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Note

High-performance liquid chromatographic enantioseparation of intermediates relating to the total synthesis of (−)-physostigmine

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(−)-Physostigmine (**1**) is a clinically useful anticholinesterase agent which has been used in the treatment of myasthenia gravis and glaucoma. More recently, selected (−)-physostigmine analogues have also shown promise as Alzheimer's disease therapeutic agents. Our interests in new (−)-physostigmine analogues have led us to develop a stereoselective synthesis of (−)-physostigmine [1] (Fig. 1). To facilitate our synthetic effort and to ensure a high optical purity of the synthetic material, we required analytical high-performance liquid chromatographic (HPLC) enantioseparation of the intermediates as shown in the scheme. We report herein the application of derivatized cellulose-based chiral stationary phase (CSP) to the separation of these intermediates.

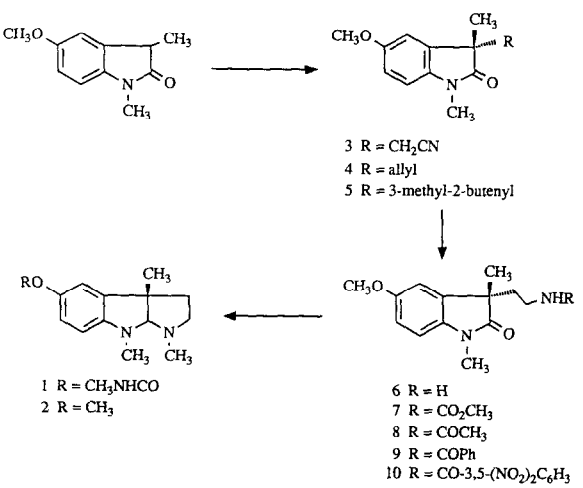

Fig. 1. Stereoselective synthesis of (−)-physostigmine. Ph = Phenyl.

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EXPERIMENTAL

Apparatus

The chromatography was performed with a M-6000A pump (Waters Assoc., Milford, MA, U.S.A.), a SF 769 variable-wavelength detector (Kratos, Ramsey, NJ, U.S.A.), and a 3390 integrator (Hewlett-Packard, Palo Alto, CA, U.S.A.).

The prepacked columns used were Daicel Chiracel OD and OJ columns (25 cm × 4.6 mm I.D.) and were purchased from Daicel Chemical Industries.

Materials

Racemic forms of compounds **1**–**10** were prepared according to literature procedures [1,2]. HPLC-grade hexanes and 2-propanol were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.).

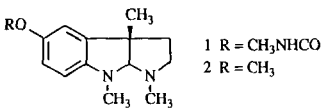
RESULTS AND DISCUSSIONS

The results and the conditions for the chromatographic separation of the intermediates on Chiralcel OD/OJ columns are presented in Tables I–III. Complete resolution can be observed for (±)-oxindoles **3**–**5** and (±)-esermethole (**2**). Attempts to separate (±)-amine **6** directly on a Crownpak CR(+) column (Daicel) have not been completely successful. Since (±)-amine **6** cannot be assayed directly on these Chiralcel columns, it is therefore derivatised as carbamate **7**, or as amides **8**–**10** before being analysed. Of these, only the carbamate **7** and the dinitro substituted benzoyl amide **10** can be resolved. It is also interesting to note that the resolution of (±)-physostigmine (**1**) and amide **10** is improved when isopropanol is replaced by ethanol as the solvent modifier.

The mechanism responsible for the separation is complex and is probably due to a combination of hydrogen bonding, and dipole-dipole interaction of the sub-

TABLE I

HPLC SEPARATION OF (±)-PHYSOSTIGMINE AND (±)-ESERMETHOLE ON CHIRALCEL COLUMNS



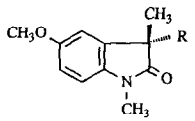
1 R = CH_3NHCO
2 R = CH_3

Mobile phases: A = isopropanol–hexane (5:95); B = ethanol–hexane (3:97); C = isopropanol–hexane (2:98). α = Separation factor; R_s = resolution factor.

Compound	Column	Mobile phase	Flow-rate (ml/min)	α	R_s	Less polar enantiomer
1	OD	A	0.5	1.11	<0.7	*S*
1	OJ	A	0.8	1.12	0.95	*S*
1	OJ	B	1.0	1.14	1.28	*S*
2	OD	C	0.6	1.36	<0.7	–
2	OJ	A	0.6	1.41	2.36	*S*

TABLE II

HPLC SEPARATION OF (±)-OXINDOLES ON CHIRALCEL COLUMNS



3 R = CH_2CN
4 R = allyl
5 R = 3-methyl-2-butenyl

Mobile phases: A = isopropanol–hexane (10:90); B = isopropanol–hexane (1:99); C = isopropanol–hexane (2:98). α = Separation factor; R_s = resolution factor.

Compound	Column	Mobile phase	Flow-rate (ml/min)	α	R_s	Less polar enantiomer
3	OD	A	1.0	1.32	3.32	*R*
3	OJ	A	1.0	1.04	<0.6	–
4	OD	B	0.7	1.0	<0.4	–
4	OJ	C	0.6	1.17	1.57	*R*
5	OJ	A	0.5	1.66	2.43	*R*

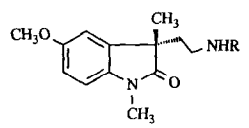
strates with the chiral stationary phases. Additional π–π interaction of the phenyl moeity of the substrate with that of the CSP may also be important for chiral recognition [3,4].

CONCLUSION

Both the Chiralcel OD and OJ columns are well suited for the analytical separation of chiral oxindoles. We have shown that by a proper choice of the CSP, enantiomers of all critical intermediates in the synthesis of (−)-physostigmine can be

TABLE III

HPLC SEPARATION OF (±)-1,3-DIMETHYL-5-METHOXYOXINDOLYLETHYLAMINE DERIVATIVES ON CHIRALCEL COLUMNS



6 = H
7 = CO_2CH_3
8 = $COCH_3$
9 = COPh
10 = CO-3,5-$(NO_2)_2C_6H_3$

Mobile phases: A = isopropanol–hexane (10:90); B = isopropanol–hexane (15:85); C = isopropanol–hexane (20:80); D = ethanol–hexane (10:90). α = Separation factor; R_s = resolution factor.

Compound	Column	Mobile phase	Flow-rate (ml/min)	α	R_s	Less polar enantiomer
7	OD	A	1.0	1.90	2.24	*R*
7	OJ	A	0.6	1.08	0.87	*R*
8	OD	B	1.0	1.00	<0.4	–
9	OD	A	1.0	1.00	<0.4	–
9	OJ	A	1.0	1.00	<0.4	–
10	OD	C	0.8	1.32	1.03	*S*
10	OD	D	1.5	1.41	3.84	*S*

separated. This chiral HPLC method will facilitate our synthetic efforts and unambiguous determination of the optical purity of the synthetic material.

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