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Direct high-performance liquid chromatographic separation of the enantiomers of methoxy and hydroxy derivatives of 3,4-dihydro-3-(dipropylamino)-2*H*-1-benzopyrans with dopaminergic activity

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Abstract

The direct HPLC resolution of the enantiomers of methoxy and hydroxy derivatives of 3,4-dihydro-3-(dipropylamino)-2*H*-1-benzopyrans and of unsubstituted amino compounds was achieved using Chiralcel OD and/or Chiralpak AD stationary phases. The position of the substituent (methoxyl or hydroxyl) in the aromatic ring has a strong effect on the enantioselectivity. Circular dichroism spectra of the single enantiomers of one compound were measured.

1. Introduction

Dopamine is an important neurotransmitter both in the central nervous system and in peripheral tissues. Malfunction of the dopaminergic system has been suggested to play a major role in diseases such as schizophrenia and parkinsonism [1]. Although treatment is available with drugs influencing the dopaminergic system, the therapy of these diseases is far from satisfactory [2].

Recently, we demonstrated that isosteric replacement of the methylene group at C-4 in the 2-aminotetralins by oxygen may play an important role in the selective recognition of the serotoninergic or dopaminergic receptors in relation to the position of the substituent (hydroxyl or methoxyl) on the aromatic ring [3,4]. The effect of the replacement of the C-4 in several mono- and dihydroxytetralins by oxygen on the dopaminergic activity has also been investigated by other groups [5,6].

An efficient synthesis of the compounds in Fig. 1 has been described [4] and its advantage lies in

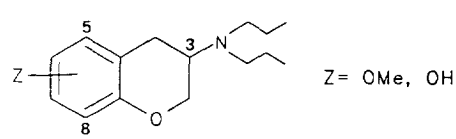

Fig. 1. Structure of compounds **1–6**, where Z = OH or OCH_3 at position 5, 6 or 8.

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the possibility of preparing both dipropylic or diallylic derivatives. From the diallylic derivatives, by enantioselective crystallization with optically pure binaphtylphosphoric acid both enantiomers of 8-hydroxy-3,4-dihydro-3-(dipropylamino)-2*H*-1-benzopyran (Z = OH) were subsequently obtained. In vitro assays of dopaminergic activity indicated highly enantioselective recognition of the D-2 sites by the (−)-enantiomer [4]. Apart from this result, the reported pharmacological evaluations refer only to racemic compounds [5,6].

This prompted us to search for a direct method of enantiomeric separation in order to obtain sizeable amounts of individual enantiomers of dipropylic or unsubstituted amino derivatives which can be submitted to pharmacological assays. Indeed, recent guidance has been given for toxicity and activity testing during the development process of chiral drugs [7].

In this paper we report the first HPLC resolution of the enantiomers of 3,4-dihydro-3-(dipropylamino)-2*H*-1-benzopyrans and of unsubstituted amino compounds.

Among various HPLC chiral stationary phases (CSPs) used by us, only polysaccharide-derived types were able to resolve the enantiomers of this class of compounds and the chiral separation depended strongly on the position of the substituent (methoxyl or hydroxyl) in the aromatic ring. The resolution factors (R_s) of some compounds were fairly good and afforded milligram separation and circular dichroism (CD) measurement of the single enantiomers.

For completeness of information, it is useful to mention the following direct methods of enantiomeric resolution of isosteric tetralin derivatives. A Chiralcel OD column was used to determine directly the enantiomeric purity of the dopamine agonist 2-(N-propyl-N-2-thienylethylamino)-5 hydroxytetralin [8]. The enantiomers of 5-hydroxy-2-(dipropylamino) tetralin were resolved by chiral ion-pair chromatography, using N-benzyloxycarbonylglycyl-L-proline as the counter ion on a carbon column [9]. The enantiomeric purity of 5,6- and 6,7-dihydroxy-2-aminotetralins was determined using an 18-crown-6 chiral crown ether coated on a silica gel column [10].

