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Note

Enantiomeric resolution of *dl-threo*-methylphenidate, U.S.P. (Ritalin®), by high-performance liquid chromatography

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There is increasing awareness that optical isomers often have markedly different biological effects, as illustrated dramatically by the discovery that the teratogenic effect of thalidomide is only associated with the *S*-(−)-enantiomer[1]. As a result, there is a need for pure enantiomers to be isolated not only for the development of enantioselective quantitative assay, but also for the investigation of potential stereoselective or stereospecific biological effects.

The chromatographic resolution of enantiomers has been well reviewed[2–4]. The two most widely researched and applied techniques are indirect and direct chromatographic resolution. Indirect chromatographic resolution involves the formation of diastereomers by reacting the mixture of enantiomers with chiral derivatizing reagents prior to separation by achiral chromatographic columns. Disadvantages associated with indirect chromatographic resolution are the unequal proportions of diastereomers may be formed as a result of different rate constants involved in the reaction with the chiral reagents[4], and erroneous results can also arise if the chiral derivatizing reagent is not enantiomerically pure. By contrast, direct chromatographic resolution involves the separation of enantiomers, without derivatization with a chiral reagents, by means of chromatographic systems which incorporate a chiral stationary phase.

dl-threo-Methylphenidate [*dl-threo*-methyl-α-phenyl-α-(2-piperidyl)acetate hydrochloride] is a mild central stimulant with pharmacological properties similar to those of dextroamphetamine[5]. This is the drug of choice for the management of children with hyperactive syndrome (attention deficit disorder). The *threo* pair enantiomers are pharmacologically more active than the *dl-erythro* isomers[6] and *d-threo*-methylphenidate is more active than its *l-threo* antipode[6]. The absolute configurations of the diastereomers have been established[7]. However, the enantiomeric resolution of *dl-threo*-methylphenidate has received little attention.

dl-threo-Methylphenidate has been resolved into dextro- and levo-rotatory isomers by fractional crystallization[6] and by using optically active ion exchangers[8]. However, these methods are laborious and involve high losses. The purpose of this paper is to communicate a high-performance liquid chromatographic (HPLC) method for the enantiomeric resolution of *dl-threo*-methylphenidate.

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EXPERIMENTAL

Materials and apparatus

dl-threo-Methylphenidate, U.S.P. (Ritalin®), was kindly donated by Ciba-Geigy (Basle, Switzerland). *d*-10-Camphorsulphonic acid (99%), triethylamine (99+%, Gold Label), diethylamine (98%), *n*-butylamine (99%) and *n*-nonylamine (98%) were obtained from Aldrich (Montreal, Canada). All the solvents used were HPLC grade and from Fisher Scientific (Edmonton, Canada).

All experiments were performed with a Model M-45 HPLC pump, a Model 480 Lambda-Max variable-wavelength UV detector (Waters Scientific, Mississauga, Canada) operated at 254 nm; a Model 7126 Rheodyne valve-loop (20 ml) injector (Technical Marketing Assoc., Calgary, Canada), and a Perkin-Elmer Model 56 linear recorder (Montreal, Canada). The column used was a Radial-Pak μBondapak C_{18} (10 cm × 5 mm I.D., 10 μm) (Waters Scientific). Circular dichroism (CD) spectra were taken in methanol at a concentration of 1.50–1.55 mg/ml (5-mm cell) with a Jasco Model J-500A spectropolarimeter interfaced with a DP-500 N data processor.

Methods

The mobile phase was dichloromethane–acetonitrile (99:1) containing 5 m*M* *d*-10-camphorsulphonic acid and 2.5 m*M* triethylamine. Degassing of the mobile phase was carried out by filtration (Millipore, Bedford, MA, U.S.A.). The column was equilibrated with the mobile phase for approximately 12 h so as to achieve reproducible retention times. *dl-threo*-Methylphenidate (30 μg) was introduced either as racemic free base or as the hydrochloride salt dissolved in the mobile phase (30 μl).

