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ISOLATION AND STEREOSPECIFIC DETERMINATION OF THE ENANTIOMERS OF OXINDAZAC BY DIRECT LIQUID CHROMATOGRAPHIC RESOLUTION ON TRIACETYLCELLULOSE

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SUMMARY

The preparation of the optically pure enantiomers of the antiphlogistic trial drug oxindazac via liquid chromatographic resolution of the corresponding *tert.*-butyl or benzyl ester on triacetylcellulose is described. Cleavage of the optically pure enantiomeric esters to the acids proceeds without significant racemization. The methyl ester of oxindazac is also completely resolved on the same chiral phase. Whereas oxindazac racemizes easily upon derivatization to diastereomers, no racemization is observed upon methylation to the corresponding methyl ester with diazomethane. An inverse isotope dilution method has been developed to determine both enantiomers of the drug in biological fluids after administration of ^{14}C-labelled oxindazac. The enantiomers are converted into their methyl esters and separated on triacetylcellulose. Quantitation is performed by on-line UV detection at 290 nm and off-line radiometry. In the analysis of plasma samples, endogenous compounds do not interfere. The recoveries of [^{14}C]oxindazac from water, rat and human plasma were 99.6 ± 1.8% for the (+)- and 96.0 ± 1.4% for the (−)-enantiomer. The plasma concentrations and urinary excretion of the two enantiomers were determined in a human volunteer who had received 200 mg of racemic ^{14}C-labelled oxindazac.

INTRODUCTION

The analgesic and antiphlogistic trial drug oxindazac*, 5-benzoyl-2,3-dihydro-6-hydroxy-1H-indene-1-carboxylic acid, is a chiral compound[1]. The centre of chirality at C-1 is in the α-position with respect to the carboxyl group and carries a very labile proton. The same structural element is present in several other non-steroidal anti-inflammatory drugs. They include arylpropionic acids such as ibuprofen, benoxaprofen and loxoprofen, and the indan-1-carboxylic acid clidanac[2–5]. In this

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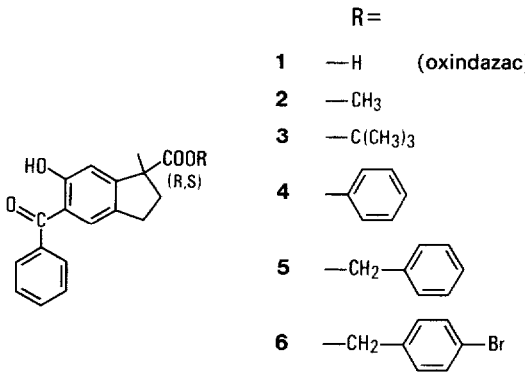


class of drugs the pharmacological activities seem to reside in the S-enantiomers[6,7]. The disposition in the organism is normally stereoselective, enzymatic inversion of the R- to the S-form being one possible mechanism[7].

Our first attempts to resolve the enantiomers of oxindazac were unsuccessful. It was not possible to apply the classical methods of preparation and resolution of diastereomers because of rapid racemization of the enantiomers, for instance upon derivatization with optically active reagents. The higher tendency to epimerize at the benzylic position of compound 1 in comparison with analogous drugs can be attributed to the combined inductive effects of the functional groups of 1.

Direct separation of enantiomers by liquid chromatography on a chiral stationary phase has become a promising alternative to the classical methods. Different types of chiral phases have been reported[8–12] and among them, microcrystalline triacetylcellulose has shown a very good stereoselective recognition for many aromatic compounds[13–17]. We found that several racemic esters of oxindazac can be completely resolved on triacetylcellulose columns. Based on this principle, we developed methods for both the preparative separation of the enantiomers of oxindazac and the determination of the enantiomeric composition of mixtures of them. The latter method, which combines liquid chromatography with inverse isotope dilution, was used in a study on the stereoselective kinetics of ^{14}C-labelled oxindazac in man. This paper describes the development and application of the new methods.

