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Note

Application of high-performance liquid chromatographic chiral stationary phases to pharmaceutical analysis

Resolution of enantiomeric barbiturates, succinimides and related molecules on four commercially available chiral stationary phases

ZHONG-YUAN YANG*, SUSAN BARKAN, CHARLOTTE BRUNNER, JOHN D. WEBER, THOMAS D. DOYLE and IRVING W. WAINER*

Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204 (U.S.A.)

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Cyclic amides and imides are widely used in therapeutic preparations as sedatives, hypnotics and anticonvulsants. Included in these drug families are barbiturates (1–4), succinimides (5–7) and related compounds (mephenytoin, 8, and glutethimide, 9) (Fig. 1). As is the case with many other classes of therapeutic agents, the molecular structures of individual members of these families may contain one or more chiral centers, with the consequent existence of one or more enantiomeric pairs. The chiral

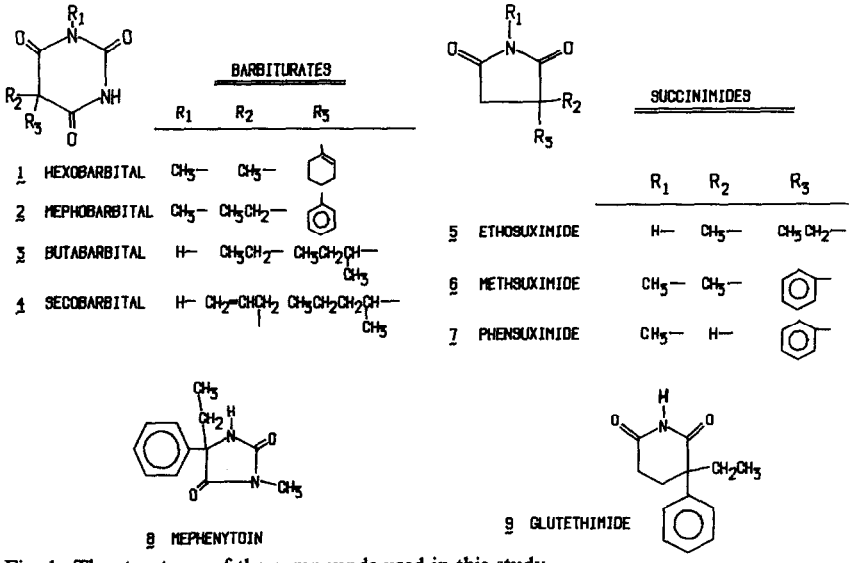

Fig. 1. The structures of the compounds used in this study.

* Food and Drug Administration International Visiting Scientist and Pan American Health Organization Fellow from the Wuhan Municipal Institute for Drug Control, People's Republic of China.

centers may be part of the ring itself (1, 2, 5–9) or part of a moiety attached to the ring (3, 4).

Enantiomers of pharmacologically active molecules often have different activities and/or metabolic pathways. The (*S*)-(+)-enantiomer of hexobarbital (1), for example, has a greater hypnotic activity than the (*R*)-(−)-isomer[1]. Similar differences have been observed in other enantiomeric barbiturates[2,3]. Metabolic differences have been observed between the enantiomers of phensuximide (7)[4], mephenytoin (8)[5,6] and glutethimide (9)[7].

The direct resolution of some of the enantiomeric members of these families has been accomplished by high-performance liquid chromatography (HPLC) on a variety of chiral stationary phases. Hexobarbital (1) was resolved by Blaschke[8] on a triacetyl cellulose chiral stationary phase and by Okamato and Shibata[9] on a poly-(triphenylmethyl)methylacrylate chiral stationary phase. The structurally related compound mephobarbital (2) was also resolved by Okamato and Shibata[9] and by Armstrong and DeMond[10]. The latter researchers used a cyclodextrin chiral stationary phase. In both of these molecules, the chiral center is part of the ring system. Enantiomeric barbiturates in which the chiral center is located in an attached alkyl moiety (3,4) have not been resolved.

Pirkle *et al.*[11] reported the resolution of aryl-substituted succinimides on an (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine chiral stationary phase. The resolutions of structurally related hydantoins[11], benzodiazepinones[12] and oxazolidones[13–16] have also been reported for this chiral stationary phase. The resolution of mephenytoin (8) has not been reported. However, glutethimide (9) and some glutethimide derivatives were resolved by Okamato and Shibata[9] on a poly(triphenylmethyl) methacrylate chiral stationary phase and by Blashke *et al.*[17] on a polyamide chiral stationary phase.