2. Experimental

2.1. Apparatus

The HPLC system consisted of a Varian Model 5060 liquid chromatograph with Valco sample loops (10 or 50 μl), a Jasco Uvidec 100-III UV spectrophotometric detector operating at 240 nm and a Varian CDS 401 data system or an Omniscribe Houston recorder for fraction collection. CD spectra were recorded on a Jasco Model 600 spectropolarimeter. The mobile phases were HPLC-grade *n*-hexane–2-propanol mixtures. The columns (25 cm × 4.6 mm I.D.) used for the experiments reported in Table 1 were packed with Chiralcel OD (cellulose tris-3,5-dimethylphenylcarbamate) and with Chiralpak AD (amylose tris-3,5-dimethylphenylcarbamate), both coated on 10-μm silica gel from Daicel (Tokyo, Japan). The columns used for some unsuccessful experiments reported in the next section were another helical phase column, Chiralpak OP(+) from Daicel, and three Pirkle phase columns, (*R*)-α-Burke 1, (*R*)-DNBPG and (*R,R*)-Whelk O-1, all from Regis Chemical (Morton Grove, IL, USA). The column void time (t_0) was measured by injection of 1,3,5-tri-*tert.*-butylbenzene as a non-retained sample. Retention times were mean values of two replicate determinations. All separations were carried out at ambient temperature.

2.2. Chemicals

The syntheses of compounds **1**, **2**, **4**, **5**, **7** and **8** have been described elsewhere [4]. They start from readily available or commercial 2-hydroxybenzaldehydes and proceed in six steps. Compounds **3**, **6** and **9** were synthesized similarly, starting from 2-hydroxy-5-methoxybenzaldehyde, as intermediate products of a unique synthetic pathway [11].

3. Results and discussion

The chromatographic results for six compounds of the general formula in Fig. 1 and for

three compounds bearing an unsubstituted NH_2 group (**7–9**) are presented in Table 1.

Among the isomeric hydroxy derivatives, two of them (**2** and **3**) were resolved on Chiralcel OD, whereas the isomeric **1** was not resolved on this phase, as reported in Table 1 and from other trials with various percentages of 2-propanol in hexane as eluent. The composition of the mobile phase was in fact critical for obtaining baseline R_s, as shown for **2** and **3**. However, the enantiomeric resolution of **1** was achieved by using a Chiralpak AD column, as shown in Fig. 2. This phase was also efficient in the resolution of **2** and **3**, although to a lesser extent by comparing the α and R_s values.

Among the isomeric methoxy derivatives, **5**

Table 1
HPLC resolution of enantiomeric compounds **1–9** on chiral stationary phases

Compound	Z[a]	R[b]	CSP	A(%)[c]	k'[d]	α	R_s
1	5-OH	$CH_2CH_2CH_3$	OD	5	1.15	NS[e]	
			AD	10	0.58	1.13	0.8
2	8-OH	$CH_2CH_2CH_3$	OD	5	1.65	1.19	1.3
			OD	10	0.99	1.15	0.8
			OD	20	0.52	1.27	0.6
			AD	10	0.67	1.09	0.8
3	6-OH	$CH_2CH_2CH_3$	OD	5	1.89	1.50	3.3
			OD	10	0.88	1.69	3.1
			OD	30	0.27	1.49	1.0
			AD	10	1.02	1.12	1.3
4	5-OCH_3	$CH_2CH_2CH_3$	OD	5	0.19	NS	
			AD	10	0.04	NS	
			AD	0	0.16	1.43	0.8
5	8-OCH_3	$CH_2CH_2CH_3$	OD	3	3.04	1.34	2.0
			OD	3[f]	2.99	1.24	1.8
			OD	5	1.60	1.26	1.7
			AD	0	0.75	NS[g]	
6	6-OCH_3	$CH_2CH_2CH_3$	OD	10	0.29	NS	
			AD	10	0.23	1.35	1.4
7	5-OCH_3	H	OD	5	4.34	1.06	0.6
			OD	5[h]	4.70	1.19	1.6
			AD	10	1.84	NS[i]	
8	8-OCH_3	H	OD	10	8.42	1.20	1.7
			OD	10[h]	7.73	1.19	1.8
			OD	20[h]	3.64	1.20	1.4
			AD	10	2.11	NS[i]	
9	6-OCH_3	H	OD	10	1.25	NS	
			OD	30	1.81	NS	
			OD	30[f]	1.62	NS	
			AD	10	2.28	1.35	5.0[j]