EXPERIMENTAL

Chemicals

Oxindazac, CGP 6258 (Lot No. 800182), was prepared by Ciba-Geigy (Basle, Switzerland). It was labelled with ^{14}C in the keto group (Batch No. Z.564.4F, Ciba-Geigy), specific radioactivity 18.9 kBq/mg and radiochemical purity *ca.* 99%, as ascertained by isotope dilution analysis and thin-layer chromatography (TLC) on silica gel (Si 60 F-254; Merck, Darmstadt, F.R.G.) in two chromatographic solvent systems: cyclohexane–dioxan–acetic acid (65:35:1, v/v/v) and *n*-heptane–1-propanol–acetic acid (85:15:1, v/v/v).

The solvents and chemicals used were all of reagent grade, obtained from Merck or Fluka (Buchs, Switzerland). Diethyl ether was distilled to remove the sta-

bilizer and was stored over magnesium sulphate; 1,2-dichloroethane was distilled before use. A phosphate buffer solution pH 6.9 (0.025 *M* disodium hydrogen phosphate + 0.025 *M* potassium dihydrogen phosphate in 1 l) and a solution of pH 2.06 (0.090 *M* potassium chloride + 0.010 *M* hydrochloric acid in 1 l) were used. Microcrystalline triacetylcellulose was prepared by acetylation of microcrystalline cellulose (Merck), following the procedure of Hesse and Hagel[18]. Diazomethane in diethyl ether was prepared in a special device[19] from N,N'-dimethyl-N,N'-dinitrosotereph-thalamide (Fluka) and 5 *M* sodium hydroxide.

Synthesis and cleavage of esters of oxindazac

The *tert.*-butyl ester 3 was prepared by reaction of 5 mmol of oxindazac with 6.1 mmol of *tert.*-butanol, 6.1 mmol of 1,1'-carbonyldiimidazole and a few drops of pyridine in 4 ml 1,2-dichloroethane at 70°C for 3 h. The reaction mixture was diluted in 20 ml 1,2-dichloroethane and washed with 1 *M* sodium hydroxide and 1 *M* hydrochloric acid, each 10 ml. The concentrated organic phase was chromatographed on a silica gel G (Merck) column of 30 × 4 cm with chloroform. The resulting yellowish oil was crystallized from methanol. Yield: 24%. M.p.: 107°C. ^{1}H NMR (deuterochloroform): 12.12 (s, OH); 7.68, 7.59 and 7.51 (m, 5H, *o*-, *p*- and *m*-benzoyl H); 7.40 (s, H-4); 7.11 (s, H-7); 3.96 (t, H-1); 2.99 and 2.80 (m, 2H, H-3); 2.44 and 2.30 (m, 2H, H-2); 1.52 (s, 9H, CH_3).

The phenyl ester 4 was prepared by reaction of 1.4 mmol of oxindazac with 20 mmol of phenol and 1.3 mmol of 1,1'-carbonyldiimidazole and a few drops of pyridine in 2 ml 1,2-dichloroethane at 70°C for 2 h. Chromatography was performed as above, with chloroform as eluent. Recrystallization was from methanol. Yield: *ca.* 67%. M.p.: 105–106°C. ^{1}H NMR (deuterochloroform): 12.12 (s, OH); 7.68, 7.60 and 7.52 (m, 5H, *o*-, *p*- and *m*-benzoyl H); 7.46 (s, H-4); 7.28 (s, H-7); 7.41, 7.25 and 7.12 (m, 5H, *m*-, *p*- and *o*-phenoxy H); 4.31 (t, H-1); 3.07 and 2.90 (m, 2H, H-3); 2.62 and 2.50 (m, 2H, H-2).

