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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF 8-AZAGONANE-12-ONE DERIVATIVES AND THEIR OXIMES

II. SEPARATION OF OPTICAL ISOMERS

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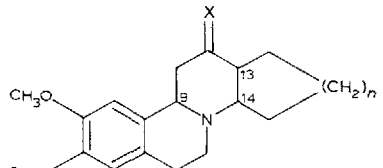
SUMMARY

The high-performance liquid chromatographic separation of optical isomers of some biologically active 8-azagonane-12-one derivatives and their oximes by a chiral complexing agent, 1(*S*)-(+)-10-camphorsulphonic acid, is presented. The enantiomeric purities obtained are compared to those measured by other methods.

INTRODUCTION

We previously described the liquid chromatographic separations of synthetic diastereomeric mixtures of racemic 8-azagonane-12-one derivatives (ketones and oximes). In this paper, a study of the separation of the optical isomers of some of these compounds is presented.

8-Azagonane-12-one derivatives, also named 8-aza-12-ketosteroids, were first obtained by Szántay *et al.*[2] as racemic mixtures of diastereomers. These compounds contain three asymmetric carbon atoms (C_9, C_{13} and C_{14}), and their oximes also exhibit *e*/*z* isomerism. Four and eight diastereomeric racemates are theoretically possible in each series (n = 1, 2, etc.) for the ketones and their oximes, respectively, but not all the configurations could be obtained due to the thermodynamic instability of certain ring system[3–6].



X = O (ketones)
X = N–OH (oximes)
n = 1, 2, ... etc.

The four different ring systems are classified in Table I, according to their B/C and C/D ring annellations.

Particular attention has recently been focused on racemic (*E*)-2,3-dimethoxy-8-aza-D-homo-9β,13α-gona-1,3,5(10)-trien-12-one oxime (EGYT-1623) due to its

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TABLE I

RING ANNELLATIONS IN 8-AZAGONANE-12-ONE DERIVATIVES

Ring system	*Ring annellation*	
	B/C	*C/D*
Normal	*trans*	*trans*
Allo	*trans*	*cis*
Epiallo	*cis*	*cis*
Pseudo	*cis*	*trans*

very interesting pharmacological properties[7]. This compound is under investigation at EGIS Pharmaceutical Works (Budapest, Hungary) as a new and potent neuroleptic. The optical isomers of EGYT-1623 and those of its parent ketone (EGYT-1623 ketone) have also been described[7] and were available to us.

This paper deals with the high-performance liquid chromatographic (HPLC) separation of racemic and partially resolved mixtures of these two compounds.

EXPERIMENTAL

Apparatus

The HPLC system consisted of Model 100 A pump (Altex, Berkeley, CA, U.S.A.), a 7125 loop injector (Rheodyne, Berkeley, CA, U.S.A.) with a 10-μl loop, a 4020 variable-wavelength UV detector (Pye-Unicam, Cambridge, U.K.), operating at 281 nm, and a 3390 A reporting integrator (Hewlett-Packard, Palo Alto, CA, U.S.A.). Optical rotations were measured with a Model 241 MC spectropolarimeter and differential scanning calorimetry (DSC) was performed on a DSC 2C calorimeter (Perkin-Elmer, Norwalk, CT, U.S.A.). The sample size was 2–2.5 mg and the temperature scan-rate, 1°C/min. No flushing gas was used.

Materials

Isooctane, chloroform and ethanol were HPLC grade (LiChrosolv, Merck, Darmstadt, F.R.G.), and diethylamine was reagent grade (Reanal, Budapest, Hungary). 1(*S*)-(+)-10-Camphorsulphonic acid was obtained from Fluka (Buchs, Switzerland).

Chromatographic conditions

The separations of optical isomers were carried out on an Ultrasphere SI 5-μm column (250 × 4.6 mm I.D.) (Beckman, Berkeley, CA, U.S.A.) with a chiral mobile phase. The isocratic elution technique was used at a flow-rate of 1.00 ml/min. The column was thermostatted at 30.0°C.

Procedure

Synthetic bulk materials were always dissolved in the solvent system used as mobile phase. The sample concentration range was 0.2–0.3 mg/ml, and 10-μl portions were injected. The mobile phase was filtered through a G-4 sintered-glass filter and sonicated before use.

RESULTS AND DISCUSSION

Different HPLC techniques can be used for the separation of optical isomers. Separation on chiral stationary phases[8–11] as well as by chiral complexing agents in the eluent[12–16] (forming diastereomeric complexes with the substrate) have been described. In our experiments, 1(*S*)-(+)-10-camphorsulphonic acid was used as chiral complexing agent[13] with non-chiral adsorption columns.

In the case of EGYT-1623 ketone samples (obtained by optical resolution of racemic EGYT-1623 ketone), the enantiomeric purities have been determined by DSC and optical rotatory dispersion (ORD) measurements.

In Fig. 1 a chromatogram of the racemic EGYT-1623 ketone is shown and in Fig. 2 a chromatogram of the racemic EGYT-1623 is presented. The identity of the peaks was established by adding pure enantiomers (obtained by chemical resolution[7]) to the corresponding racemates.