In this paper we report the chromatography of nine enantiomeric members of these pharmacologically important series of compounds on four commercially available Pirkle-type chiral stationary phases. The chiral stationary phases were the ionically and covalently bonded (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine phase (chiral stationary phase I and chiral stationary phase II, respectively) and the ionically and covalently bonded (*S*)-N-(3,5-dinitrobenzoyl)leucine phase (chiral stationary phase III and chiral stationary phase IV, respectively). The resolution factors and other chromatographic parameters for compounds 1–9 varied from one chiral stationary phase to another, but analytically useful separations were obtained on at least one chiral stationary phase for all of the pharmaceuticals investigated.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 3500 liquid chromatograph equipped with an SP 4000 data system and an SP 8200 UV detector set at 254 nm. The columns were stainless-steel (25 cm × 4.6 mm I.D.) packed with commercially available ionically and covalently bonded forms of the Pirkle-type (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine and (*S*)-N-(3,5-dinitrobenzoyl)leucine chiral stationary phases (Regis Chemical, Morton Grove, IL, U.S.A.). A Perkin-Elmer (Norwalk, CT, U.S.A.) Model 241MC spectropolarimeter was also used in the study.

Materials

The racemic pharmaceuticals were USP Reference Standards (United States Pharmacopeia, Rockville, MD, U.S.A.). The HPLC solvents were purchased from Burdick & Jackson (Muskegon, MI, U.S.A.).

Chromatographic conditions

The mobile phases consisted of various mixtures of hexane–isopropanol–acetonitrile. A flow-rate of 0.6 ml/min was maintained throughout the study. The chromatographic studies were performed at ambient temperature with UV detection at 254 nm. The solutions used in the study were prepared by dissolving 10 mg of material in 10 ml of a hexane–isopropanol (90:10) solution. Each column passed a suitability test using racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol as the test solute. A column was deemed suitable if the separation factor (α) and the resolution factor (R) of the test solute were at least 1.4 and 3, respectively.

Determination of the order of elution

The resolved enantiomers from the chromatography of racemic hexobarbital and glutethimide were individually collected and concentrated under reduced pressure and their direction of rotation (+ or −) was determined by using a microcell.

RESULTS AND DISCUSSION

The nine solutes were chromatographed on each of the four chiral stationary phases by using the same mobile phase [hexane–isopropanol (90:10)]; the results are presented in Table I. In most, but not all cases, the leucine-based chiral stationary phases (III and IV) demonstrated a higher enantiomeric selectivity than the phen-

TABLE I

RESOLUTION OF ENANTIOMERS ON FOUR CHIRAL STATIONARY PHASES (CSPs), USING A HEXANE–ISOPROPANOL (90:10) MOBILE PHASE

CSP I = ionic phenylglycine; CSP II = covalent phenylglycine; CSP III = ionic leucine; CSP IV = covalent leucine.

Compound	*CSP I*		*CSP II*		*CSP III*		*CSP IV*	
	k_1'*	α**	k_1'*	α**	k_1'*	α**	k_1'*	α**
1 Hexobarbital	5.37	1.07	3.14	1.04	4.21	1.12	4.11	1.09
2 Mephobarbital	7.51	1.00	4.89	—***	7.61	1.00	7.39	1.05
3 Butabarbital	9.74	1.00	4.20	1.00	—§	—§	7.75	1.00
4 Secobarbital	8.15	1.06	3.46	1.00	10.91	1.10	6.10	1.00
5 Ethosuximide	2.15	1.04	2.29	1.02	1.31	1.00	1.78	1.00
6 Methsuximide	4.91	1.07	4.81	1.00	2.48	1.14	3.65	1.15
7 Phensuximide	4.02	1.04	2.95	1.00	3.70	1.05	3.20	1.04
8 Mephenytoin	2.01	1.04	1.82	1.00	1.29	1.00	2.32	1.09
9 Glutethimide	9.78	1.11	4.68	1.00	4.98	1.12	3.54	1.04

* Capacity factor of the first eluted enantiomer.

** Separation factor, $\alpha = k_2'/k_1'$.

*** Second peak observed as a shoulder.

§ No peaks observed in the chromatogram.

ylglycine-based chiral stationary phases (I and II). A notable exception was methsuximide (6), for which the phenylglycine chiral stationary phase was clearly superior. In the cases of ethosuximide (5) and glutethimide (9), the results for the two chiral stationary phase types were comparable.

The selectivity also varied within the chiral stationary phase types. The separation factors (α) observed for secobarbital (4) and for glutethimide (9) were greater for the ionic form of the leucine chiral stationary phase (III) than for the covalent form (IV). The reverse was true for mephobarbital (2) and mephenytoin (8). The ionic form of the phenylglycine chiral stationary phase (I) had a consistently higher selectivity than the covalent form of the chiral stationary phase (II), for all the compounds, with the possible exception of mephobarbital.