The benzyl ester 5 was prepared by reaction of 21.3 mmol of oxindazac with 87.7 mmol of benzyl bromide and 49.9 mmol of triethylamine in 40 ml 1,2-dichloroethane at 50°C for 15–20 min. The reaction mixture was diluted in 20 ml 1,2-dichloroethane, washed twice with water and the concentrated organic phase was chromatographed as described above, but with 1,2-dichloroethane as eluent. Recrystallization was from methanol or ethanol. Yield: *ca.* 90%. M.p.: 106–107°C. ^{1}H NMR (deuterochloroform): 12.08 (s, OH); 7.67, 7.60 and 7.51 (m, 5H, *o*-, *p*- and *m*-benzoyl H); 7.40 (s, H-4); 7.38 (br s, 5H, phenyl H of benzyl); 7.11 (s, H-7); 5.23 and 5.18 (AB system, J = 13 Hz, OCH_2); 4.10 (t, H-1); 3.00 and 2.83 (m, 2H, H-3); 2.50 and 2.37 (m, 2H, H-2).

The *p*-bromobenzyl ester 6 was prepared by reaction of 0.7 mmol of oxindazac, dissolved in 2 ml acetone, with *ca.* 1.5 mmol of triethylamine and 0.7 mmol of *p*-bromobenzyl bromide in 3 ml acetone at 50°C for about 15 min and then for 4 h at room temperature. The reaction mixture was poured onto 50 ml water and then extracted with *ca.* 100 ml 1,2-dichloroethane. After drying over magnesium sulphate, the organic solvent was concentrated and the yellowish oil was crystallized from methanol. Crystals formed only on storing at −20°C. Yield: *ca.* 80%. M.p. 70–72°C. ^{1}H NMR (deuterochloroform): 12.09 (s, OH); 7.66, 7.60 and 7.51 (m, 5H, *o*-, *p*- and *m*-benzoyl H); 7.52 and 7.25 (AA'BB' system, *p*-bromophenyl H); 7.42 (s, H-4); 7.08

(s, H-7); 5.18 and 5.11 (AB system, J = 13 Hz, OCH_2); 4.10 (t, H-1); 3.00 and 2.83 (m, 2H, H-3); 2.47 and 2.38 (m, 2H, H-2).

For the preparation of the methyl ester 2 on an analytical scale, 0.003–0.03 mmol of oxindazac were treated with a large excess of freshly prepared diazomethane (see above) in diethyl ether for about 15 min in an ultrasonic bath. For spectroscopic analysis, the ester was chromatographed on a LiChrosorb Si 60 column (see below) with cyclohexane–1,2-dichloroethane–tetrahydrofuran–acetic acid (55:40:4:1, v/v/v/v) as eluent. Yield: > 90%. M.p.: 89–90°C. ^{1}H NMR (deuterochloroform): 12.10 (s, OH); 7.66, 7.59 and 7.51 (m, 5H, *o*-, *p*- and *m*-benzoyl H); 7.41 (s, H-4); 7.11 (s, H-7); 4.07 (t, H-1); 3.77 (s, OCH_3); 3.00 and 2.82 (m, 2H, H-3); 2.47 and 2.37 (m, 2H, H-2).

For hydrolysis of the *tert.*-butyl ester of oxindazac, 0.14 mmol of the ester 3 were dissolved in 10 ml 1,2-dichloroethane, *ca.* 1 mmol of trimethylsilyl iodide added and the mixture allowed to react for 10–15 min at room temperature. The deep red solution was shaken together with 50 ml water in a separating funnel for about 5 min, to hydrolyse the intermediate silyl ester. The water phase was extracted with 1,2-dichloroethane and the organic solvent was concentrated to dryness. After a second extraction the yield was nearly quantitative. The product was recrystallized from methanol.

The benzyl ester of oxindazac was cleaved by hydrogenation. A 0.7-mmol amount of the ester 5 was dissolved in 10 ml tetrahydrofuran, 20 mg of 5% palladium on carbon were added and hydrogen was bubbled through the mixture at 25°C and normal pressure for about 3 h. The gas consumption was 125% of theoretical. The catalyst was separated on a filter frit, and the concentrated organic solution was chromatographed on a silica gel G column (30 × 2 cm) with cyclohexane–1,2-dichloroethane–ethanol (50:40:10, v/v/v). Recrystallization was from 1,2-dichloroethane and a few drops of heptane. Yield: 45–65%.

