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Note

Formation of diastereomeric derivatives of 2-arylpropionic acids using L-leucinamide

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To investigate the stereoselective drug disposition of chiral 2-arylpropionic acids, α-methylbenzylamine is mostly used as an optically active coupling component following activation of the carboxylic moiety with thionyl chloride or 1,1'-carbonyldiimidazole [1–3]. Other reagents have seen described (e.g. refs. 4–6), of which the most important seems to be *S*-(−)-1-(naphthen-1-yl)ethylamine, which was applied for the separation of several non-steroidal antiinflammatory drugs (NSAIDs) from the group of 2-arylpropionic acids by Hutt and co-workers [5,6].

In 1985 Björkman [7] described the formation of diastereomeric derivatives of indoprofen and in 1987 [8] of ketoprofen following formation of the mixed anhydride with a chloroformate and further reaction with the amino acid derivative L-leucinamide, a reaction that is known from protein synthesis. The general reaction scheme is given in Fig. 1. As the proposed procedure seemed to be easy to perform and leads to derivatives with suitable chromatographic properties on reversed-phase systems, the applicability of the procedure was investigated for a series of compounds from the group of 2-arylpropionic acids (Fig. 2). In the present paper the chromatographic behaviour of these derivatives is described. Furthermore, the method was applied to biological material for some of the investigated compounds.

EXPERIMENTAL

Chemicals and reagents

R/S-Carprofen was obtained from Hoffmann-La Roche (Basle, Switzerland), *R/S*-pirprofen from Brunnengräber (Lübeck, F.R.G.), *R/S*-benoxaprofen from

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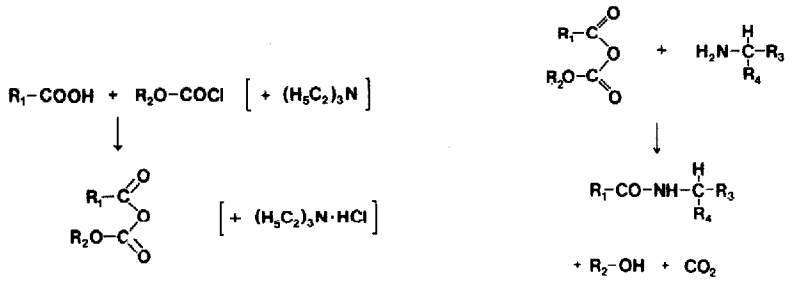

Fig. 1. Reaction scheme characterizing the derivatization of 2-arylpropionic acids (R_1-COOH) with a chloroformate (in this case R_2-=-CH_2CH_3) and further reaction of the mixed anhydride with an amine (which is the amino acid derivative L-leucinamide, R_3-=-CO-NH_2, R_4-=-$CH_2CH(CH_3)_2$) forming the diastereomeric reaction product (amide).

Eli Lilly (Bad Homburg, F.R.G.) and *R/S*-indoprofen from Farmitalia Carlo Erba (Freiburg, F.R.G.) before they were withdrawn from the market, *S*-naproxen from Grünenthal (Stolberg, F.R.G.), *R*-naproxen from Syntex (Palo Alto, CA, U.S.A.), *R/S*-cicloprofen from Squibb (Princeton, NY, U.S.A.), *R/S*-flurbiprofen from Thomae (Biberach, F.R.G.) and *R/S*-, *R*- and *S*-flunoxaprofen from Ravizza (Muggio, Italy). Carprofen enantiomers were prepared according to ref. 9, and benoxaprofen enantiomers according to ref. 10. The enantiomeric purities *e* were 0.88 for *S*-carprofen, 0.97 for *R*-carprofen, 0.93 for *S*-benoxaprofen and 0.94 for *R*-benoxaprofen. Those for *S*- and *R*-flunoxaprofen were 0.93 and 0.92, respectively.