After collection of appropriate fractions of the HPLC eluate, the enantiomers of *threo*-methylphenidate were recovered from the mobile phase after decomposition of the ion pairs with 0.1 *M* sodium hydroxide solution, and subsequently, salting out the free bases by addition of sodium chloride. The recovered free bases of the enantiomers were then converted to amorphous hydrochloride salts, by addition of a cold solution of hydrogen chloride gas in diethyl ether. Elemental analysis of each enantiomer agreed closely with the calculated value. Analysis: calculated for *dl-threo*-methylphenidate $C_{14}H_{19}NO_2 \cdot HCl$: C, 62.33; H, 7.47; N, 5.19. Found *dl-threo*-methylphenidate: C, 62.82; H, 7.71; N, 5.32; *d-threo*-methylphenidate: C, 62.00; H, 7.35; N, 5.18; *l-threo*-methylphenidate: C, 62.41; H, 7.26; N, 5.18.

3,5-Dinitrobenzoyl-*dl-threo*-methylphenidate was synthesized according to the procedure reported for amino acids[9]. The reaction product was extracted with diethyl ether without acidification of the reaction mixture. Elemental analysis of 3,5-dinitrobenzoyl-*dl-threo*-methylphenidate agreed with the calculated value. Analysis: calculated $C_{21}H_{21}N_3O_7$: C, 59.01; H, 4.95; N, 9.83. Found: C, 58.73; H, 5.05, N, 9.80.

RESULTS AND DISCUSSION

The use of chiral HPLC columns for the resolution of racemic drugs containing amino acid, amino alcohol or amine functions, often requires the formation of derivatives with a reagent such as 3,5-dinitrobenzoyl chloride[10] in order to promote separation of enantiomers. This method is not suitable for the preparation of pure enantiomers of methylphenidate, however, as hydrolysis of the ester function and

possibly racemization, may occur during hydrolysis of the amide linkage of 3,5-dinitrobenzoyl derivatives. Hence, we developed an HPLC procedure where hydrolysis of the ester functional group and racemization can be avoided during the recovery of the enantiomers.

Our HPLC system differed fundamentally from recently published systems for the separation of enantiomers[11–13]. Previous workers employed polar bonded stationary phases such as cyanopropyl silica or silica-diol, in conjunction with mobile phases considered to be less polar than the stationary phases. Our system employs a non-polar stationary phase (μBondapak C_{18}) and a mobile phase of greater polarity (dichloromethane–acetonitrile) containing *d*-10-camphorsulphonic acid and a competing base. No separation of the enantiomers of methylphenidate was observed when a moderately polar stationary phase such as cyanopropyl silica was used, and there was no resolution on a non-polar stationary phase (μBondapak C_{18}) in the absence of a competing base. The present system, however, separated the enantiomers efficiently. CD spectra of the respective peaks, collected after eluting from the column, show that *d-threo*-methylphenidate eluted before the *l-threo* antipode (Fig. 1). The CD spectra were identical when scanned from wavelength 185 to 285 nm, except that opposite Cotton effects were exhibited.

Figs. 2–4 show that the efficiency of the enantiomeric resolution, as indicated by the separation factor (α), is affected by *d*-10-camphorsulphonic acid concentration, triethylamine concentration and phase composition. The separation factor decreased when the ratio of *d*-camphorsulphonic acid to triethylamine was increased (Fig. 2). Optimum enantiomeric resolution is obtained when the ratio of *d*-camphorsulphonic acid to triethylamine is approximately 2:1 as shown in Figs. 2 and 3. With respect to the mobile phase composition, the separation factor decreased with increasing acetonitrile content in the mobile phase (Fig. 4).

The capacity factors (k') were found to be affected less by *d*-camphorsulphonic

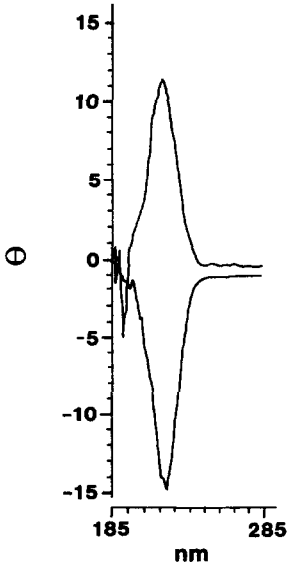

Fig. 1. CD spectra of *d*- and *l-threo*-methylphenidate. θ is the molecular ellipticity.