Chromatographic conditions

Preparative chromatographic resolution of the *tert.*-butyl ester 3 was performed on a 60 × 5 cm glass column (Buechi, Uster, Switzerland). This column was slurry-packed with swollen microcrystalline triacetylcellulose (particle size 40–70 μm) in 95% ethanol at room temperature. A pressure of 4 kg cm^{-2} (flow-rate 300 ml h^{-1}) was applied and the eluate was detected by a UV-spectrophotometer (UV-120-02; Shimadzu, Tokyo, Japan) at 290 nm in series with a polarimeter (241 MC; Perkin-Elmer, Norwalk, CT, U.S.A.) at 365 nm. The benzyl ester 5 was resolved under the same conditions. Traces of base, which may cause racemization, have to be eliminated by washing the glassware with acetic acid and then acetone before use.

For the analytical chromatographic resolution of the esters of oxindazac, the same triacetylcellulose (particle size 25–32 μm) packed in a 18 × 1.3 cm or 60 × 1.3 cm glass column (Buechi) was used. The mobile phase, 95% ethanol, was pumped (Altex pump, Model 110; Kontron, Zürich, Switzerland) through the column at a flow-rate of 0.5 ml min^{-1}, so that a pressure of about 1.5 kg cm^{-2} was reached. The compound was detected and quantified on-line with a UV-detector at 290 nm (Uvikon 725, Kontron) connected to an integrating recorder (W & W 1100, Kontron).

To separate oxindazac from endogenous constituents in extracts of body fluids, chromatography on LiChrosorb Si 60 (Merck, 10 μm) was used. A stainless-

steel column (25 × 1 cm) was connected to a pre-column (4 × 0.4 cm), both packed with the same material. The chromatographic solvent cyclohexane–1,2-dichloroethane–acetic acid (80:18:2, v/v/v) was pumped through the column at a flow-rate of 4 ml min^{-1}. The pump, detection and quantification were as described above; the wavelength was set at 350 nm.

Polarimetry

The optical rotation was measured with a Model 241 MC polarimeter (Perkin-Elmer) equipped with a 5-ml cell of length 10 cm. The solvents used were chloroform and ethanol.

Inverse isotope dilution analysis (IDA)

To a 1-ml plasma sample containing ^{14}C-labelled oxindazac was added an aqueous solution (1 ml) of the non-labelled racemic oxindazac (*ca.* 500 μg). After a homogeneous solution had been obtained, the sample was extracted with 100–150 ml dichloromethane at pH 2.06, and after concentration the analyte was treated with diazomethane as described above. The diethyl ether was evaporated and the dry residue was dissolved in 95% ethanol for separation on triacetylcellulose (column: 18 × 1.3 cm). The peaks corresponding to the two enantiomeric forms of the methyl ester were integrated and collected for off-line measurement of radioactivity. When urine samples were analysed, or when enzymatic hydrolysis with β-glucuronidase preceded the determination, an additional chromatographic purification on LiChrosorb Si 60 (see above) had to be included before the reaction with diazomethane and separation on triacetylcellulose.

Enzymatic hydrolysis

Enzymatic hydrolysis of conjugates in plasma and urine was performed with β-glucuronidase (*Escherichia coli*; Sigma, St. Louis, MO, U.S.A.) at pH 6.9 and 37°C for 3 h. For extraction with dichloromethane, the samples were adjusted to pH 2.06, and the extracts were chromatographed on LiChrosorb Si 60 (see above).

Calibration of the LC method used in IDA

Replicate samples containing various amounts of the racemic methyl ester of oxindazac were injected onto the triacetylcellulose column. The amounts ranged from 113 to 273 μg of each enantiomer. The two peaks were integrated, their areas summed and then divided by two. The range of the concentrations was chosen to encompass the expected concentrations of biological samples mixed with the carrier substance. Peak areas were determined automatically by the UV-detector and the integrator. Calibration curves were fitted by means of a computer program using a linear regression analysis.