[a] See the general formula in Fig. 1.
[b] Substituents on the nitrogen atom.
[c] Percentage of 2-propanol in *n*-hexane at a flow-rate of 1 ml/min for Chiralcel OD (t_0 = 3.3 min) and at a flow-rate of 0.5 ml/min for Chiralpak AD (t_0 = 7.2 min), unless specified otherwise.
[d] Capacity factor of the first-eluted enantiomer.
[e] Not separated.
[f] Flow-rate 1.3 ml/min, t_0 = 2.7 min.
[g] Shoulder on the rising edge.
[h] 2-Propanol doped with 0.5% of diethylamine.
[i] Flat-topped peak.
[j] Tailed peaks.

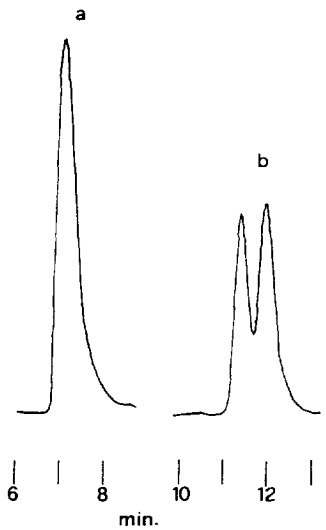

Fig. 2. HPLC behaviour of **1** on (a) Chiralcel OD with mobile phase *n*-hexane–2-propanol (95:5) at a flow-rate of 1 ml/min and (b) Chiralpak AD with mobile phase *n*-hexane–2-propanol (9:1) at a flow-rate of 0.5 ml/min.

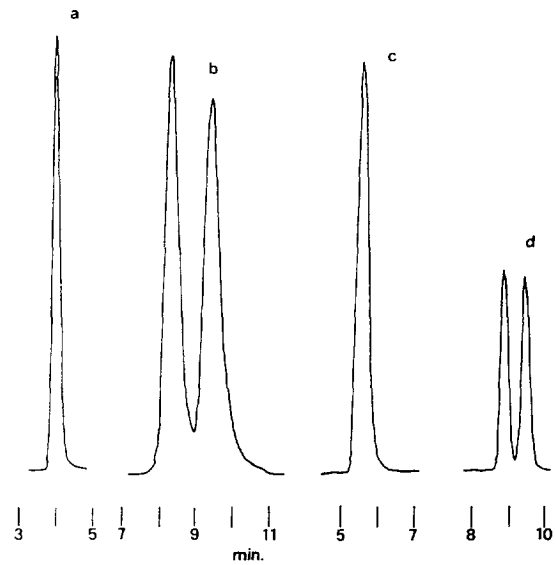

Fig. 3. HPLC separation of (a) **4**, (b) **5** and (c) **6** on Chiralcel OD with mobile phase *n*-hexane–2-propanol (95:5) at flow-rate of 1 ml/min and (d) **6** on Chiralpak AD with mobile phase *n*-hexane–2-propanol (9:1) at a flow-rate of 0.5 ml/min.

was efficiently resolved into its enantiomers by the Chiralcel OD column but the isomers **4** and **6** were not separated, as shown in Table 1 and Fig. 3. The enantiomeric resolution of the latter compounds was instead obtained using a Chiralpak AD column. The good resolution factor obtained for **5** afforded a separation of its enantiomers by repeated 50-μl injections of racemic **5** and collection of the eluates from the chromatographic peaks. The CD spectra of both eluates were measured and they were mirror images of each other, as shown in Fig. 4, indicating that the two eluates are optical isomers. Analytical HPLC re-runs on the eluates indicated an enantiomeric purity of 100% for the first peak and 95% for the second peak. Their UV spectra were also identical.

