Journal of Chromatography, 179 (1979) 370–375

CHROM. 12,183

Note

Valofan diastereoisomers: their analysis by high-performance liquid chromatography and interconversion with Proxibarbal in aqueous solution

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(Received July 3rd, 1979)

The metabolism of Proxibarbal [5-allyl-5-(2-hydroxypropyl)barbituric acid, I, Fig. 1] has been examined under various experimental conditions by several workers. In organic and aqueous solutions[1,2] as well as in humans[3] and animals[2,4] the drug undergoes hydrolysis of the barbiturate ring followed by lactonization to give Valofan (α-allophanyl-α-allyl-γ-valerolactone, II, Fig. 1).

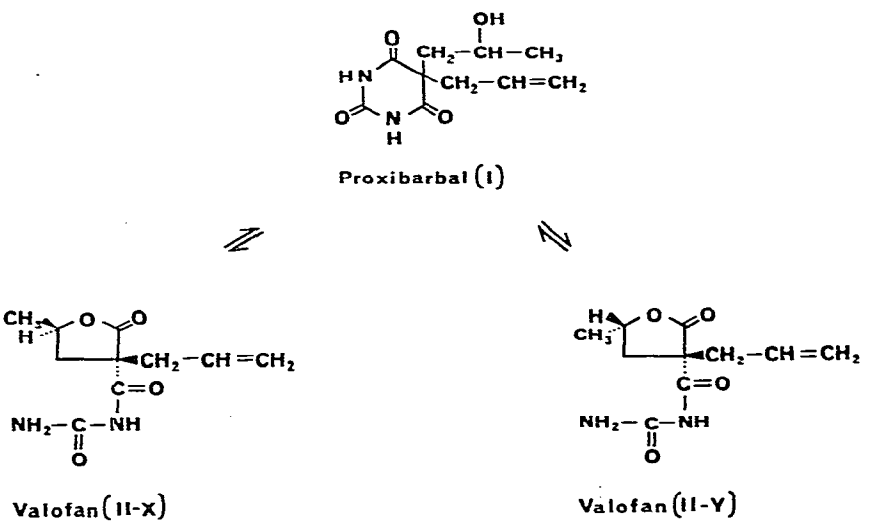

Fig. 1. Structures of the compounds investigated. Only one enantiomer of II-X and II-Y is shown.

This paper reports a high-performance liquid chromatographic (HPLC) method for the separation of Proxibarbal and the two diastereoisomeric forms of Valofan. The method has been applied to a study of the chemical interconversion of the three isomers.

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EXPERIMENTAL

A Siemens S 101 high-performance liquid chromatograph equipped with a Orlita pump (Type DMP-AE 10.4) was used. The detector was a Zeiss PM 2 DLC spectrophotometer with a measuring cell volume of 8 μl.

All reagents were of analytical-reagent grade and were purchased from E. Merck (Darmstadt, G.F.R.). Proxibarbal and Valofan were kindly supplied by Hommel (Adliswil, Switzerland).

All columns were prepared in our laboratory by a modified slurry packing method[5].

For the analytical discrimination of the three compounds a 250 mm × 6 mm O.D. × 3 mm I.D. stainless-steel column packed with LiChrosorb RP-8, 10 μm (Merck), was used with water–methanol (70:30) as the mobile phase and a detector wavelength of 200 nm. For the preparative separation of the two diastereoisomers a 500 mm × 9 mm O.D. × 6 mm I.D. stainless-steel column packed with LiChrosorb SI 60, 10 μm (Merck) was used with *n*-hexane–ethanol (96:4) as the mobile phase at a detector wavelength of 210 nm.

For the preparative separation of the two diastereoisomers, compound II was dissolved in ethanol to give a 20 m*M* solution. The injected volumes were 250 μl.

The kinetic studies were performed in a thermostated water-bath (Type 1420; Braun, Melsungen, G.F.R.) at 37 $\pm$ 0.05°. The concentration of compound I was 3 m*M* and that of compound II 2.5 m*M*. The pH of the solution was adjusted to 7.40 $\pm$ 0.05 using a 0.05 *M* triethanolamine buffer. 1,4-Di(hydroxymethyl)benzene (10 μM) was used as an internal standard.

The solution to be analysed was driven continuously by a peristaltic pump (Pharmacia P3) across a pneumatic six-channel valve (Siemens) equipped with a 10-μl loop. A complete passage through the circuit took less than 20 sec with negligible cooling of the solution.

