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COMMUNICATIONS

Enantiomer resolution of nefopam hydrochloride, a novel analgesic: a study by liquid chromatography and circular dichroism spectroscopy

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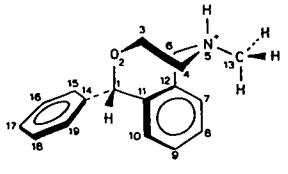
Abstract—Nefopam, a potent analgesic, has been completely resolved into enantiomers on a preparative scale by low-pressure liquid chromatography on swollen, microcrystalline triacetylcellulose. The enantiomerically pure hydrochlorides were prepared from the base, and the circular dichroism spectra of the free base and the hydrochloride are reported.

Nefopam hydrochloride (5-methyl-1-phenyl-3,4,5,6-tetrahydro-1*H*-2,5-benzoxazocine hydrochloride, I) (Klohs et al 1972) is an established potent analgesic, pharmacologically distinct from the opioid narcotics and non-steroidal anti-inflammatory drugs (Heel et al 1980), and is prescribed as the racemate (±-I). Although resolution into its enantiomers ((+)- and (−)-I × H_2O) has been reported using dibenzoyltartaric acid, by Bolt et al (1974), the enantiomeric excess and purity of the resolved samples have not been determined. There is evidence that the enatiomers differ in metabolic behaviour, as analgesics, and, circumstantially, as antidepressant agents (Bolt et al 1974; Hole et al 1984; Smith 1986; Fasmer et al 1987; Lingjaerde personal communication). As measured by [^{14}C]5-HT uptake inhibition in human blood platelets, the (+)-I enantiomer is 7–32 times more potent than the (−)-I enantiomer, both in protein-free medium and in diluted platelet-rich plasma (Lingjaerde personal communication; Smith 1986). (+)-I is also the more potent analgesic after both systemic and central administration (Vonvoightlander et al 1983; Fasmer et al 1987).



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The advent of new preparative chromatographic techniques for enantiomer resolution prompted the present investigation, with a view to developing a simple preparative method for resolving (±)-I into its enantiomers, with enantiomeric excess = 100%.

For this purpose, medium pressure liquid chromatography on swollen microcrystalline triacetylcellulose (TAC, Mannschreck et al 1985 and references therein) was investigated. Exploratory experiments with the racemic hydrochloride showed that while injection of an ethanolic solution of the hydrochloride into the TAC system did not give satisfactory resolution, an ethanol solution of the racemic free base gave excellent resolution and was chosen for the preparative resolution.

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Methods

The racemic hydrochloride was treated with an excess of 5% aqueous sodium bicarbonate solution, and the liberated non-crystalline base was taken up in diethyl ether. After drying and evaporation of the mixture, the resulting oil was dissolved in 96% ethanol to a concentration of ca 100 mg/15 mL. This solution was injected, 15 mL for each run, into the TAC system which consisted of two 40 × 2·5 cm columns in series, packed with microcrystalline triacetylcellulose, 25–40 μm, and with a 15 mL loop for the sample injection. The eluate was passed through a UV and a polarimeter detector, and the outputs from these were fed to a two-channel recorder (see Fig. 1). Further details on the instrumentation are given by Isaksson & Roschester (1985).

Ultraviolet spectra were recorded with 96% ethanol as solvent.

Circular dichroism spectra were recorded with a JASCO Model J-500A spectropolarimeter. The spectra of the enantiomers of the free base were obtained directly on the eluates from the TAC column, and the concentrations were estimated spectrophotometrically from the absorbance at 258 nm.

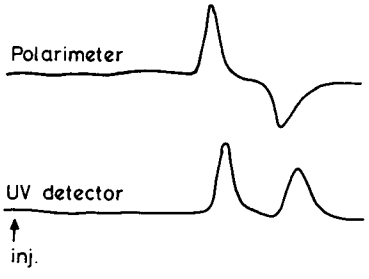

FIG. 1. Chromatogram from TAC resolution of (±)-nefopam in 96% ethanol.

Results

Fig. 1 shows that complete baseline separation was achieved in one passage of the columns: data describing the resolution are given in Table 1. If an ethanol solution of the racemate was allowed to stand for several days, peaks due to small amounts of other chiral material appeared on the chromatogram.

To obtain the enantiomers of the hydrochloride, the respective ethanolic eluates were evaporated, the residues were dissolved in dichloromethane, and the solutions were shaken twice with water, dried with anhydrous magnesium sulphate, and evaporated. The residues were dissolved in dry diethyl ether, and dry HCl gas was bubbled through the solutions until no more precipitates were formed. The hydrochlorides were obtained as colourless microcrystals in quantitative yield. The (+)-base (E_1) gave the (+)-hydrochloride.

Table 1. Separation data.

