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Short Communication

High-performance liquid chromatographic detection of enantiomeric amino alcohols after derivatization with *o*-phthaldialdehyde and various thiosugars

ALEXANDR JEGOROV*
Institute of Entomology, Czechoslovak Academy of Sciences, Branišovská 31, 370 05 České Budějovice (Czechoslovakia)
TOMÁŠ TRNKA
Department of Organic Chemistry, Charles University, Albertov 6, 128 40 Prague (Czechoslovakia)
and
JOSEF STUCHLÍK
Galena Co., 147 70 Opava, Komárov (Czechoslovakia)
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ABSTRACT

A series of thiosugars are described as the new pre-column derivatization agents for chiral resolution of 2-amino-1-alcohols. The isoindole derivatives were formed by reaction of 2-amino-1-alcohols with *o*-phthaldialdehyde and thiosugars in aqueous solution and at a mildly basic pH. The reaction was complete within 1 min. The diastereomers formed can efficiently be resolved on conventional reversed-phase columns and measured with a fluorescence detector which provides detection limits of less than 10 pmol per 10-μl injection.

INTRODUCTION

Amino alcohols are present as a terminal group in some fungal metabolites, *e.g.* in peptide antibiotics (peptaibols) [1,2], as well as in various natural compounds produced by other organisms [3]. Amino alcohols are also used as chiral synthones for the preparation of a number of biologically active compounds, *e.g.* ergot alkaloids such as ergometrin or methylergometrin [4,5], which are nowadays produced synthetically. In both cases, an appropriate analytical method is necessary to identify or to control the enantiomeric purity of either natural or synthetic compounds, as it is well known that compounds constituted from different enantiomers usually possess different biological activity.

Whereas a number of liquid chromatographic methods have been described for

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the resolution of amino acid enantiomers, apparently little attention has been paid to the resolution of amino alcohols. Resolution of some amino alcohols was recently achieved after derivatization with various chiral isocyanates or isothiocyanates [6,7]. Another method, which was primarily developed for the analysis of amino acid enantiomers, is based on the pre-column derivatization of amino groups with *o*-phthaldialdehyde (OPA) and chiral thiols. Compared with other reagents, the analysis of amino compounds after derivatization with the OPA–thiol reagents is quick, convenient and relatively independent of the presence of impurities in the sample. Diastereomeric isoindole derivatives thus formed are easily separable on a reversed-phase high-performance liquid chromatographic (HPLC) column and can be easily detected using fluorimetry. Boc-L-cysteine, N-acetyl-L-cysteine and N-acetyl-penicillamine were used also for the derivatization of amino alcohols, and good resolution was obtained for all amino alcohols tested with at least one chiral reagent [8]. Since no reagent has general applicability, we describe here an alternative method for the resolution of amino alcohol enantiomers, which is based on their derivatization with *o*-phthaldialdehyde and various thiosugars.

EXPERIMENTAL

Reagents and chemicals

The sodium salts of 1-thio-β-D-glucose [9], 1-thio-β-D-galactose [10] and 1-thio-β-D-mannose [11] were prepared as described previously; OPA (Calbiochem, Los Angeles, CA, USA), amino alcohol enantiomers (Fluka, Buchs, Switzerland and Sigma, St. Louis, MO, USA), methanol, boric acid, potassium hydroxide and sodium acetate (Lachema, Brno, Czechoslovakia) were used.

Chromatographic systems

A Varian Vista 5500 liquid chromatographic system equipped with a Fluorichrom filter fluorescence detector was used. The excitation wavelength has a maximum at 360 nm, while the emission wavelength has a bandpass above 420 nm. The analytical column used was a Separon SGX C_{18}, 7 μm (250 × 4 mm I.D.) from Tessek (Prague, Czechoslovakia). Solvent A was 0.1 *M* sodium acetate (pH 7.3, adjusted with dilute acetic acid) and solvent B was a 1:9 mixture of sodium acetate (pH 7.3) and methanol, respectively. A linear gradient elution of 30–70% B over 40 min was used. A constant flow-rate of 1 ml/min was maintained during the analysis. A back-pressure terminator (Varian, Sunnyvale, CA, USA), set at 0.6 MPa, was used to prevent formation of bubbles. Stability studies were performed with a Bio-Rad AS-100 HRLC automatic sampling system (Richmond, CA, USA) and a Hypersil ODS, 3 μm (60 × 4.6 mm I.D.) column from Hewlett Packard (Amstelveen, Netherlands) under isocratic elution with 0.1 *M* sodium acetate (pH 7.2)–methanol (6:4, v/v); the flow-rate was 1.0 ml/min, and evaluation was based on comparison of peak areas of baseline-separated peaks.

