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Note

Preparative separation of diastereoisomeric 2-arylpropionic acid derivatives by centrifugal thin-layer chromatography

Comparison with preparative liquid chromatography

JEAN-MICHEL MAÎTRE, GILLES BOSS, BERNARD TESTA* and KURT HOSTETTMANN
School of Pharmacy, University of Lausanne, CH-1005 Lausanne (Switzerland)
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Metabolic studies of chiral drugs and other xenobiotics can be performed using the racemates or the isolated enantiomers. The latter can be obtained by stereospecific synthesis or by resolution. Although resolution by crystallization of diastereoisomeric salts or other derivatives is frequently used, it is time consuming and does not usually yield enantiomeric percentages much greater than 95%.

The separation of diastereoisomers by liquid chromatography (LC) provides a valuable alternative to fractional crystallization. Preparative LC is considered to be the method of choice for the gram-scale separation of stereoisomers[1], although it requires expensive equipment and large volumes of solvents. In this study, we found that centrifugal thin-layer chromatography[2] (CTLC) allows the rapid separation of diastereoisomers on the decigram scale with good yields and minimal solvent consumption. To the best of our knowledge, this is the first time that this technique has been used for the preparative separation of stereoisomers.

The compounds investigated were chiral 2-arylpropionic acids, including ibuprofen and cicloprofen, which are non-steroidal antiinflammatory agents. The resolution of these drugs is of biomedical interest as they display enantioselective activity[3] and metabolism[4].

EXPERIMENTAL

Chemicals

The structures of the 2-arylpropionic acids investigated are shown in Fig. 1. 2-(2-Naphthyl)propionic acid (III) and 2-(4-biphenyl)propionic acid (V) have been described previously[5]. Racemic ibuprofen and cicloprofen were generous gifts from Boots (Nottingham, U.K.) and Squibb (Princeton, NJ, U.S.A.), respectively.

The (−)-(*S*)-1-phenylethylamides of these acids were synthesized by reaction of the acyl chloride with the amine in chloroform or toluene (for ibuprofen)[6,7]. The amides were recrystallized from *n*-hexane–ethanol (95:5) or methanol (for the cicloprofen amide). The NMR spectra confirmed the identities and purities of the compounds. However, the amides of III and V were contaminated with *ca.* 10% of the phenylethylamide of 2-naphthylacetic acid and 4-biphenylacetic acid, respectively.

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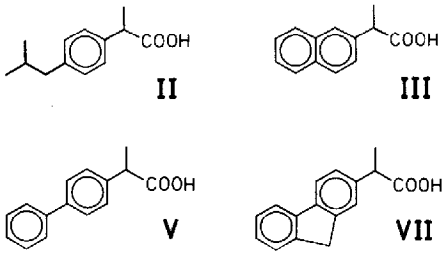

Fig. 1. 2-Arylpropionic acids investigated: (II) ibuprofen: (III) 2-(2-naphthyl)propionic acid; (V) 2-(4-biphenyl)propionic acid; (VII) cicloprofen. The numbering of compounds is taken from a previous study[5].

The chromatographic solvents (Merck, Darmstadt, F.R.G.) were of analytical-reagent grade for centrifugal TLC and of purum grade for preparative LC.

Centrifugal TLC

A Chromatotron Model 7924 (Harrison Research, Palo Alto, CA, U.S.A.) equipped with an FMI Model RPG 150 pump was used. The silica layer was 2 mm thick and was prepared from a mixture of 60 g of silica gel 60 GF_{254}, 8 g of calcium sulphate hemihydrate and 120 ml of water. The plates were dried at 70°C for 12 h, then rinsed and stabilized with the eluent (chloroform–cyclohexane–tetrahydrofuran, 54.2:45:0.8, v/v). The mixture to be separated (0.100 g) was dissolved in 1.5 ml of the eluent. The flow-rate of the eluent was maintained at 3.5 ml/min and the separation on the plates was monitored with a UV lamp. Samples (4–6 ml) were collected and directly analysed by analytical high-performance liquid chromatography (HPLC) as described previously[5].

Preparative LC

A Jobin-Yvon Chromatospac instrument equipped with an LKB 2238 Uvicord SII UV detector operating at 254 nm and an LKB 2210 two-channel recorder was used. The column (500 mm × 40 mm I.D.) was freshly prepared before each separation using silica H60 for TLC (15 μm) (Merck). The eluent was cyclohexane–isopropanol (97:3, v/v). The flow-rate was 23 ml/min corresponding to a pressure of 9 bar. Samples to be analysed (1.0–1.2 g) were dissolved in 10 ml of the eluent. Fractions of various volumes were collected and directly analysed by analytical HPLC[5].

