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CHROMATOGRAPHIC RESOLUTIONS

XIV*. OPTICAL RESOLUTION OF THE RACEMIC ANTICANCER DRUG IFOSFAMIDE AND OTHER CHIRAL OXAZAPHOSPHORINES

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SUMMARY

Ifosfamide, a chiral anticancer drug with phosphorus as chiral centre, was resolved into its enantiomers by chromatography on polyamides with bonded optically active substituents. Both enantiomers were isolated and characterized, the enantiomeric purity being at least 95% (^{31}P nuclear magnetic resonance in the presence of an optically active shift reagent) and 99% (analytical chromatography), respectively. Experiments on the resolution of other oxazaphosphorines including the therapeutic drugs cyclophosphamide and trofosfamide are described.

INTRODUCTION

Ifosfamide (Holoxan®, 1), cyclophosphamide (Endoxan®, 2) and trofosfamide (Ixoten®, 3) are important and widely used drugs in anticancer therapy, in the treatment of autoimmune diseases and after organ transplantation[2]. All these compounds are chiral because of the asymmetrically substituted phosphorus atom of the 1,3,2-oxazaphosphorine heterocycle. These drugs are administered clinically as racemates.

The enantiomers of compounds 1–3 have already been synthesized via diastereoisomeric intermediates, which were separated by recrystallization or by liquid chromatography on silica gel. After separation of the diastereoisomers, the optically active residue was cleaved to give the enantiomers[3–5]. However, these are no appropriate routes for the synthesis of large amounts or, in particular, for the preparation of radioactively labelled optical isomers.

The direct chromatographic resolution of racemates on polyamides with bonded optically active substituents[6,7] has, on the other hand, proved to be a successful method for the isolation of enantiomers in high enantiomeric purity. We now report experiments on the resolution of compounds 1–3 as well as the oxazaphosphorines 4–13 (see Table I). The compounds 4, 5, 8 and 9 are of importance as chiral metab-

* For part XIII, see ref. 1.

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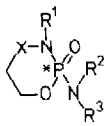
TABLE I

1,3,2-OXAZAPHOSPHORINES



Compound	R^1	R^2	R^3	X
1	CH_2CH_2Cl	CH_2CH_2Cl	H	CH_2
2	H	CH_2CH_2Cl	CH_2CH_2Cl	CH_2
3	CH_2CH_2Cl	CH_2CH_2Cl	CH_2CH_2Cl	CH_2
4	CH_2CH_2Cl	H	H	CH_2
5	H	CH_2CH_2Cl	H	CH_2
6	H	H	H	CH_2
7	CH_2CH_2Cl	$R^2 = R^3 = CH_2CH_2$		CH_2
8	H	CH_2CH_2Cl	CH_2CH_2Cl	CO
9	CH_2CH_2Cl	CH_2CH_2Cl	H	CO
10	CH_2CH_2Cl	$COCH_2Cl$	H	CH_2
11	CH_2CH_2Cl	CH_2CH_2OH	H	CH_2
12a,b*	H	CH_2CH_2Cl	CH_2CH_2Cl	$CHN(OH)CONH_2$
13a,b*	CH_2CH_2Cl	CH_2CH_2Cl	H	$CHN(OH)CONH_2$

* The diastereoisomer a is eluted more rapidly than the isomer b when chromatographed on silica gel.

olites, 10 and 11 as potential metabolites of the anticancer oxazaphosphorines, especially with regard to studies of the enantioselective drug metabolism.

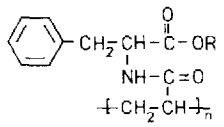
EXPERIMENTAL

Materials

The 1,3,2-oxazaphosphorine racemates were obtained from ASTA-Werke (Bielefeld, F.R.G.) The optically pure lanthanide shift reagent Tris[3-(trifluoromethyl-hydroxymethylene)-*d*-camphorate-europium(III)] [$Eu(tfc)_3$] was purchased from EGA Chemie (F.R.G.). The bulk solvents toluene and dioxane were distilled before use; all other chemicals were of analytical quality.