Derivatization procedure

Stock solutions were prepared weekly with 50 mg of sodium salts of thiosugars in 1 ml of water, 50 mg of OPA in 1.25 ml of methanol and amino alcohols (4 m*M* in water). Borate buffer was prepared by dissolving 0.50 g of boric acid in 19 ml of water

and adjusting the pH to 9.30 (8.20 or 10.00) with potassium hydroxide solution (45 g of potassium hydroxide in 100 ml of water). A 10-μl sample of the amino alcohol solution, 100 μl of the borate buffer 50 μl of the thiosugar solution and 50 μl of the OPA solution (thiosugar/OPA ratio = 2.2) were introduced consecutively into a small glass vessel, and the mixture was stirred. After 60 s, a 10-μl aliquot was analysed. For the studies of fluorescence response, both racemic mixtures and individual enantiomers were used.

RESULTS AND DISCUSSION

We have recently shown that thiosugars can be employed for the chiral resolution of amino acids [12,13]. By analogy with the reactions of other thiols [14–16], 1-isoindolyl-(1-thio-β-D-glycosides) are assumed to be formed in the course of a reaction of an amino acid with OPA and a thiosugar. This type of derivatization can possibly be applied to all kinds of primary amines and to a wide range of mercaptans. Accordingly, all amino alcohols reacted with OPA and thiosugars in alkaline conditions yield highly fluorescent isoindole derivatives. The reactions occurred rapidly and quantitatively at ambient temperature, reaching their maximum fluorescence within 1 min (Fig. 1). In the course of the reaction, only one highly fluorescent derivative was formed with each amino alcohol enentiomer. Similarly, as in the case of OPA–2-mercaptol derivatives [17–20], thiosugar-substituted isoindoles are unstable (Fig. 1). Their stability was found to decrease with decreasing pH. The stability of OPA–thiosugar mixtures themselves is also limited, hence the use of separate stock solutions is recommended. Solutions of sodium salts of thiosugars are stable for months with the exception of the sodium salt of 1-thio-β-D-mannose, a fresh solution of which should be prepared every day.

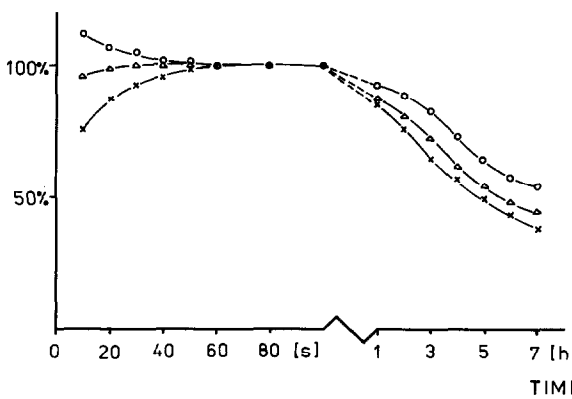

Fig. 1. Fluorescence response of the OPA–1-thio-β-D-galactose derivatization as a function of reaction time and pH [(×) 8.2, (△) 9.3, (○) 10.0] and stability of the selected derivative in the borate buffer at 20°C [mixture: 10 μl of 3 m*M* (*S*)-(+)-2-amino-1-butanol, 200 μl of borate buffer, 30 μl of thiosugar solution, 30 μl of OPA solution]; relative percentages refer to the area of the chromatographic peak obtained by the analysis of the reaction mixture injected after 60 s (100%). Conditions: Hypersil ODS, 3 μm (60 × 4.6 mm I.D.) column, isocratic elution with 0.1 *M* sodium acetate (pH 7.2)–methanol (6:4, v/v), flow-rate 1.0 ml/min.