Recovery of enantiomers from samples spiked with racemic [^{14}C]oxindazac

Samples of 1 ml water, or 1 ml rat or human plasma, were spiked with about 7 or 11 μg of ^{14}C-labelled oxindazac and submitted to IDA. The results obtained were compared with the amount of racemic [^{14}C]oxindazac added to each sample to determine the recovery. It was assumed that synthetic [^{14}C]oxindazac is a 1:1 mixture of the ^{14}C-labelled (+)- and (−)-enantiomers.

Application

A single oral 200-mg dose of racemic ^{14}C-labelled oxindazac in a gelatine capsule was administered to a healthy male volunteer[20]. Blood was withdrawn at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing. Urine was collected in fractions during 2 days. Plasma was prepared from the heparinized blood samples and the carrier substance (non-labelled racemic oxindazac) was added immediately. Plasma specimens and urine were stored at –20°C until analysis.

Measurement of radioactivity

Radiometry was done by liquid scintillation counting as described elsewhere[21].

RESULTS AND DISCUSSION

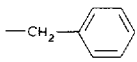
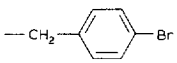
Preparation of the optically pure forms of oxindazac

The free acid 1 is only partially resolved by chromatography on triacetylcellulose. By contrast, several esters of 1 are completely resolved on the same support. The results are summarized in Table I. For a preparative isolation of the optically pure forms of oxindazac, via direct chromatographic resolution of an ester derivative, the *tert.*-butyl or benzyl ester is particularly suitable. They are easily cleaved under mild conditions to yield again the free acid without racemization.

TABLE I

CHROMATOGRAPHIC RESOLUTION OF ESTERS OF OXINDAZAC ON TRIACETYLCELLULOSE

Column: 60 × 1.3 cm. Eluent: 95% ethanol. Flow-rate: 30 ml h^{-1}.

Compound	*R*	*Capacity factors*		*Separation factor, α*	*Resolution factor, R_s*
		k_1*	k_2		
2	$-CH_3$	2.67 (−)	4.16	1.56	2.1
3	$-C(CH_3)_3$	1.10 (−)	1.72	1.55	1.5
4		4.13 (−)	8.27	2.00	3.0
5		4.15 (−)	7.94	1.91	2.9
6		3.68 (−)	15.23	4.14	2.1

* Sign of the optical rotation of the first eluted enantiomer.

Chromatographic resolution of these two esters, 3 and 5, on a preparative scale (100–200 mg) gives in both cases the pure enantiomeric forms in very high yield (Fig. 1). The optical purity of the isolated enantiomeric esters was tested by re-injection of a sample on an analytical column. Only one peak was detected, indicating the optical purity and stability of the enantiomers. The properties of the enantiomers of the *tert.*-butyl and the benzyl ester are included in Table II.

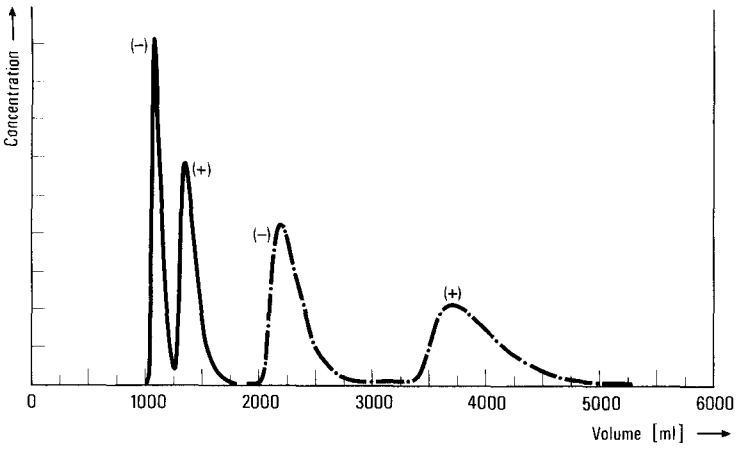


Fig. 1. Chromatographic resolution of 100 mg of compound 3 (——) and 200 mg of 5 (· – · – ·) on a triacetylcellulose column (60 × 5 cm).