Analytical scale liquid chromatography

Resolutions were performed with a low-pressure (3 bar) liquid chromatography system consisting of a Duramat membrane pump, a Perkin-Elmer polarimeter Model 241 with an 80-μl micro flow cell and a Knauer differential refractometer Model 5100. The glass columns (Pharmacia) were packed with either 5.0 g of Po-



	R
14	C_2H_5
15	CH_2–C$_6$H$_5$

ly[(*S*)-N-(ethoxycarbonyl-2-phenylethyl)acrylamide] (14) (29.5 cm × 1.0 cm)[7,8] or with 5.1 g of Poly[(*S*)-N-(benzyloxycarbonyl-2-phenylethyl)acrylamide] (15) (31 cm × 1.0 cm)[1], as optically active stationary phases. For chromatography about 5 mg of each of the racemic compounds 1–5 and 7–11 were dissolved in 0.5 ml of the solvent (6 in 2.0 ml dioxane) and injected via a loop injector (Latek, F.R.G.). Toluene–dioxane (1:1) was used as the mobile phase. The flow-rate varied from 10 to 15 ml/h. The racemic diastereoisomers 12a, b and 13a, b of low solubility were chromatographed on 6.2 g of adsorbent 14 (column 42.0 cm × 1.0 cm) with toluene–dioxane–methanol (44:44:12) as the mobile phase at a pressure of 0.5 bar.

Preparative isolation of ifosfamide enantiomers

Resolution of 250, 255 and 260 mg ifosfamide (1) was performed using a glass column (78.5 cm × 3.3 cm) packed with 235 g of adsorbent 14 and with toluene–dioxane (1:1) as eluent. Elution of the enantiomers started after 1050 ml of the eluent had been passed; in each case the resolution was almost complete (separation factor, $\alpha = 1.41$). The fractions of the eluate containing the corresponding optically pure isomers were pooled and evaporated *in vacuo* (yield: 97%). The colourless, oily residues were dissolved in diethyl ether. When the solutions were cooled to −70°C, both enantiomers crystallized as white needles. Recrystallization from diethyl ether at −20°C yielded 216 mg of (+)-ifosfamide, m.p. 72°C. (Found: C, 31.95; H, 5.84; N, 10.75%), $[\alpha]_{365}^{25} = +140.2$, $[\alpha]_{D}^{25} = +44.9$ [*c* (g/100 ml) = 1.01, methanol] and 231 mg of (−)-ifosfamide, m.p. 72°C (Found: C, 32.04; H, 5.80; N, 10.80%), $[\alpha]_{365}^{25} = -139.6$, $[\alpha]_{D}^{25} = -44.4$ (*c* = 1.03, methanol). $C_7H_{15}Cl_2N_2O_2P$ requires: C, 32.17; H, 5.74; N, 10.73%.

^{31}P Nuclear magnetic resonance (NMR)

A 300-MHz NMR spectrometer (Bruker WM 300) was used with 85% orthophosphoric acid as the external standard. ^{31}P NMR experiments were performed as in the following examples. To a solution of 10.0 mg of racemic ifosfamide in a mixture of 1.8 ml tetrachloromethane and 0.2 ml [$^2H_6$]benzene, 80.9 mg of optically active $Eu(tfc)_3$ were added (molar ratio 2.38:1). The spectrum revealed the presence of two almost completely resolved resonances at −67.0 and −68.5 ppm in a 1:1 ratio.

RESULTS AND DISCUSSION

Analytical chromatographic resolution of 1,3,2-oxazaphosphorines

The racemic compounds 1–10 (structures given in Table I) were chromatographed under similar conditions on the optically active polyacrylamide stationary phase 14 the chirality of which is based on the (*S*)-phenylalanine ethyl ester side chains. In the case of the N-hydroxyureido derivatives 12a,b and 13a,b, insufficiently soluble in the standard solvent toluene–dioxane, the more polar mixture toluene–dioxane–methanol was used as the mobile phase. In addition, some of these racemates were chromatographed on adsorbent 15 which differs from 14 in bearing (*S*)-phenylalanine benzyl ester residues at the polymer backbone. The results are presented in Table II.