RESULTS

Resolution and configuration of Valofan diastereoisomers

Under various experimental conditions, substance II gave two peaks in the chromatograms. These two peaks were designated as corresponding to compounds II-X and II-Y and their ratio was found to be 40:60. Valofan contains two asymmetric carbon atoms and we therefore postulated that II-X and II-Y should be diastereoisomeric forms. To confirm this, it was necessary to isolate the two peaks. Under optimized chromatographic conditions (LiChrosorb SI 60, 10 μm, see Experimental) complete separation of I, II-X and II-Y was obtained (Fig. 2).

Repeated collections of the fraction corresponding to II-X and II-Y yielded 35 mg of X and 45 mg of Y. These two compounds were analysed by NMR spectrometry at 360 MHz by Dr. Fuhrer, Ciba-Geigy Corp., Basle, Switzerland. Salient features of the 360 MHz spectra of II-Y and II-Y are given in Table I.

The NMR spectra showed II-X and II-Y to be contamined with each other to a limited extent, less than 5% in the former instance and less than 10% in the latter. The spectra further demonstrated II-X and II-Y to be devoid of other contaminants with the exception of trace amounts of the HPLC solvents. A pre-

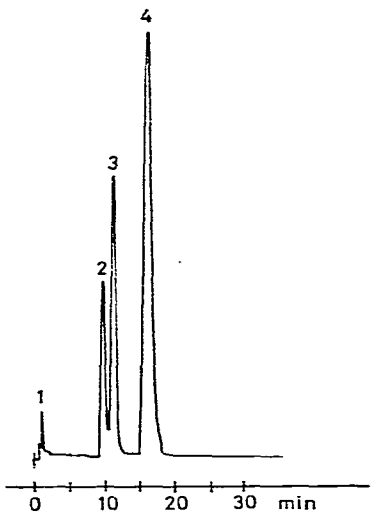
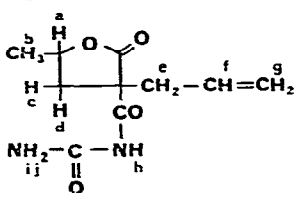


Fig. 2. Separation of I, II-X and II-Y on LiChrosorb SI 60, 10 μm. Pressure 160 bar; flow-rate, 2.8 ml/min; mobile phase, *n*-hexane–ethanol (96:4); chart speed, 120 mm/h. Peaks: 1 = solvent; 2 = II-X; 3 = II-Y; 4 = I.

liminary configurational assignment based on the chemical shifts reported in Table I indicates that II-X and II-Y are indeed diastereoisomers, with II-X being the form having an allophanyl-methyl *trans* relationship, while II-Y is the diastereoisomer with a *cis* relationship (*r*-3-allophanyl-3-allyl-*trans*-5-methyl-2-oxo- and *r*-3-allophanyl 3-allyl-*cis*-5-methyl-2-oxotetrahydrofuran, respectively).

TABLE I

CHEMICAL SHIFTS IN THE 360-NHz ^{1}H NMR SPECTRA OF VALOFAN DIASTEREOISOMERS



II-X		*II-Y*	
Atom	δ	*Atom*	δ
b	1.47	b	1.45
d	1.80	d	2.43
e	2.67	c	2.43
c	3.00	e	2.64
a	4.57	a	4.65
g	5.23	g	5.26
f	5.62	f	5.72
i	5.74	i	5.80
j	7.89	j	7.91
h	8.70	h	9.26

Reversed-phase separation of Proxibarbal and Valofan diastereoisomers

The analytical method for the separation of II-X and II-Y is not suitable for the routine analysis of the two constituents owing to rapid inactivation of the column by micro-injections of the aqueous solvents used in the kinetic study.

Therefore, a reversed-phase method was developed (see Experimental), which allowed the complete resolution of compounds I, II-X, II-Y and the internal standard, as shown in Fig. 3.

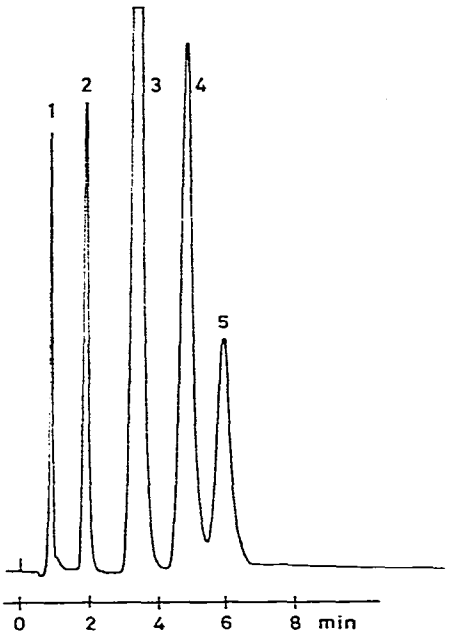


Fig. 3. The separation of I, II-X and II-Y on LiChrosorb RP 8, 10 μm. Pressure, 170 bar; flow-rate, 2 ml/min; mobile phase, water–methanol (70:30); chart speed, 600 mm/h. Peaks: 1 = buffer; 2 = internal standard; 3 = I; 4 = II-Y; 5 = II-X.