Ethyl chloroformate, triethylamine and L-leucinamide were purchased from Fluka (Buchs, Switzerland). All solvents were analytical grade and from E. Merck

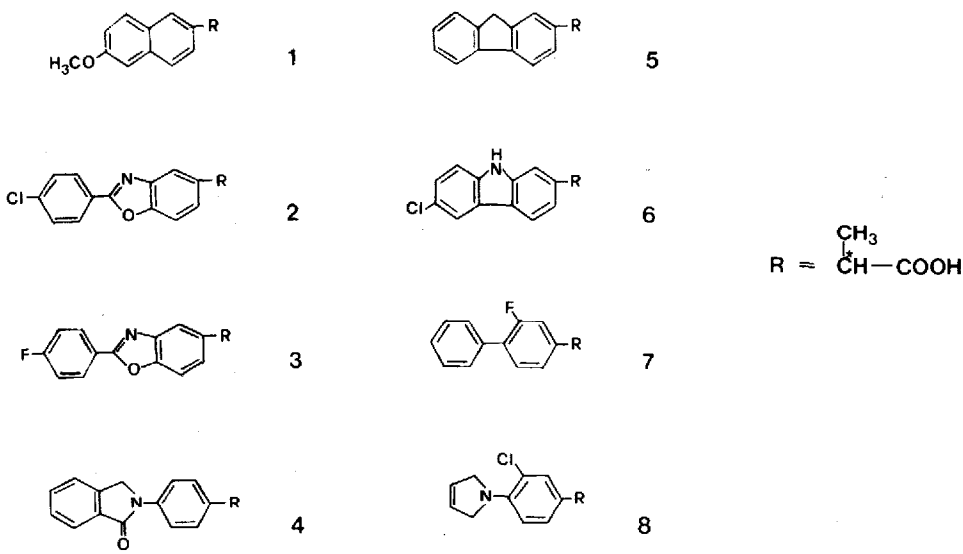

Fig. 2. Chemical structures of the investigated 2-arylpropionic acids. 1 = Naproxen; 2 = benoxaprofen; 3 = flunoxaprofen; 4 = indoprofen; 5 = cicloprofen; 6 = carprofen; 7 = flurbiprofen; 8 = pirprofen.

(Darmstadt, F.R.G.). Acetonitrile was dried over a molecular sieve (4 Å) before use in the preparation of the reagent solutions.

Equipment

Separations were performed on a Zorbax ODS column (DuPont, Wilmington, DE, U.S.A.; 25×0.46 cm I.D., 5 μm particle size). The high-performance liquid chromatographic (HPLC) system consisted of a Knauer HPLC pump, a Rheodyne injection valve, a Knauer variable-wavelength UV spectrophotometer and a Knauer recorder.

Chromatographic conditions

The stationary phase was octadecylsilane (ODS), the mobile phase acetonitrile–10 m*M* phosphate buffer (pH 6.5) (55:45, v/v), the flow-rate 1.2 ml/min and the temperature ambient. The UV detection wavelengths were: benoxaprofen and flunoxaprofen, 309 nm; carprofen and indoprofen, 280 nm; all other compounds, 254 nm. A considerable improvement in sensitivity (for assaying biological samples) can be achieved by measuring the fluorescence (if possible) instead of the UV absorbance.

Derivatization of pure reference compounds

To 5 μg of racemic drug, 100 μl of a solution of triethylamine in dried acetonitrile (50 m*M*) were added, and the tube was vortexed briefly. Then 50 μl of a solution of ethyl chloroformate in dried acetonitrile (60 m*M*) were added and after 2 min 50 μl of a solution of L-leucinamide (1 *M*) and triethylamine (1 *M*) in methanol were added. After 3 min the reaction was stopped with 0.2 ml of 0.25 *M* hydrochloric acid. The products were extracted with 5 ml of ethyl acetate. After evaporation of the solvent, the residue was dissolved in 200 μl of methanol, and 3 μl were injected.

Biological samples

Plasma and urine samples after oral administration of racemic carprofen were investigated. The samples had been adjusted to pH 3 to prevent hydrolysis of the ester glucuronides and acyl migration. Carprofen enantiomers were extracted from plasma (0.2 ml) at pH 5 with dichloromethane–diethyl ether (1:4, v/v) (5 ml). In urine, alkaline hydrolysis (using 1 *M* sodium hydroxide) was performed prior to the extraction at pH 5. After evaporation of the dichloromethane–diethyl ether, 50 μl of toluene were added and evaporated to remove traces of water. Then the derivatization was carried out as described above.

Reproducibility

The coefficients of variation (C.V.) were determined for derivatization of the pure compounds and after extraction from biological material in the cases of carprofen and flunoxaprofen. Furthermore, the addition of an internal standard (*S*-naproxen) and its influence on the C.V. were tested.