TABLE I

RESOLUTION AND RETENTION TIMES OF VARIOUS OPA–THIOSUGAR DERIVATIVES

Column: Separon SGX C_{18}, 7 μm (250 × 4 mm, I.D.), for elution conditions see the Experimental section; t_0 = 1.1 min. Resolution $R = (t_{R2} - t_{R1})/(w_1 - w_2)$ where w_1 and w_2 are the peak width at half height of the first and the second eluted peaks, respectively. t_R values corresponding to derivatives with fluorescence response higher from the enantiomeric couple are underlined.

Sample	Derivatization reagent OPA–thiosugar											
	1-Thio-β-D-glucose				1-Thio-β-D-galactose				1-Thio-β-D-mannose			
	t_{r1}	t_{r2}	R	Elution order	t_{r1}	t_{r2}	R	Elution order	t_{r1}	t_{r2}	R	Elution order
2-Amino-1-propanol	9.8		0	–	8.8		0	–	12.1	12.9	0.53	*R, S*
2-Amino-1-butanol	16.5	18.4	1.50	*S, R*	15.0	16.2	0.87	*S, R*	17.5	19.8	1.36	*R, S*
2-Amino-1-pentanol	23.4	25.5	1.60	*S, R*	21.9	23.3	1.02	*S, R*	24.1	26.4	1.34	*R, S*
2-Amino-1-hexanol	29.3	31.5	1.56	*S, R*	28.1	29.6	1.02	*S, R*	30.1	32.2	1.24	*R, S*
Valinol	21.2	23.0	1.47	*S, R*	20.3	21.8	1.19	*S, R*	21.5	23.6	1.52	*R, S*
Leucinol	29.0	31.4	1.61	*S, R*	27.8	29.6	1.27	*S, R*	29.1	31.1	1.20	*R, S*
α-Phenylglycinol	23.4	25.9	2.18	*S, R*	23.5	25.0	1.00	*S, R*	23.7		0	–
Phenylalaninol	23.8	27.4	3.20	*S, R*	23.6	27.1	2.10	*S, R*	22.6	24.3	1.29	*S, R*

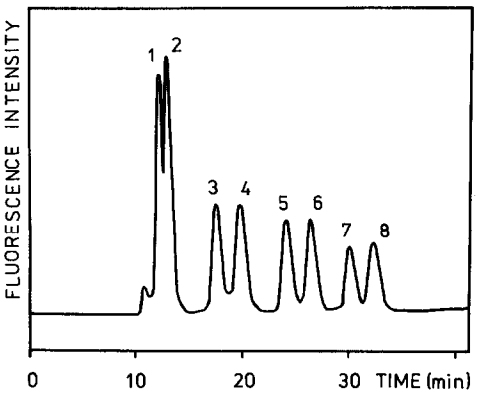

Fig. 2. Separation of amino alcohol enantiomers after pre-column derivatization with OPA–1-thio-β-D-mannose. Column, Separon SGX C_{18}, 7 μm (250 × 4 mm I.D.); mobile phase A, 0.1 *M* sodium acetate (pH 7.3); mobile phase B, 0.1 *M* sodium acetate–methanol (1:9, v/v); gradient 30–70% B in 40 min; flow-rate, 1.0 ml/min. Peaks: 1, 2 = (*R*)-, (*S*)-2-amino-1-propanol; 3, 4 = (*R*)-, (*S*)-2-amino-1-butanol; 5, 6 = (*R*)-, (*S*)-2-amino-1-pentanol; 7,8 = (*R*)-, (*S*)-2-amino-1-hexanol.

Fig. 2 shows an example of the separation of four amino alcohols derivatized with OPA–1-thio-β-D-mannose. Resolution and elution orders of other derivatives are given in Table I. As for the role of individual thiosugars, elution orders obtained with 1-thio-β-D-mannose were mostly reversed compared with that of 1-thio-β-D-glucose or 1-thio-β-D-galactose. This indicates that the configuration of the OH group at the C-2 atom plays an important role in the interaction between the sugar moiety and OH group of an amino alcohol. As is evident from Table I, as well as from, for example, space models of the isoindole, the OH group at the C-4 atom (glucose *vs.* galactose) could not participate in this case. However, this remote OH group might participate in chiral recognition if suitable polar groups are present on the side chain of an amino compound, as was documented, for example, in the differential resolution of Lys, Asp and Glu [21].