Application to kinetic studies

The suitability of the reversed phase separation for kinetic studies was demonstrated by investigating the interconversion of Proxibarbal and Valofan in aqueous solution. After dissolution of I the progressive appearance of II-X and II-Y was detected. Under physiological conditions (pH 7.40, 37°), equilibrium was reached after 3 h consisting of 12% II-Y, 6% II-X and 82% I (± 1%). Results of a typical experiment are shown in Fig. 4.

When compound II was dissolved in the buffer, a II-X to II-Y ratio of 34:66 was calculated at time zero. The progressive appearance of compound I led in 3 h to an equilibrium identical with that noted above. Results of a typical experiment are shown in Fig. 5.

CONCLUSION

The results demonstrate the value of HPLC in examining the relatively fast

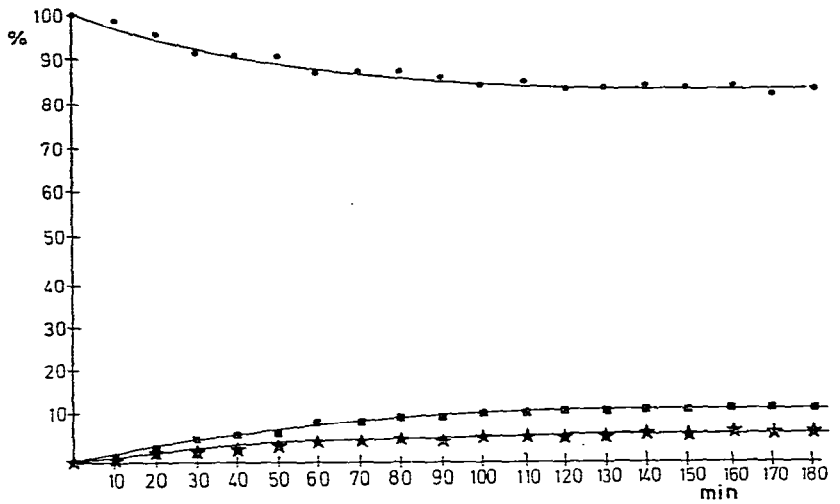


Fig. 4. Reversible isomerization of Proxibarbal to Valofan in aqueous solution at pH 7.40 and 37°. ●, I; ★, II-X; ■, II-Y.

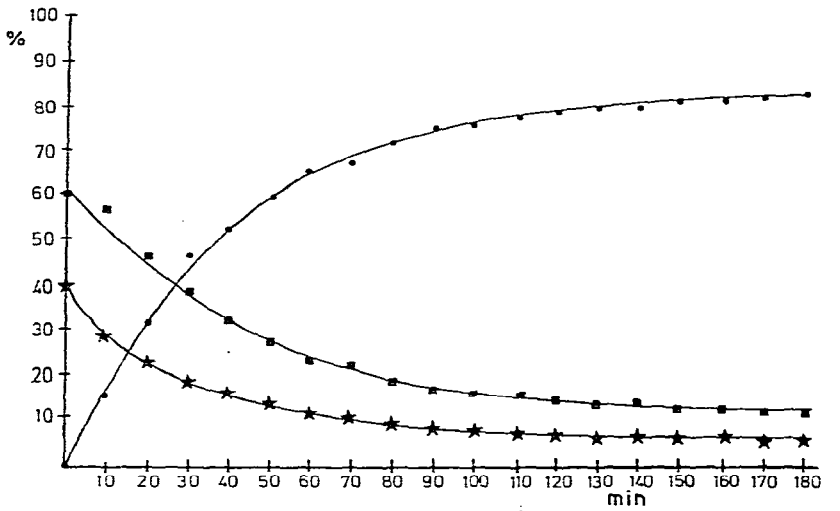


Fig. 5. Reversible isomerization of Valofan diastereoisomers to Proxibarbal in aqueous solution at pH 7.40 and 37°. Symbols as in Fig. 4.

interconversion of isomers. The method is now being applied to a detailed kinetic study to the isomerization and breakdown of Proxibarbal and Valofan.

ACKNOWLEDGEMENTS

We thank Hommel AG for the gift of compounds, and Dr. Fuhrer for the NMR spectra.

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