Chromatograms of racemic ifosfamide (1) and 2-dechloroethyl ifosfamide (4) obtained on adsorbent 14 are shown in Fig. 1. Of the three therapeutically used

TABLE II

RESOLUTION OF RACEMIC 1,3,2-OXAZAPHOSPHORINES ON THE OPTICALLY ACTIVE POLYACRYLAMIDES 14 AND 15

k' = Capacity factor; α = separation factor; R_s = resolution.

Compound	*Adsorbent*	*Enantiomer most strongly* retained*	k'	α	R_s
1	14	+ (*R*)	0.57/0.78	1.37	1.13
2	14	− (*S*)	0.77	−**	−**
3	14	NR***	0.12	−	−
4	14	+ (*R*)	1.64/2.51	1.53	2.60
5	14	+ (*R*)	3.50	−	−
6	14	+	8.74/9.43	1.08	−
7	14	− (*S*)	0.19	−	−
8	14	+ (*R*)	0.64/0.76	1.19	−
9	14	+ (*R*)	0.63/0.70	1.11	−
10	14	+	0.87/1.09	1.25	−
11	14	+	1.33/1.72	1.29	0.96
12a	14	−	0.28	−	−
12b	14	+	0.11	−	−
13a	14	+	0.02	−	−
13b	14	−	0.06	−	−
1	15	+ (*R*)	0.56/0.75	1.34	0.74
2	15	NR***	0.72	−	−
4	15	+ (*R*)	1.38/2.42	1.75	2.25
5	15	NR***	3.12	−	−
8	15	+ (*R*)	0.54/0.70	1.30	−
9	15	+ (*R*)	0.42	−	−

* The sign of the rotation in the mobile phase is given. In cases where the absolute configuration is known, this is added.

** α and/or R_s values could not be calculated due to weak resolutions.

*** NR = No measurable resolution.

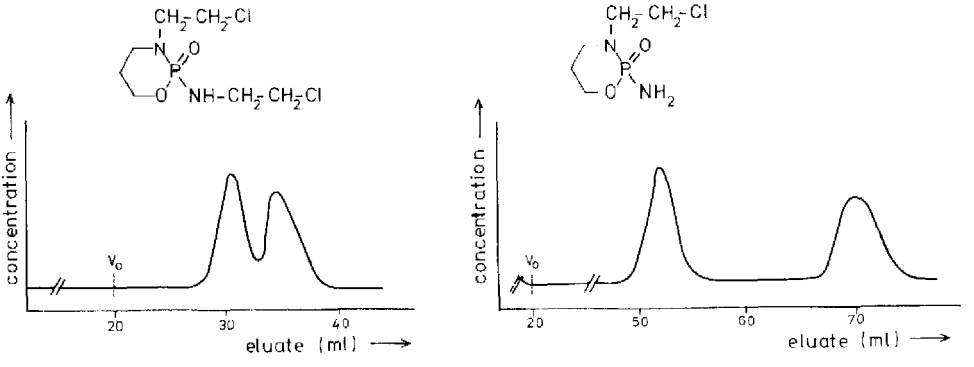


Fig. 1. Low-pressure liquid chromatography of ifosfamide (1) (left) and 2-dechloroethyl ifosfamide (4) (right) (5.0 mg of each racemate) on 5.0 g of adsorbent 14. Column: 29.5 cm × 1.0 cm. Mobile phase: toluene–dioxane (1:1). The corresponding rotation curves have been omitted. V_0 = Void volume of the column, determined with xylene.

oxazaphosphorines, ifosfamide (1) was almost completely resolved on adsorbent 14 (α = 1.37, R_s = 1.13). Cyclophosphamide (2), a structural isomer of 1 having an unsubstituted endocyclic nitrogen atom, was partially resolved though the retention times of both compounds were similar.