TABLE II

PROPERTIES OF THE ENANTIOMERS OF OXINDAZAC (1) AND ITS ESTERS 3 AND 5

Compound	*m.p. (°C)*	$[\alpha]_D^{24}$	$[\alpha]_{578}^{24}$	$[\alpha]_{546}^{24}$
1 (−)*	155–157	−32 ± 2**	−34.8 ± 2**	−43.2**
1 (+)*	154–156	+32 ± 2***	+34.5 ± 2***	+43.2***
3 (−)	106–107	−37.6 ± 2§	−40.6 ± 2§	−50 ± 2§
3 (+)	106–107	+39.5 ± 2§§	+42.6 ± 2§§	+52 ± 2§§
5 (−)	119–123	−66 ± 2§§§	−71.3 ± 2§§§	−85.6 ± 2§§§
5 (+)	119–122	+70 ± 2†	+73.7 ± 2†	+88.2 ± 2†

* The absolute configuration is still unknown.
** *c* 0.373, chloroform.
*** *c* 0.368, chloroform.
§ *c* 0.946, ethanol.
§§ *c* 0.902, ethanol.
§§§ *c* 0.188, ethanol.
† *c* 0.186, ethanol.

By cleavage of the optically pure enantiomers of the *tert.*-butyl ester with trimethylsilyl iodide, or of the benzyl ester by hydrogenation, up to 90% of the corresponding pure enantiomeric forms of the free acid 1 are obtained.

Properties of the enantiomers of oxindazac

The properties of the enantiomers of oxindazac are given in Table II. The optical purity of the isolated enantiomers of oxindazac was confirmed as follows: reaction of the individual enantiomers with diazomethane gave the corresponding methyl esters. Upon chromatography on an analytical triacetylcellulose column, only one peak is observed for the (−)-enantiomer (Fig. 2b) and for the opposite (+)-enantiomer. The racemic methyl ester is completely resolved on the same column

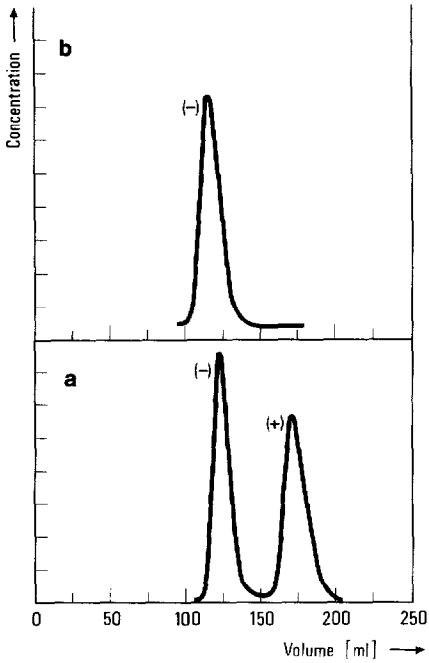


Fig. 2. Chromatography of the methyl ester of racemic oxindazac (a) and its (−)-enantiomer (b) on a triacetylcellulose column (60 × 1.3 cm).

(Fig. 2a). This demonstrates that cleavage of the *tert.*-butyl and benzyl esters, and esterification with diazomethane, proceed without significant racemization.

IDA method for measurement of the enantiomers of oxindazac

With the IDA method described here, it is possible to determine the enantiomeric composition of samples containing ^{14}C-labelled oxindazac. This method combines direct chromatographic resolution of the methyl ester 2, as shown in Fig. 2a, with the principle of inverse isotope dilution. Racemic, unlabelled oxindazac is added to the sample, acting as an ideal internal standard which allows correction for all losses preceding the actual measurement. The drug is extracted from the sample and treated with diazomethane. Ester formation is virtually quantitative, and no by-products are formed. The methyl ester is resolved by chromatography. The amount of each enantiomer can be determined from the spectrophotometric and radiometric results.