$k'_+ = [(V)_+ - V_0]/V_0 = 0{\cdot}55$ (1*S* enantiomer)
$k'_- = 1{\cdot}04$ (1*R* enantiomer)
$\alpha = k'_-/k'_+ = 1{\cdot}89$
R_s[a] $= (t_2 - t_1)/[0{\cdot}5(tw_1 + tw_2)] = 1{\cdot}46$

[a] t_1 and t_2 are the peak retention times, tw_1 and tw_2 the base widths of the respective peaks.

CD spectra of the free base enantiomers are virtually mirror images (Fig. 2), substantiating the completeness of the chromatographic resolution. The spectra in the region 200–300 nm consist of two bands with the same sign, corresponding to the ^1L_a and ^1L_b transitions in the two benzene chromophores (Platt 1949). The (+)-hydrochloride in 0·14 M ethanolic hydrogen chloride gives a similar spectrum (Fig. 3), although with ca 65% higher intensity and slight wavelength shifts (Table 2).

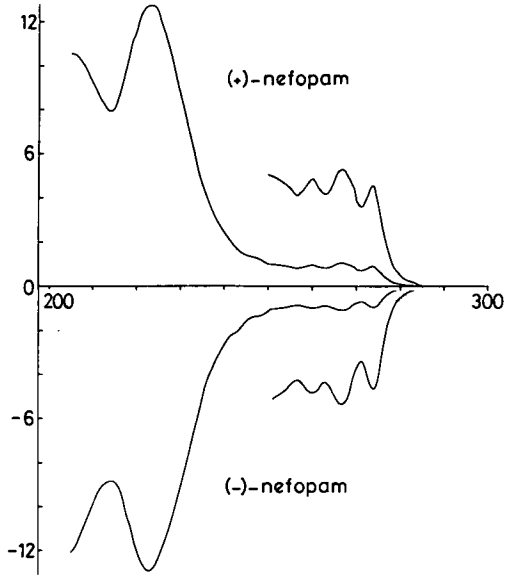

FIG. 2. CD spectra (λ nm, Δε) of (+)- and (−)-nefopam in 96% ethanol. Expansion ×5.

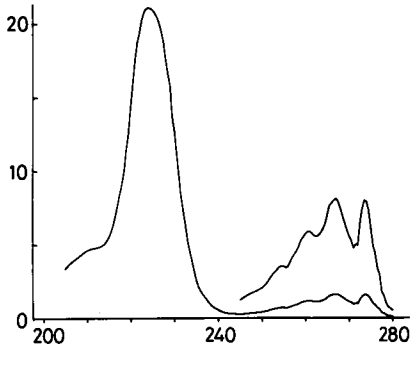

FIG. 3. CD spectrum (λ nm, Δε) of (+)-nefopam in 0·14 M HCl in absolute ethanol. Expansion ×5.

Table 2. CD maxima.

Compound	Solvent	λ_{max} nm (Δε)
(+)-Nefopam	EtOH 96%	273·5 (+0·91), 266·5 (+1·06), 260 (+0·94), 223·5 (12·5)
(−)-Nefopam	EtOH 96%	273·5 (−0·92), 266·5 (−1·07), 260 (−0·94), 223·5 (−12·6)
(+)-Nefopam[a]	EtOH, abs., HCl (0·14 M)	274·5 (+1·57), 267·5 (+1·62), 261 (+1·10), 253·5 (+0·74), 225 (+20·5)

[a] This solution is stable for at least three months.

The UV spectrum of the hydrochloride displayed a distinct band with vibrational fine structure, centered at 266 nm (ε 720) and with the long-wavelength peak at 274 nm (ε 610). At shorter wavelength the absorbance increased continuously without maxima or shoulders to λ 200 nm. The spectrum of the free base was similar, but the long-wavelength band shifted to shorter wavelength, and appeared on the base of the strong absorption. The strongest vibrational peak, at 258 nm (ε 720), was used for spectrophotometric concentration estimation.

The rotational strengths of the near-UV transitions originate in interactions between the transitions in the benzene chromophores (one of toluene and one of *o*-xylene type), and in chiral perturbations of these transitions by the other ring elements. The absence of couplet character in the 224 nm band indicates that the coupling between the ^1L_a transitions is weak. (+)-I has the (1*S*, 5*S*)-configuration (Glaser et al 1986), and it follows from the above that the (+)-free base has the (1*S*)-configuration.

Samples of (+)-I and (−)-I provided by Riker Laboratories were shown by TAC medium pressure liquid chromatography to contain a chiral impurity but to be in essence enantiomerically pure.

It should be noted that 'enantiomerically pure' only refers to the stereogenic Cl. The chromatographic resolution only fixed the configuration at N5 to the extent that the ratio of the diastereomers (1*S*, 5*R*)/(1*S*, 5*S*) in (+)-I is the same as the ratio (1*R*, 5*S*)/(1*R*, 5*R*) in (−)-I. These qualifications should be borne in mind when considering the results of biological testing carried out with the resolved species of I. As to the topology of protonated nefopam at the receptor site, there are effectively two different molecules of each nefopam enantiomer being presented to the receptor at any given instant. Preliminary molecular mechanics calculations (MMP2, Allinger & Yuh 1983) on the protonated base predict a conformer, close to that found in the X-ray crystallography studies (Hansen et al 1984; Glaser et al 1986; Klüfers et al 1986), to have the lowest energy, with the analogue with quasiaxial *N*-methyl group 0·5 kcal mol^{-1} higher in energy Glaser, Frenking and Loew have carried out the first empirical force-field calculations (MOLMEC) on protonated nefopam (R. Glaser private communication).