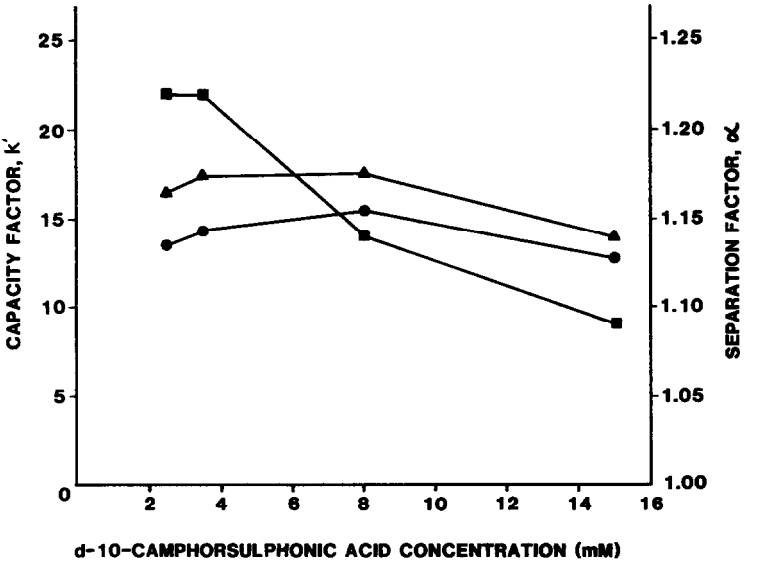

Fig. 2. Influence of *d*-10-camphorsulphonic acid concentration in the eluent on separation and capacity factors of enantiomers of *threo*-methylphenidate using constant triethylamine concentration. The separation factor $\alpha = k'_l/k'_d = (V_l - V_0)/(V_d - V_0)$, where k'_l and k'_d are the capacity factors and V_l and V_d the retention times of *l*- and *d-threo*-methylphenidate, respectively, and V_0 is the retention time of the void volume. Conditions: Radial-Pak μBondapak C_{18} (10 cm × 5 mm I.D., 10 μm); eluent, dichloromethane–acetonitrile (99:1) containing 1.25 m*M* triethylamine; flow-rate, 1 ml/min; detection at 254 nm. ■ = α; ▲ = k'_l; ● = k'_d.

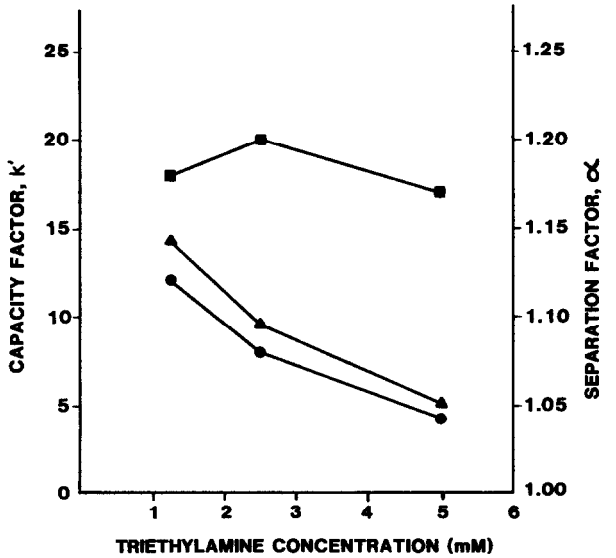

Fig. 3. Influence of triethylamine concentration in the eluent on the separation and capacity factors of enantiomers of *threo*-methylphenidate using constant *d*-10-camphorsulphonic acid concentration (5.0 m*M*). Other conditions and symbols as in Fig. 2.

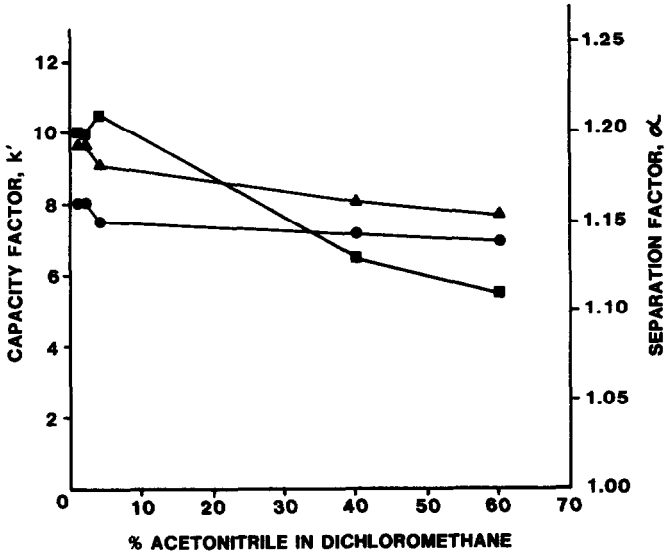

Fig. 4. Influence of percentage of acetonitrile in the eluent on separation and capacity factors of enantiomers of *threo*-methylphenidate using 5.0 m*M* and 2.5 m*M* of *d*-10-camphorsulphonic acid and triethylamine, respectively. Other conditions and symbols as in Fig. 2.