HPLC resolution

The enantiomeric resolution was characterized by calculating the separation and resolution factors, α and R, respectively. Peak parameters were calculated as follows: capacity, $k' = (t_p - t_0)t_0$; separation factor, $\alpha = k'_2/k'_1$; resolution, $R = 2(t_2 - t_1)/(w_2 - w_1)$; where t_0 = retention time of the unretained compound; t_p = retention time of peak (t_1 for peak 1, t_2 for peak 2); $w_{1,2}$ = width of peaks 1 and 2 (min).

RESULTS AND DISCUSSION

The method turned out to be easy to perform, usually leading to very good separations of the diastereomeric products. The separation and resolution factors are summarized in Table I for the given chromatographic separation system. An example of the resolution is shown in Fig. 3. In all cases the time required for one analysis was less than 20 min. For carprofen, naproxen, benoxaprofen and flunoxaprofen the enantiomers were investigated separately, and it was shown that the derivative of the *R*-enantiomer was eluted earlier than the one of the *S*-enantiomer from a reversed-phase column.

Investigation of the C.V. confirmed that an internal standard is always useful. For carprofen and flunoxaprofen, for example, a suitable internal standard is *S*-naproxen. Using the internal standard, the C.V. could be reduced from 15 to 8% for flunoxaprofen and from 16 to 7% for carprofen. These values are the means of the values calculated for the *R*- and the *S*-enantiomers.

Application of the method to human plasma and urine samples (after oral administration of racemic carprofen) showed it to be suitable for the enantiospecific assay of this drug in biological material. The concentrations found in plasma were higher for the *S*-enantiomer, and the amount excreted in urine was slightly higher for *S* than for *R*.

In summary, the procedure that was proposed by Björkman [7] for the quantification of indoprofen enantiomers is applicable for almost all the tested 2-arylpropionic acids. One of the major advantages of the method is the high enantiomeric stability and enantiomeric purity of the chiral coupling component.

TABLE I

HPLC RESOLUTION OF L-LEUCINAMIDE DERIVATIVES OF SEVERAL 2-ARYLPROPIONIC ACIDS

Compound	k'_1	k'_2	α	R
Benoxaprofen	14.8	15.1	1.02	0.45
Carprofen	9.0	10.5	1.17	3.89
Cicloprofen	8.8	10.0	1.14	2.82
Flunoxaprofen	6.6	7.2	1.09	2.18
Flurbiprofen	8.5	10.0	1.18	3.26
Indoprofen	2.2	3.0	1.36	3.10
Naproxen	4.2	5.3	1.26	1.16
Pirprofen	7.2	8.2	1.13	3.60

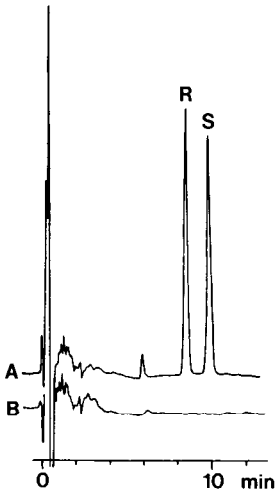

Fig. 3. HPLC resolution of the L-leucinamide derivatives of *R*- and *S*-flurbiprofen: (A) 37.5 ng per peak, (B) blank; produced by mixing the reagents without addition of a 2-arylpropionic acid.

The enantiomeric purity *e* of L-leucinamide was determined to be > 0.98, i.e., an impurity could hardly be detected. Using the chromatographic conditions described in Experimental, the peaks of the diastereomeric products were almost symmetrical and baseline separation was always obtained, the resolution factors being higher than 1.1, except for benoxaprofen, where a separation sufficient for the assay of enantiomers was not achieved. Interestingly, flunoxaprofen showed completely different behaviour on the reversed-phase column, although it differs from benoxaprofen in one substituent only. For benoxaprofen, however, very good resolution occurred on a silica gel column with dichloromethane–methanol as mobile phase. A method for the determination of benoxaprofen enantiomers in biological material from humans and rats using L-leucinamide as the coupling component, as well as a method for the quantification of carprofen enantiomers will be described in detail elsewhere [9,11]. With few exceptions the separation system described in Experimental could be used for the assay of biological samples, without or with minor modification.

Thus the general applicability of the proposed method is confirmed. From all these results it can be concluded that the method is a possible and significant alternative to the usually applied chiral derivatization with α-methylbenzylamine. The quality of resolution of the diastereomeric derivatives compares well with that obtained by Hutt et al. [5], who proposed *S*-(−)-1-(naphthen-1-yl)ethylamine as coupling component.

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