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Distribution of 7-hydroxymethotrexate in human blood

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Abstract—We have examined the in-vitro distribution of 7-hydroxymethotrexate (7-OH-MTX), a cytotoxic metabolite of methotrexate (MTX), in human blood, and its protein binding in serum. The distribution of 7-OH-MTX (10^{-6} M) in fresh samples of whole blood was studied at 37 °C and pH 7·51 ± 0·05 (mean ± s.d.), and its protein binding was assessed by equilibrium dialysis of serum against Krebs Ringer phosphate buffer at 37 °C and pH 7·41 ± 0·07 (mean ± s.d.). 7-OH-MTX had a mean cell/plasma concentration ratio of 0·03 (range 0–0·27, n = 18). It was extensively bound in human serum, with a bound fraction of 90·4 ± 3·3% (mean ± s.d.) in healthy volunteers (n = 11), and significantly lower, 82·3 ± 4·0% (mean ± s.d.), in hypoalbuminaemic surgical patients (n = 7). The binding of 7-OH-MTX was correlated with serum albumin (HSA) concentrations ($r = 0·72$, $P < 0·0007$, n = 18). Blood distribution data support the contention that 7-OH-MTX has a small volume of distribution, and HSA appears to be mainly responsible for the high degree of its protein binding in serum.

After high-dose intravenous therapy with the antineoplastic agent methotrexate (MTX), high concentrations of the metabolite 7-hydroxymethotrexate (7-OH-MTX) are detected in the blood (Breithaupt & Kuenzlen 1982; Slørdal et al 1986a). The distribution of any drug in the body is a function of lipophilicity and binding (Øie 1986). Unlike MTX, the distribution and protein binding of which have been extensively studied, little information is available on 7-OH-MTX. We have examined 7-OH-MTX distribution in whole blood and its binding in human serum. As important serum binding proteins, such as albumin (HSA) and α_1-acid glycoprotein (AAG) are recognized to fluctuate with certain situations and diseases (Tillement et al 1978; Øie 1986), the study was carried out with samples from participants where substantial variation in these blood constituents were expected.

Materials and methods

Chemicals. 7-OH-MTX was obtained by preparative high pressure liquid chromatography (HPLC) of urine from a patient given intravenous high-dose MTX therapy. Chromatography was performed essentially according to Slørdal et al (1986b), but with fraction sampling, and further purification by HPLC using a mobile phase of distilled water, pH 6·2, and subsequent wash-out of retained substance with methanol and water (50 : 50, v/v). After freeze-drying and resuspension, the product on HPLC showed a single peak, identical with 7-OH-MTX obtained from Dr W. E. Evans, St Jude Children's Research Hospital, Memphis, TN, USA. All other reagents were of analytical grade.

Whole blood and serum. Blood was obtained by venipuncture of 18 volunteers, 10 females and 8 males, aged 25–83 years, with a mean age of 48 years. Eleven were healthy, and seven had recently undergone major surgery of the hip. For experiments using whole blood, samples were drawn in EDTA tubes (Vacutainer, Grenoble, France). Serum was prepared by leaving venous samples at room temperature (20 °C) for 1 h before centrifugation at 2000*g* for 10 min.

Distribution in whole blood in-vitro. 200 µL of a solution of 7-OH-MTX in 0·9% NaCl (saline) was added to 1·8 mL aliquots of freshly obtained EDTA blood, to a concentration of 10^{-6} M. In open polyethylene tubes, the samples were gently shaken at 37 °C in an atmosphere of air containing 5% (v/v) CO_2. Initial studies had established that the cell/plasma (C/P) distribution ratio of 7-OH-MTX did not vary with time from 20 to 120 min (data not shown). After 30 min incubation, the samples were centrifuged at 1000*g* and 37 °C for 10 min, using a bench type cooling centrifuge (Sigma 2KD, Sigma Laborzentrifugen GmbH, Osterode am Harz, FRG) fitted with a thermistor, heating device and regulator capable of maintaining the temperature inside the centrifuge bowl within ±0·5 °C of preset values. The samples were then transferred to new tubes, and pH was measured (Radiometer, Copenhagen, Denmark). Calculation of the cellular concentrations of 7-OH-MTX was based on the haematocrit (Cellokrit 2, Linson Instrument AB, Stockholm, Sweden) in the samples, the concentrations of 7-OH-MTX in reference samples (1·8 mL of EDTA plasma samples spiked with the same amount of 7-OH-MTX as the whole blood samples) and 7-OH-MTX

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