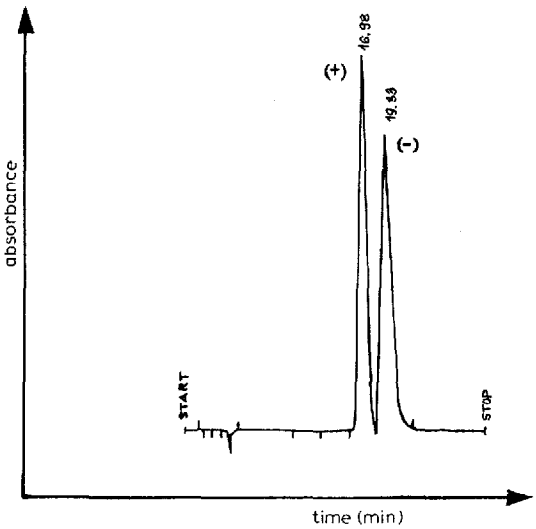

Fig. 1. Separation of optical isomers of EGYT-1623 ketone. Conditions: column, Beckman Ultrasphere SI, 5 μm, 250 × 4.6 mm I.D.; mobile phase, isooctane–chloroform–ethanol–diethylamine (30:65:5:0.01) containing 10^{-3} *M* (+)-10-camphorsulphonic acid; flow-rate, 1.00 ml/min; detection ast 281 nm.

The eluent composition, particularly the concentration of (+)-10-camphorsulphonic acid and that of diethylamine, is important for the separation of the enantiomers.

In Table II, capacity factors, separation factors and enantiomer peak area ratios are given for racemic EGYT-1623 and EGYT-1623 ketone.

By this method, less than 1% of the other optical isomer present as impurity can be detected. The enantiomeric purity (mole fraction of the more abundant enantiomer) of partially resolved samples can be calculated from the peak area ratio.

In case of a chemically resolved EGYT-1623 ketone sample, other methods such as DSC and ORD measurements have also been applied to monitor the enantiomeric purities (Table III).

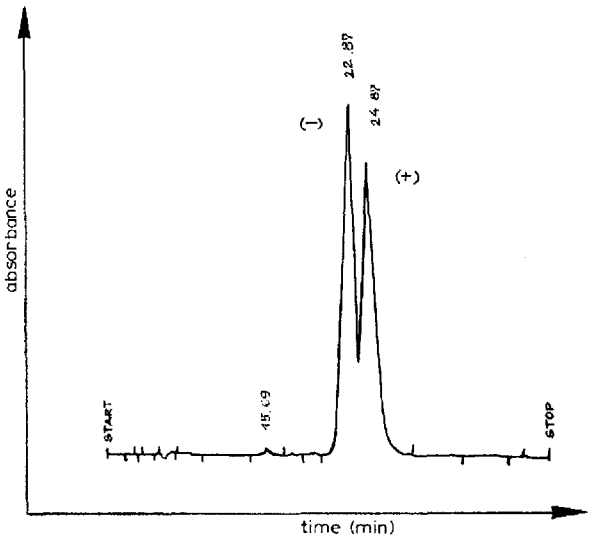

Fig. 2. Separation of (±) EGYT-1623 racemate. For chromatographic conditions see Fig. 1.

TABLE II

CAPACITY FACTORS, SEPARATION FACTORS AND PEAK AREA RATIOS OBTAINED FOR (±) EGYT-1623 AND (±) EGYT-1623 KETONE

Compound	*Capacity factor*		*Separation factor (α)*	*Peak area ratio*
	k_1	k_2		
(±) EGYT-1623 ketone	5.46 (+)	6.35 (−)	1.13	0.99
(±) EGYT-1623	7.81 (−)	8.58 (+)	1.10	0.98

Without going into details of the DSC techniques used, we cite an excellent publication dealing with the principles and limitations of these methods[18]. Detailed results will be published elsewhere.

From Table III, we can conclude that:

(i) HPLC and DSC of sample 1 show that it can be considered to be the pure (+)-enantiomer of EGYT-1623 ketone.

(ii) In cases of high enantiomeric purities (samples 1–3) the experimental results obtained by different techniques are in very good agreement with each other.

(iii) For samples in which impurities, *i.e.* substances other than the enantiomer of opposite sign, are present (samples 4 and 6, see remark a and b in Table III), only HPLC can give the real molar composition with certainty.

(iv) Due to the very high enantiomeric purity of the (+) EGYT-1623 ketone (sample 1), the measured optical purities (except those of samples 4 and 6) can be considered as correct.

TABLE III

ENANTIOMERIC PURITY OF OPTICALLY RESOLVED EGYT-1623 KETONE SAMPLES

EE = Enantiomeric excess; X* = mole fraction for the more abundant enantiomer.

Sample no.	$[\alpha]_D^{20}$ *(c = 1, chloroform)*	*Optical purity calculated from* $[\alpha]_D^{20}$		*HPLC X* (%)*	*Differential scanning calorimetry*	
		EE (%)	*X* (%)*		*X* (%) from van 't Hoff plot*	*X* (%) from calculated binary phase diagram**
1	+40.2	standard ref. material		100	99.32	–
2	−39.0	97.0	98.5	99.2	98.52	99.0
3	−38.0	94.5	97.0	96.8	–	96.0
4	+19.8	49.2	74.6	71.7**	–	80.0
5	−26.5	65.9	83.0	87.0	–	88.3
6	+31.7	78.9	89.5	89.3***	–	–

* The binary phase diagram is calculated from T_0 (DSC melting point) and the molal heat of fusion of the pure enantiomers and that of the racemic compound using the Schröder–van Laar[16] and Prigogine–Defay equations[17].

** Contains about 4% of "pseudo" ketone (see Table I).

*** Contains about 5% of "normal" ketone (see Table I).

CONCLUSIONS

The separation factors and peak area ratios obtained for racemic EGYT-1623 and EGYT-1623 ketone show that HPLC is a good method for determining the enantiomeric purity in partially resolved samples.

Comparing the results for EGYT-1623 ketone obtained by various methods, it can be concluded that HPLC may become the method of choice. It can be applied in cases where interference, *e.g.*, the presence of impurities other than the enantiomer of opposite sign, cause serious limitations for the other methods.

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