The relative sample standard deviations of retention times were 1.5% [1-thio-β-D-glucose-(*S*)-(+)-2-amino-1-pentanol, $n = 6$] for the gradient method (Fig. 2) or 1.4% [1-thio-β-D-galactose-(*R*)-(−)-2-amino-1-butanol, $n = 24$, within 2 days] for the isocratic method (Fig. 3). The average relative deviation of the peak area for individual derivatives was less than 4% for between-day assays and less than 3% for within-days assays, which shows that the present system is reproducible. The detection limit for (*S*)-(+)-2-amino-1-butanol, based on a signal-to-noise ratio of 2, was less than 10 pmol. The analysis of amino alcohols after conversion to isoindoles is relatively easy when large samples are available (> 100 pmol), which is the case for most analysis of enantiomeric purity of synthetic chemicals. Problems associated with the analysis increase with diminishing sample size and are largely due to impurities in reagent and the inherent lack of chemical stability of the isoindoles examined in the reaction. Much of this background interference was shown to derive from OPA contaminants and old samples of thiosugar solutions.

As in the case of other chiral thiols, individual enantiomers formed derivatives having slightly different specific fluorescence intensity (Table I). In this particular

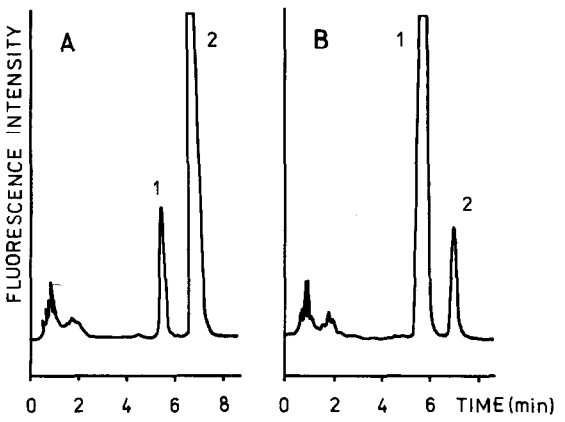

Fig. 3. Separation of 2-amino-1-butanol derivatized with OPA–1-thio-β-D-glucose. Conditions: Hypersil ODS, 3 μm (60 × 4.6 mm I.D.) column, isocratic elution with 0.1 *M* sodium acetate (pH 7.2)–methanol (6:4, v/v), flow-rate 1.0 ml/min. (A) (*S*)-(+)-2-Amino-1-butanol (3 m*M*) with 1% (*R*)-(−)-2-amino-1-butanol impurity; (B) (*R*)-(−)-2-amino-1-butanol (3 m*M*) with 1% (*S*)-(+)-2-amino-1-butanol impurity Peaks: 1, 2 = (*S*)-, (*R*)-2-amino-1-butanol; column efficiency = 81 200 plates/m [plate number = 5.54 $(t_{r2}/w)^2$] for peak 2.

case, (*S*)-enantiomers formed derivatives whose chromatographic peaks had about 5–20% greater area regardless of whether they were eluted first or second in the enantiomeric pair. Since derivatives of quite different amino compounds possess almost identical absorbance and fluorescence spectra and usually also exhibit comparative fluorescence responses [14,15], differences in the fluorescence intensity of various enantiomers seem likely to originate from the local arrangement of substituents around the isoindole moiety.

Most of the amino alcohols showed sufficient resolution with each of the three derivatization reagents. Baseline separation usually allowed simultaneous determination of trace impurities of one enantiomer in the other with one thiosugar (Fig. 3). For a particular case, separation can alternatively be achieved using isocratic elution. Resolution is fairly dependent on the quality of the column used; in our experience, poorly end-capped or old columns usually suffer particularly from loss of resolution. As the buffer–methanol mixture causes relatively high back-pressure on reversed-phase columns, the use of short-length columns seems to be reasonable.

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