acid concentration or phase composition than by triethylamine concentration. There was about a three-fold decrease in the k' values when the ratio of triethylamine to *d*-10-camphorsulphonic acid was increased from 0.25:1.0 to 1.0:1.0 (Fig. 3). Preliminary studies on the effect of different competing bases indicated that the largest separation factor was obtained with triethylamine (Table I). Therefore, the use of triethylamine in preparative-scale HPLC gave the optimum loading capacity. The use of *n*-butylamine and *n*-nonylamine as competing bases gave poorer resolution and poor symmetry.

In the present experiments, peak symmetry appeared to improve with increasing concentrations of acetonitrile in the mobile phase. For example, in systems containing a constant ratio of *d*-10-camphorsulphonic acid to triethylamine, better peak symmetry was obtained with mobile phase containing the greater proportion of ac-

TABLE I

EFFECT OF DIFFERENT COMPETING BASES IN THE MOBILE PHASE

Mobile phase: 5 m*M* *d*-camphorsulphonic acid and 2.50 m*M* competing base in dichloromethane–acetonitrile (99:1).

Competing base	*Capacity factor*		*Separation factor* (α)
	k'_d	k'_l	
Triethylamine	8.06	9.68	1.20
Diethylamine	2.32	2.66	1.15
n-Butylamine	6.38	7.23	1.13
n-Nonylamine	17.15	18.88	1.10

etonitrile (Fig. 5A and B). By contrast, the use of diethylamine as the competing base led to good peak symmetry, even in a mobile phase containing a low proportion of acetonitrile (Fig. 5C). Also, there was no detrimental effect on peak symmetry by the water content of the solvent used. These observations emphasize that the present system is fundamentally different from that of Pettersson and Schill[11]. This means that the mechanism responsible for the separation of enantiomers is also likely to be different.

It is thought that chiral separation of racemates by HPLC can be accounted for by Dalgliesh's three-point rule[11,14–16]. In general, each enantiomer of the racemate would appear to interact with the chiral eluent, to form diastereomers which can, therefore, be separated by an appropriate HPLC system. Dalgliesh's three-point rule[17] postulates that the diastereomers are formed by interaction at three points in the vicinity of the chiral carbon atom. The interactions are of a labile, reversible nature involving hydrogen bonding, hydropohobic interactions, electrostatic attractions, charge transfer, inclusion fit, etc.