RESULTS

Preliminary studies

Preliminary studies were necessary in order to choose suitable eluents for both centrifugal TLC and preparative LC. (a) The compounds to be separated must have good solubility in the eluent system, typically 1 part or more in 10 parts. (b) TLC (employing the same or similar stationary phases) must be used to optimize the eluent composition. The migration of the solutes must lie within the range $0.1 < R_F < 0.3$, with $\Delta R_F > 0.05$. Thus, on TLC plates made with 60 F_{254} silica, the following ΔR_F values were obtained with chloroform–cyclohexane–tetrahydrofuran (54.2:45:0.8) eluent: amides of 2-(2-naphthyl)propionic acid, 0.05; amides of

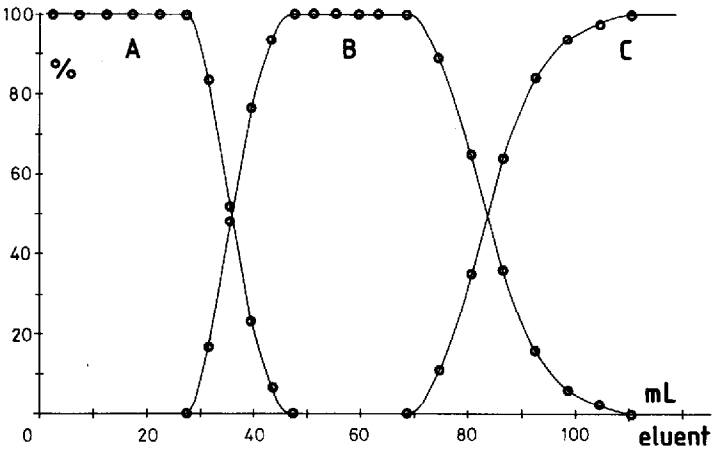

Fig. 2. Centrifugal TLC separation of the (*S*)-1-phenylethylamides of (*R*)- and (*S*)-2-(2-naphthyl)propionic acid and 2-naphthylacetic acid (A, B and C, respectively). The results are expressed in terms of the percentage composition (A + B + C = 100%) in each 4–6 ml fraction collected. The origin of the abcissa (0 ml of eluent) is arbitrarily fixed at the appearance of A.

2-(4-biphenyl)propionic acid, 0.07; and amides of cicloprofen, 0.06. For the amides of ibuprofen in the cyclohexane–isopropanol (97:3) eluent, the ΔR_F value was 0.08. The final R_F values in this study were between 0.12 and 0.25.

Centrifugal TLC

In a single passage (70–90 min, 250–300 ml of eluent), the Chromatotron allowed a good separation of diastereoisomeric phenylethylamides. When the mixture contained the amide of the arylacetic acid as an impurity, the latter eluted last and was also well separated. Fig. 2 exemplifies a typical separation obtained with 2-(2-naphthyl)propionic acid (A and B) contaminated with 2-naphthylacetic acid.

For the three arylpropionamides investigated, *ca.* 80% of the starting material

TABLE I

TYPICAL YIELDS AND STEREOISOMERIC PERCENTAGES OF THE DIASTEREOISOMERIC (−)-(*S*)-1-PHENYLETHYLAMIDES OF 2-ARYLPROPIONIC ACIDS SEPARATED BY CENTRIFUGAL TLC

Sample: 100 mg.

(−)-(S)-1-Phenylethylamide	*Yield (mg)*	*Stereoisomeric percentage*
(*R*)-2-(2-Naphthyl)propionic acid	40	99.8
(*S*)-2-(2-Naphthyl)propionic acid	35	99.0
(*R*)-2-(4-Biphenyl)propionic acid	40	99.4
(*S*)-2-(4-Biphenyl)propionic acid	40	98.9
(*R*)-Cicloprofen	41	99.8
(*S*)-Cicloprofen	38	99.1

TABLE II

TYPICAL YIELDS AND STEREOISOMERIC PERCENTAGES OF THE DIASTEREOISOMERIC (−)-(*S*)-1-PHENYLETHYLAMIDES OF IBUPROFEN SEPARATED BY PREPARATIVE LC

Sample: 1200 mg.

Run	*Parameter*	*Amide*	
		(R)-Ibuprofen	*(S)-Ibuprofen*
First	Yield (mg)	400	250
	Stereoisomeric percentage	99.8	99.5
Second	Yield (mg)	150	80
	Stereoisomeric percentage	99.2	98.4

was consistently recovered after separation, each fraction being contaminated with 1% or less of its diastereoisomer. Typical results are presented in Table I.

Preparative LC

The 1-phenylethylamides of ibuprofen were separated by preparative LC. Samples of 1–1.2 g were used. After a first run (*ca.* 60 min, and 1 l of eluent), *ca.* 50% of the sample was recovered as two pure fractions. The impure fractions were pooled and recycled (*ca.* 60 min, 0.8 l of eluent), yielding two pure fractions amounting to *ca.* 40% of the recycled material. Results of a typical experiment are given in Table II.

DISCUSSION

The two chromatographic methods have their advantages and disadvantages. In this study, the resolving power of preparative LC appears to be inferior to that of centrifugal TLC, although we did not compare the same pairs of diastereoisomers. The amides of ibuprofen showed the best separation in the preliminary TLC analyses; nevertheless, their separation by preparative LC was only partial and necessitated a second cycle in order to achieve yields (75–80%) comparable to those obtained with centrifugal TLC.

The volumes of solvent needed to separate 1 g of starting material are comparable for the two techniques, namely 2–3 l. However, preparative LC consumes much larger amounts of stationary phase.

It therefore appears that centrifugal TLC is a rapid, efficient and cost-effective technique for the decigram-scale separation of diastereoisomers. For gram-scale separations, preparative LC has advantages.

The separated diastereoisomers have to be hydrolysed if the purpose of the work is to obtain pure enantiomers of 2-arylpropionic acids. Acid hydrolysis (*e.g.*, with sulphuric acid–dioxane) is time-consuming and always leads to racemization[8]. For example, we found the racemization to be moderate with cicloprofen, but complete with of ibuprofen. The method of choice, which destroys the amine but leaves the acid enantiomerically pure, is diazotation with N_2O_4 followed by cleavage of the amide bond[9–12].

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