Among the isomeric methoxy derivatives of the primary amines, again we note a marked difference in the enantiomeric selectivity of the two CSPs on the same compound. Compound **9** was in fact resolved only by using a Chiralpak AD phase, as reported in Table 1 and in Fig. 5. The isomers **7** and **8** were instead better resolved by using the Chiralcel OD column. Addition of diethylamine to the mobile phase was effective in

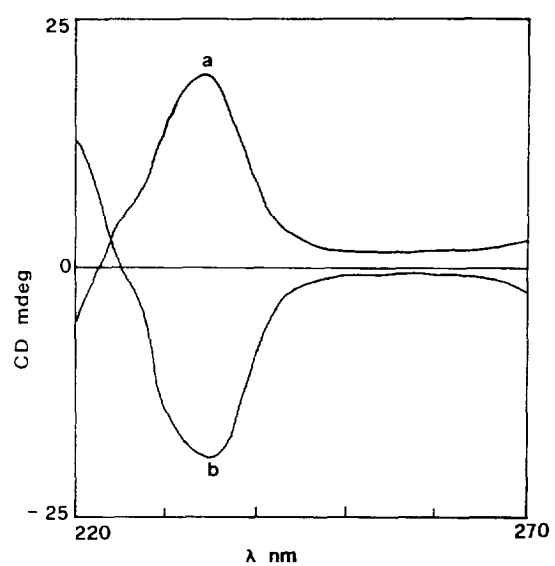

Fig. 4. CD spectra of the enantiomers of **5** obtained from (b) the first and (a) the second HPLC-eluted peaks.

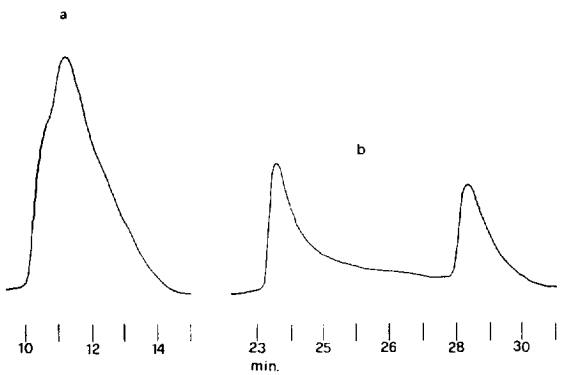

Fig. 5. HPLC behaviour of **9** with two CSPs: (a) Chiralcel OD and (b) Chiralpak AD. Mobile phase, *n*-hexane–2-propanol, (a) 7:3 at a flow-rate 1 ml/min and (b) 9:1 at a flow-rate 0.5 ml/min.

the enhancement of the enantiomeric resolution of **7–9**, as expected for primary amines. The effect is very strong for **7**, as shown in Fig. 6.

Hence very fine effects tune the interaction of the enantiomers of these derivatives of 3,4-dihydro-3-amino-2*H*-1-benzopyrans with the polar carbamate moiety of the Chiralcel OD and Chiralpak AD phases. This isomeric effect was also found in other classes of chiral compounds [12]. Also, the difference in the size of the helical cavity of the cellulose- and amylose-derived phases plays a significant role in the chiral recognition of the same compound.

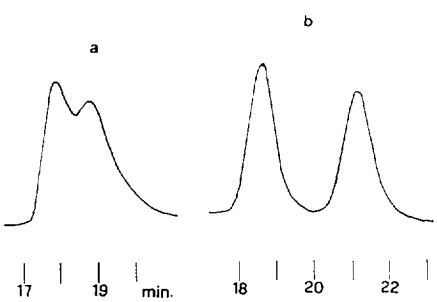

Fig. 6. HPLC resolution of **7** on Chiralcel OD at a flow-rate of 1 ml/min with mobile phase (a) *n*-hexane–2-propanol 95:5 and (b) *n*-hexane–2-propanol doped with 0.5% of diethylamine (95:5).

The good values of the resolution factors open the way to the rapid semi-preparative HPLC isolation of individual enantiomers and subsequent in vitro dopaminergic activity assays on them.

Finally, we should mention that several trials were made to obtain enantiomeric resolution of **2**, **3**, **6** and **9** using various Pirkle CSPs, reported under Experimental. However, although these phases differ in the kind and number of stereogenic centres, unsatisfactory results were obtained.

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