The analysis of blank samples of plasma from rat and man revealed that endogenous compounds do not interfere with the UV-detection of the enantiomeric esters. However, urine samples and plasma samples treated with β-glucuronidase gave rise to some interference. In these cases, the analyte extracted from the biological sample has to be purified on a LiChrosorb Si 60 column before methylation.

To demonstrate the absence of racemization, the optically pure enantiomers of compound 1 were taken through the individual steps of the analytical procedure. These steps include extraction at pH 2.06, enzymatic hydrolysis at pH 6.9, chro-

matography on LiChrosorb Si 60 and reaction with diazomethane. When checking the optical purity of the (+)- or the (−)-isomer, the content of the respective antipode was found to be 3% at the most.

Calibration and validation of the IDA method

Calibration of the spectrophotometric determination was performed with the racemic methyl ester 2. It was shown that both enantiomers possess the same photometric response. The calibration curves were linear; the reproducibility of the method expressed as the coefficient of variation (C.V.) was 0.4% for both enantiomers ($n = 7$).

Samples of water, rat plasma and human plasma, spiked with the usually occurring amounts of ^{14}C-labelled oxindazac, were analysed by the IDA assay for accuracy. The recoveries were 99.6 ± 1.8% for the (+)- and 96.0 ± 1.4% for the (−)-enantiomer ($n = 7$). The data were calculated with the assumption that synthetic [^{14}C]oxindazac is a 1:1 mixture of the two enantiomers. In fact, the sum of the individual values of (+)- and (−)-oxindazac resulted in means ± C.V. of 97.8 ± 1.4% for the recovery of racemic oxindazac. The sensitivity of the method would be around 0.01 μg/g (0.04 nmol/g) when a specific radioactivity of *ca.* 19 kBq/mg is applied.

Kinetics of the enantiomers of ^{14}C-labelled oxindazac in man

The analytical assay was used to determine the pharmacokinetics of the enantiomers of oxindazac in a healthy human volunteer. He had received a single oral dose of 200 mg of racemic ^{14}C-labelled oxindazac. Plasma samples were analysed for total ^{14}C-labelled compounds and the (+)- and (−)-enantiomers (Fig. 3). The two optical isomers were determined in individual urine fractions and in the pooled urine (0–48 h) before and after enzymatic hydrolysis with β-glucuronidase (Table III). The results show stereoselective disposition of oxindazac in man; the underlying mechanism is still being unexplored.

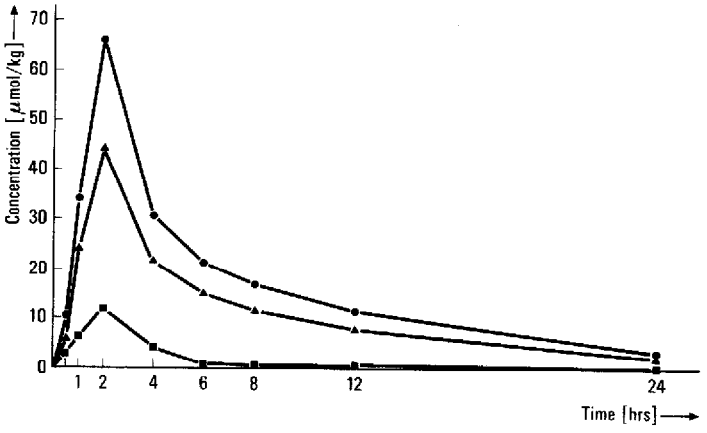


Fig. 3. Plasma concentrations of total ^{14}C-labelled compounds (●) and of the (+)- (■) and (−)-enantiomers (▲) of oxindazac after a single oral dose (200 mg) of ^{14}C-labelled drug to a healthy volunteer.