In the present case, interaction between protonated methylphenidate and *d*-

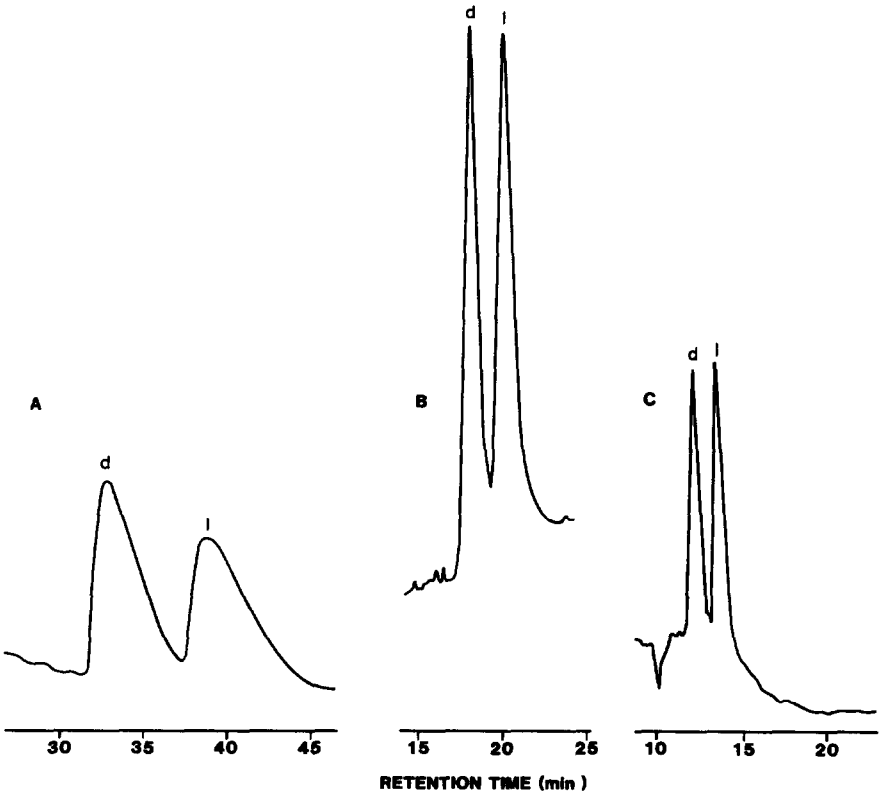

Fig. 5. Enantiomeric resolution of *dl-threo*-methylphenidate by HPLC. (A) Eluent, dichloromethane–acetonitrile (99:1) containing 5.0 m*M* *d*-10-camphorsulphonic acid and 2.5 m*M* triethylamine. (B) Eluent, dichloromethane–acetonitrile (60:40) containing 5.0 m*M* *d*-10-camphorsulphonic acid and 2.5 m*M* triethylamine. (C) Eluent, dichloromethane–acetonitrile (99:1) containing 5.0 m*M* *d*-10-camphorsulphonic acid and 2.5 m*M* diethylamine. Other conditions as in Fig. 2.

10-camphorsulphonate anion could occur through bonding between hydrophobic regions of the respective molecules, in addition to electrostatic attraction between their charged centres. A third point of interaction could be accounted for by weak hydrogen bonding between the acidic methine proton of methylphenidate and the oxo group of *d*-10-camphorsulphonate. Hydrogen bonding involving an atom other than oxygen, nitrogen or fluorine (*e.g.*, carbinyl hydrogen) has been reported as one of three interaction points in the separation of racemates by chiral stationary phases[14]. Thus, it appears likely that methylphenidate does obey Dalgliesh's three-point rule in its interaction with *d*-10-camphorsulphonate, although exceptions have been noted in other cases[12,13].

The lack of resolution of 3,5-dinitrobenzoyl-*dl-threo*-methylphenidate (Fig. 6) lends further support to the "three-point" chiral recognition model, which requires that at least one of the interactions must be stereochemically dependent and attractive in nature. Hence, the amino group of methylphenidate has been converted into an amide which cannot be protonated. Consequently, stereochemically dependent electrostatic attraction with *d*-10-camphorsulphonate is impossible.

The use of *d*-10-camphorsulphonic acid in HPLC for the resolution of racemic drugs has been well documented[11–13], although individual chromatographic systems differ considerably. For example, no competing base was necessary in the separation of some enantiomeric amino alcohols[11], whereas in the present study, the absence of a competing base led to extremely long and erratic retention times for methylphenidate. This, together with the observed effect of competing base concentration on capacity factors (Fig. 3), suggests that the mechanism of separation involves more than the simple partitioning of methylphenidate–*d*-10-camphorsulphonate ion pairs

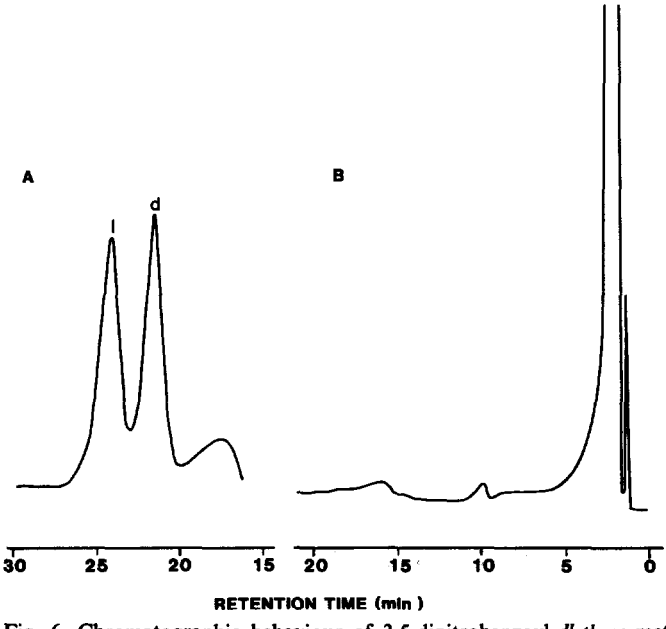

Fig. 6. Chromatographic behaviour of 3,5-dinitrobenzoyl-*dl-threo*-methylphenidate (B) and *dl-threo*-methylphenidate (A). Other conditions as in Fig. 2.