The more strongly retained 2-dechloroethyl ifosfamide (4) was completely separated into its enantiomers (α = 1.53, R_s = 2.6). In contrast, the analogous 3-dechloroethyl ifosfamide (5), having increased retention time due to the adsorptive effect of the additional NH group, is only weakly resolved. Thus, the hydrogen-bonding interaction of the endocyclic NH function of 5 with the stationary phase obviously increases the adsorption strength, but does not affect the resolution. Compound 6, unsubstituted at both nitrogens, was eluted very slowly, but only a poor enrichment of the enantiomers was observed.

Both trofosfamide (3) and the aziridine derivative 7 were eluted very rapidly because there is no adsorptive hydrogen bonding due to complete substitution of the nitrogen atoms. They were not, or only weakly resolved, respectively.

Replacement of the chloro atom of the side chain of the exocyclic nitrogen of ifosfamide by a hydroxyl group (compound 11) causes an increase in retention times, but merely influences the separation. Thus, an additional adsorptive functional group distant from the chiral centre has no effect on the resolution. Compounds 9 and 10 bearing a carbonyl group within the chloroethyl side chain and the heterocycle, respectively, showed similar k' values but smaller α values than for ifosfamide. In contrast, 4-ketocyclophosphamide (8) is almost completely resolved because of the interaction of the additional carbonyl group with the stationary phase.

The chiral N-hydroxyureido compounds 12a,b and 13a,b were weakly retained and hence not resolved when using the more polar solvent containing methanol.

In comparison with these results on adsorbent 14, a somewhat better resolution was achieved for the oxazaphosphorines 4 and 8 on the stationary phase 15. However, in the case of compound 4, smaller R_s values are observed due to a tailing of the completely separated enantiomers (α = 1.75). The structural isomer 5 was eluted slowly without any resolution. While 4-ketocyclophosphamide (8) could be separated almost completely (α = 1.30), only poor enrichment of the optical isomers of 9 was observed in the first and last volume fractions of the eluate. The resolution also deteriorated in the cases of ifosfamide (1) and cyclophosphamide (2). However, the capacity factors, k', of all the oxazaphosphorines tested were similar on both stationary phases.

Preparative isolation of the enantiomers of ifosfamide

The analytical resolution method described above was also applied to the isolation of enantiomerically pure optical isomers on a preparative scale. This is demonstrated for the anticancer drug ifosfamide (1). In total, 765 mg of the racemate 1 were chromatographed in three experiments on a column packed with 235 g of adsorbent 14. The separation of the enantiomers was almost complete under these conditions. After recrystallization from diethyl ether at low temperatures, analytically pure (−)- and (+)-ifosfamide were obtained.

Determination of the enantiomeric purity of the ifosfamide enantiomers

The enantiomeric purity of ifosfamide enantiomers synthesized previously had

been determined by ^{31}P NMR spectroscopy in the presence of optically active lanthanide shift reagents[9]. With the racemate, the best resolution of the ^{31}P NMR signals was obtained when a molar ratio of $Eu(tfc)_3$/ifosfamide of 2.4 was used. The signal of (−)-ifosfamide was shifted downfield to a greater extent.

Both enantiomers isolated by chromatography showed only one signal in the corresponding NMR spectra. The accuracy of these ^{31}P NMR measurements was estimated to be ±5%, thus indicating an enantiomeric purity of at least 95% for both optical isomers.

Chromatography of samples of the isolated ifosfamide enantiomers, using the analytical column with adsorbent 14, proved to be a more sensitive method for the determination of enantiomeric purity. The (+)- and (−)-ifosfamide, respectively, revealed only one peak in the polarimeter and refractometer curves of the corresponding chromatograms. The addition of 1% of the racemate to the isomers could still be clearly detected. Thus, the enantiomeric purity of the (+)- and (−)-ifosfamide probes was at least 99%.

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