TABLE III

EXCRETION OF THE (+)- AND (−)-ENANTIOMERS OF OXINDAZAC IN URINE AFTER A SINGLE ORAL DOSE OF 200 mg OF ^{14}C-LABELLED DRUG TO A HEALTHY VOLUNTEER

Results are expressed as % of urinary ^{14}C.

Treatment of urine	*Compound determined*	*% of urinary ^{14}C in individual urine fractions*				
		0–6 h	*6–12 h*	*12–24 h*	*24–48 h*	*0–48 h (Pool)*
Untreated	(+)-Enantiomer	7.2	−*	–	–	7.7
	(−)-Enantiomer	5.4	–	–	–	5.9
	Total	12.6				13.6
Hydrolysed	(+)-Enantiomer	35.2	28.1	34.3	25.9	30.7
	(−)-Enantiomer	28.7	22.3	28.0	22.6	28.1
	Total	63.9	50.4	62.3	48.5	58.8

* Not determined.

The plasma concentrations of total oxindazac, calculated by summing the values for the two isomers, fit well the concentration patterns obtained in an earlier study in man[1]. In the latter case, oxindazac was determined by a non-stereospecific gas–liquid chromatographic method.

CONCLUSION

By direct resolution of enantiomeric esters on triacetylcellulose, it is possible to isolate the optically pure forms of racemic oxindazac on a preparative scale. For analytical purposes, this chromatographic resolution is used together with the isotope dilution technique. The method is suitable for stereospecific measurement of the enantiomers of oxindazac in body fluids.

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REFERENCES

1 P. H. Degen and W. Schneider, *J. Chromatogr.*, 277 (1983) 361.
2 R. F. N. Mills, S. S. Adams, E. E. Cliffe, W. Dickinson and J. S. Nicholson, *Xenobiotica*, 3 (1973) 589.
3 C. H. Cashin, W. Dawson and E. A. Kitchen, *J. Pharm. Pharmacol.*, 29 (1977) 330.
4 S. Naruto, Y. Tanaka, R. Hayashi and A. Terada, *Chem. Pharm. Bull.*, 32 (1984) 258.
5 S. Tanayama, E. Tsuchida and Z. Suzuoki, *Xenobiotica*, 3 (1973) 643.
6 S. Tamura, S. Kuzuna and K. Kawai, *J. Pharm. Pharmacol.*, 33 (1981) 29.
7 A. J. Hutt and J. Caldwell, *J. Pharm. Pharmacol.*, 35 (1983) 693.

8 D. W. Armstrong, *J. Liq. Chromatogr.*, 7, Suppl. 2 (1984) 353.
9 S. Allenmark, *J. Biochem. Biophys. Methods*, 9 (1984) 1.
10 I. W. Wainer and T. D. Doyle, *LC, Liq. Chromatogr. HPLC Mag.*, 2 (1984) 88.
11 P. H. Pirkle and J. Finn, in J. Morrison (Editor), *Asymmetric Synthesis*, Vol. 1, Academic Press, New York, 1983, p. 87.
12 G. Blaschke, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 13.
13 G. Hesse and R. Hagel, *Justus Liebigs Ann. Chem.*, (1976) 996.
14 K. Schloegl and M. Widhalm, *Chem. Ber.*, 115 (1982) 3042.
15 K. Schloegl and M. Widhalm, *Monatsh. Chem.*, 115 (1984) 1113, and refs. cited therein.
16 G. Blaschke and H. Markgraf, *Arch. Pharm. (Weinheim Ger.)*, 317 (1984) 465.
17 H. Koller, K. H. Rimböck and A. Mannschrek, *J. Chromatogr.*, 282 (1983) 89.
18 G. Hesse and R. Hagel, *Chromatographia*, 6 (1973) 277.
19 H. M. Fales, T. M. Jaouni and J. F. Babashak, *Anal. Chem.*, 45 (1973) 2302.
20 H. Stierlin, J. W. Faigle, W. Küng and W. Theobald, in preparation.
21 W. Dieterle and J. W. Faigle, *J. Chromatogr.*, 259 (1983) 301.