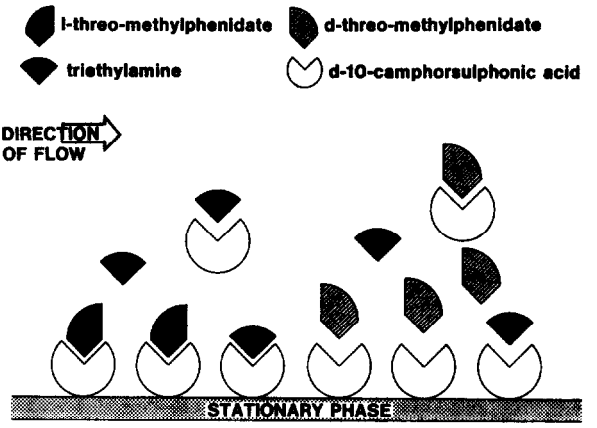

Fig. 7. Model of dynamic ion-exchange mechanism of *dl-threo*-methylphenidate with *d*-10-camphorsulphonate–triethylamine ion pairs on the stationary phase.

between the mobile and stationary phases. It would seem more likely that competing base–*d*-10-camphorsulphonate ion pairs saturate the non-polar stationary phase through hydrophobic interactions during the equilibration process. An equilibrium between the free and immobilized ion pairs was attained. The enantiomers of protonated methylphenidate would then take part in ion exchange with protonated competing base, as shown schematically in Fig. 7. *d-threo*-Methylphenidate eluted before *l-threo* antipode which seems to suggest that *d-threo*-methylphenidate is more strongly solvated.

CONCLUSION

We have developed an HPLC system for resolving *dl-threo*-methylphenidate into its dextro- and levo-rotatory isomers. *d-threo*-Methylphenidate eluted from the HPLC column before the *l-threo* antipode.

Optimum chiral separation was obtained when the ratio of *d*-10-camphorsulphonic acid to triethylamine was 2:1 (5 m*M*:2.5 m*M*) in a mobile phase containing 1% acetonitrile in dichloromethane. A dynamic ion-exchange mechanism seem to be involved in the stereoselective retention of methylphenidate. However, other retention models cannot be totally excluded.

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REFERENCES

1 G. Blaschke, H. P. Kraft, K. Fickentscher and F. Köhler, *Arzneim.-Forsch.*, 29 (1979) 1640.
2 G. Blaschke, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 13–24.
3 C. H. Lochmüller and R. W. Souter, *J. Chromatogr.*, 113 (1975) 283–302.
4 I. S. Krull, *Advan. Chromatogr.*, 16 (1978) 175.
5 N. J. Giarman, in J. R. DiPalma (Editor), *Drill's Pharmacology in Medicine: Antidepressant Drugs*, McGraw-Hill, New York, 3rd ed., 1965, p. 368.
6 R. Rometsch, *U.S. Pat.*, 2 839 519 (1958).
7 A. Shafiée and G. Hite, *J. Med. Chem.*, 12 (1969) 226–270.
8 C. G. Kratchanov and M. I. Popova, *J. Chromatogr.*, 241 (1982) 197–203.
9 F. G. Mann and B. C. Sauders, *Practical Organic Chemistry*, Longmans, London, 4th ed., 1960, pp. 379–380.
10 W. H. Pirkle and M. H. Hyun, *J. Org. Chem.*, 49 (1984) 3043–3046.
11 C. Pettersson and G. Schill, *J. Chromatogr.*, 204 (1981) 179–183.
12 G. Szepesi, M. Gazdag and R. Iváncsics, *J. Chromatogr.*, 241 (1982) 153–167.
13 G. Szepesi, M. Gazdag and R. Iváncsics, *J. Chromatogr.*, 244 (1982) 33–48.
14 W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143–158.
15 I. W. Wainer and T. D. Doyle, *J. Chromatogr.*, 259 (1983) 465–472.
16 C. Gilon, R. Leshem and E. Grushka, *J. Chromatogr.*, 203 (1981) 365–375.
17 C. E. Dalgliesh, *J. Chem. Soc.*, (1952) 3940.