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Note

Separation of (*R*)- and (*S*)-proxyphylline as diastereoisomeric camphanates by reversed-phase liquid chromatography

MERETE RUUD-CHRISTENSEN* and BJARNE SALVESEN
Department of Pharmacy, University of Oslo, Blindern, 0316 Oslo 3 (Norway)
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The bronchodilator proxyphylline [(±)-3,7-dihydro-7-(2-hydroxypropyl)-1,3-dimethyl-1*H*-purine-2,6-dione] is currently used in the racemic form[1]. The absolute configuration of proxyphylline has recently been reported, and optical resolution of the drug as diastereoisomeric camphanates (Fig. 1) has been carried out by thin-layer chromatography (TLC) and recrystallization from methanol[2]. No high-performance liquid chromatographic (HPLC) method for the resolution of (*R,S*)-proxyphylline has been described.

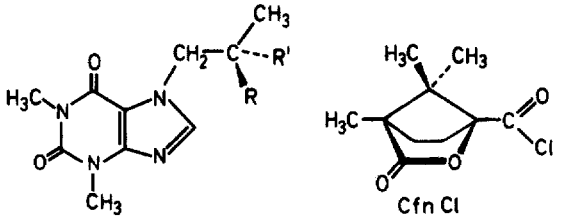

Fig. 1. The chemical structures and absolute configuration of (*R*)-proxyphylline (R = OH, R′ = H), (*S*)-proxyphylline (R = H, R′ = OH), (*R*)-proxyphylline camphanate (R = OCfn, R′ = H), (*S*)-proxyphylline camphanate (R = H, R′ = OCfn) and (−)-camphanoyl chloride (Cfn Cl).

Recently we reported methods for syntheses of (*R*)- and (*S*)-proxyphylline[2,3]. The optical purity of the products was determined by nuclear magnetic resonance (NMR) spectroscopy of the corresponding camphanoyl derivatives. A more convenient method for quantification of the camphanates was desirable. In the HPLC method described here, traces of the optically pure proxyphylline enantiomers are easily detected and quantified as corresponding camphanates.

EXPERIMENTAL

Chemicals and reagents

All solvents were of p.a. quality. (±)-Proxyphylline was purchased through Norsk Medisinaldepot (Oslo, Norway). (*S*)-(+)- and (*R*)-(−)-proxyphylline were obtained either by synthesis from theophylline and optically active propylenoxide or by optical resolution of racemic proxyphylline [2–4]. (−)-Camphanoyl chloride was obtained from Fluka (Buchs, Switzerland).

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Preparation of derivatives

The derivatives were prepared by reaction between proxyphylline and (−)-camphanoyl chloride with anhydrous pyridine as a catalyst[2,5,6].

Chromatography

The HPLC equipment consisted of a Beckman 112 solvent delivery module pump (Berkeley, CA, U.S.A.), a Rheodyne No. 7125 sample valve (Berkeley, CA, U.S.A.) and a LDC UV III Monitor 1203 detector (Riveria Beach, FL, U.S.A.). An Autolab minigrator from Spectra-Physics (Santa Clara, CA, U.S.A.) was used for calculation of peak areas.

Proxyphylline and (*R*)- and (*S*)-proxyphylline camphanates were separated on a 250 × 4.2 mm I.D. RP-18 Spheri-5 column (5 μm, C_{18} reversed phase; Brownlee Labs., Santa Clara, CA, U.S.A.) with isocratic elution at a flow-rate of 1.0 ml/min. A 34-mm guard column LC-18 Pellicular packing (the packing material were obtained from Supelco, Bellefonte, PA, U.S.A.) was used between the injector and the main column. The eluent was sulphuric acid (6.6 m*M*)–aqueous sodium sulphate (50 m*M*)–methanol–acetonitrile–isopropanol (41:17:20:19:3.4). The analyses were performed at room temperature. The injected volume was 20 μl, and the detection took place at 254 nm.

Standards

For calculation of response factors, standard solutions in the mobile phase corresponding to 20-μl injections of between 50 ng and 5 μg of proxyphylline and (*R*)- and (*S*)-proxyphylline camphanates, were made. The area response factors for the two proxyphylline camphanates were calculated from four solutions with mass ratios between 1.0 and 0.01 for the two compounds. The response factors for the two diastereoisomeric camphanates were identical (2% relative standard deviation). The area response for proxyphylline was calculated relative to (*R*)-proxyphylline camphanate using six solutions with mass ratios of proxyphylline to the ester in the range 0.02–3.3, giving a ratio of 1.9 between area response factors of proxyphylline and the camphanate (3% relative standard devation).

For all quantitative analyses the calculations were based on ratios of the compounds of two or three. No internal standard was added.

RESULTS AND DISCUSSION

This HPLC method gave a complete separation of proxyphylline, and (*R*)- and (*S*)-proxyphylline camphanates (Fig. 2). The remaining small amounts of camphanic acid or pyridine, with retention times between those of proxyphylline and (*R*)-proxyphylline camphanate, did not interfere with the results.

This is thus an appropriate method for detection and quantitative analysis of optical impurities of (*R*)-proxyphylline camphanate in (*S*)-proxyphylline camphanate or *vice versa*. For quantitative determination of the optical purity, response factors based on peak areas were more accurate than those based on peak heights because the latter showed a tendency to vary when the concentration ratio between the esters exceeded 10:1.

We used the method to investigate the optical purity of several synthetic (*R*)-

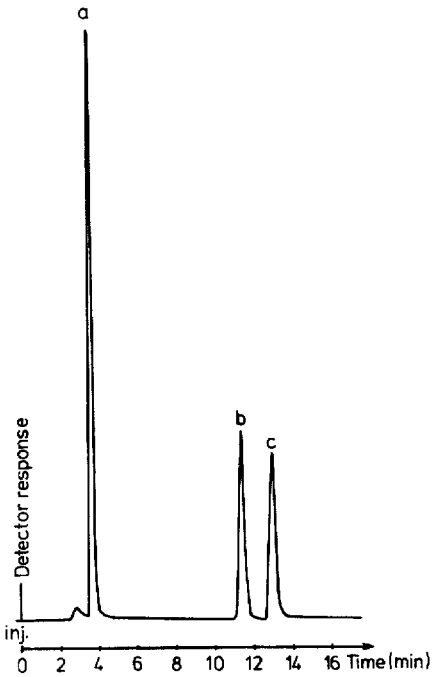

Fig. 2. Chromatogram after injection of 200 ng of (*R,S,*)-proxyphylline (a; 3.8 min), (*R*)-proxyphylline camphanate (b; 11.6 min) and (*S*)-proxyphylline camphanate (c; 13.1 min). Area responses a:b:c is 1.9:1.0:1.0.

and (*S*)-proxyphylline as corresponding camphanates, by direct injection of a solution of the product in the mobile phase (20 μl) and determination of the optical purity by means of the respective response factors. The results obtained (98–99% optical purity) were in good agreement with previous results obtained by NMR analysis[2,3]. A drawback of the NMR method is the requirement of a TLC-purified product. This reversed-phase HPLC method requires only a single injection of the product in the mobile phase to give the optical purity as well as the amount of underivatized proxyphylline still present.

The method can also be adopted to semi-micro-scale isolation of absolutely pure enantiomers by chromatography of synthetic mixtures of the diastereoisomeric camphanates, separate collection of the optical pure camphanates, and regeneration of the optical pure proxyphylline enantiomers after hydrolysis[2].

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