The selectivity and efficiency of the chromatographic resolutions were optimized individually for each of the nine solutes by varying both the chiral stationary phase and the composition of the mobile phase. The results of this optimization process are presented in Table II. The ionic modification of the leucine chiral stationary phase was superior for five solutes, and the covalent leucine chiral stationary phase was superior for three solutes; a phenylglycine chiral stationary phase was selected for only one solute, methsuximide (6).

TABLE II

CHROMATOGRAPHIC CONDITIONS FOR OPTIMUM RESOLUTION OF ENANTIOMERS

Compound	*CSP**	*Mobile phase***	k'_1	α	R***
1 Hexobarbital	III	C	7.02	1.12	1.90
2 Mephobarbital	IV	A	7.39	1.05	—§
3 Butabarbital	III	B	9.46	1.05	—§
4 Secobarbital	III	D	14.09	1.09	1.31
5 Ethosuximide	I	A	2.15	1.04	0.46§§
6 Methsuximide	IV	B	2.85	1.11	1.75
7 Phensuximide	III	C	5.80	1.05	0.80§§
8 Mephenytoin	IV	B	1.96	1.06	0.69§§
9 Glutethimide	III	C	7.02	1.10	1.76

* See Table I for definitions.

** A = hexane–isopropanol (90:10); B = hexane–isopropanol–acetonitrile (89:10:1); C = hexane–isopropanol–acetonitrile (94.5:5:0.5); D = hexane–isopropanol–acetonitrile (93.1:4.9:2).

*** $R = (t_2 - t_1)/(1/2)(w_2 + w_1)$; t = retention time; w = base width.

§ Incalculable.

§§ Estimates for moderately overlapping peaks.

It was found empirically that addition of minimal amounts of acetonitrile to the mobile phase markedly enhanced the efficiency and reduced tailing. However, this improvement was accompanied by measurable loss of selectivity and rapid decrease of capacity factors. The optimum ratios of hexane:isopropanol:acetonitrile given in Table II were determined empirically.

Selected chromatograms produced by these optimum systems are presented in Fig. 2. It is noteworthy that the resolution of secobarbital was not previously achieved by HPLC on a chiral stationary phase. The site of chirality in the secobar-

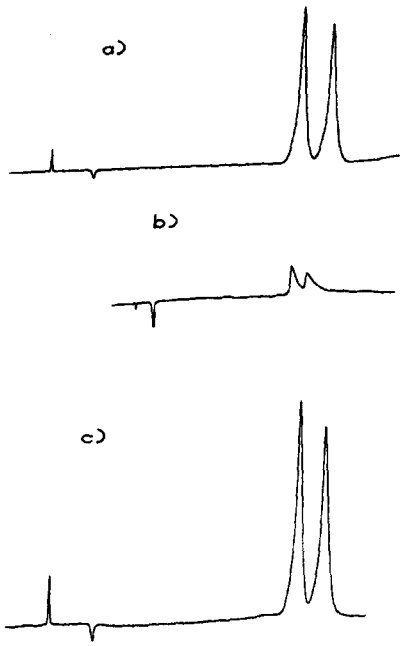

Fig. 2. Selected chromatograms: (a) hexobarbital (1), chiral stationary phase III, mobile phase C; (b) secobarbital (4), chiral stationary phase IV, mobile phase B; (c) glutethimide (9), chiral stationary phase III, mobile phase C.

bital molecule resides in the side chain and can be viewed essentially as a difference in orientation of a methyl and an *n*-propyl group. Butabartital (3) was also resolved, but with a lower separation factor; a methyl and an ethyl group are the substituents attached to the chiral carbon in the butabarbital molecule. These chromatographic results highlight the subtle stereochemical discriminations of which the Pirkle-type chiral stationary phases are capable.

Milligram amounts of the individual enantiomers of hexobarbital and glutethimide were isolated by cumulative collection of the resolved components, followed by evaporation of the mobile phase. Polarimetric measurement then established the sign of rotation for each enantiomer. Re-injections of these isolates on each of the four chiral stationary phases established the relative elution order on each column. (This process also demonstrated that the isomeric purity of the isolated materials was greater than 95%.) In this manner it was shown that the (*S*)-(−)-enantiomer of glutethimide and the (*S*)-(+)-enantiomer of hexobarbital eluted first on the (*R*)-phenylglycine chiral stationary phases (when resolution was observed). The elution order was reversed for both compounds on both modifications of the (*S*)-leucine chiral stationary phase.

CONCLUSIONS

The commercially available Pirkle-type (*R*)-phenylglycine and (*S*)-leucine chiral stationary phases have a wide applicability in the chromatographic resolution of

enantiomeric molecules. However, it is not yet possible to predict in advance which column will perform best for a given solute. This must still be determined on a case-by-case basis. It is clear, however, that the analytical potential of chiral stationary phases has been demonstrated for determination of enantiomeric identity and purity and for direct, detailed and accurate elucidation of the stereochemistry of drug substances.

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