OF (*R,R*)-DBTAQ ISOMERS

	Minor enantiomer	
	S	*R*
Slope, *k* (eqn. 1)	0.801 ± 0.044	1.234 ± 0.036
Fischer ratio, *F*	325	1200
Significance level, *p*	<0.001	<0.001
Square of the correlation coefficient, r^2	0.967	0.991
Number of points, *n*	12	12

In conclusion four different diastereomeric derivatization schemes were applied to mixtures of 3-aminoquinuclidine enantiomers followed by HPLC separation of the diasteromers. The best separation was achieved in the case of the O,O-dibenzoyltartaric acid derivatives which allowed detection of the minor enantiomer down to 0.001 enantiomeric ratio.

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