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endo-1,4,5,6,7,7-HEXACHLOROBICYCLO[2.2.1]HEPT-5-ENE-2-CARBOXYLIC ACID, A SUPERIOR RESOLVING AGENT FOR THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS OF HYDROXYLATED DERIVATIVES OF TWO AZAAROMATIC HYDROCARBONS

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SUMMARY

The high-performance liquid chromatographic (HPLC) separation of enantiomers of oxide and hydroxy derivatives of dibenz[a,j]acridine and 7-methylbenz[c]acridine was investigated on a chiral stationary phase chromatography column using commercially available columns. In most cases either poor or no separation of enantiomers was achieved. Normal-phase separation of diastereoisomeric ester derivatives of the hydroxy compounds, prepared from commercially available (−)-menthoxyacetic acid or (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, was investigated. No separation of the diastereoisomeric esters of *trans*-3,4-dihydroxy-3,4-dihydrodibenz[a,j]acridine was observed. However, diastereoisomeric esters prepared from (+)-*endo*-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2-carboxylic acid [(+)-HCA] were easily separated. Using the three chiral acids, diastereoisomers were prepared from sixteen hydroxy derivatives of dibenz[a,j]acridine and 7-methylbenz[c]acridine. (+)-HCA esters gave good to excellent HPLC separations which were superior to those achieved using other chiral acids in most cases. The enantiomeric composition of *trans*-3,4-dihydroxy-3,4-dihydrodibenz[a,j]acridine formed as a major rodent liver microsomal metabolite of dibenz[a,j]acridine was determined using (+)-HCA.

INTRODUCTION

Studies of the stereochemistry of metabolism and bioactivation of polycyclic aromatic hydrocarbons [1] have necessitated the conversion of enantiomeric mixtures into diastereoisomers which may be preparatively separated and quantified chromatographically [2]. The diastereoisomers have been separated by normal-phase or sometimes reversed-phase high-performance liquid chromatography (HPLC). More recently direct separation of enantiomers by HPLC on a chiral stationary phase (CSP) has been used. Derivatives of chrysene [3,4],

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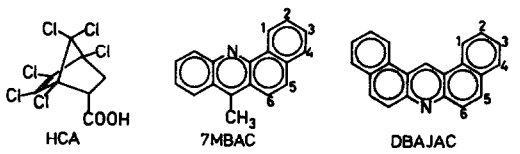


Fig. 1. Structures of *endo*-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2-carboxylic acid (HCA), 7-methylbenz[c]acridine (7MBAC) and dibenz[a,j]acridine (DBAJAC).

benz[a]anthracene [4–6], 3-methylcholanthrene [6], benzo[a]pyrene [6,7] and phenanthrene [4], some of which were methylated [5–7] and which were *cis*- and *trans*-dihydrodiols, *trans*-tetrahydrodiols, K-region arene oxides [8] and monohydroxytetrahydro derivatives [7,9], were separated into enantiomers on covalently (-C) and ionically (-I) bonded optically active N-(3,5-dinitrobenzoyl)phenylglycine (DNBPG) or N-(3,5-dinitrobenzoyl)leucine (DNBLeu) columns. Direct separation of the enantiomers of some benzo[a]pyrene and benz[a]anthracene derivatives was previously reported on a covalently bonded 2-(2,4,5,7-tetranitrofluorideneaminooxy)propionic acid (TAPA) column [10]. Two chiral acids, (−)-menthoxyacetic acid (MOA) [11–22] and α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) [11,13–17,19,23], have been extensively used to prepare and separate diastereoisomeric esters of *trans*-dihydrodiols and *trans*-tetrahydrodiols of benz[a]anthracene [11,13,15,18], benzo[a]pyrene [12,14,18,24], phenanthrene [13], 7-methylbenz[a]anthracene [16], chrysene [23], 7,12-dimethylbenz[a]anthracene [18], dibenz[c,h]acridine [17] and benzo[c]phenanthrene [18] and other compounds. MTPA was developed to allow quantification of enantiomers by integration of chemically non-equivalent proton signals in the NMR in mixtures of diastereoisomeric ester derivatives [25].

On occasions, these chiral acids and CSP columns [3,5–9] have failed to give satisfactory separations [17,18] for either preparative or quantitative work. In additions, reactions of MTPA and its acid chloride are slow. The failure of some polycyclic azaarene derivatives to separate as their (−)-MOA derivatives (see below) and the availability of the optically pure isomers of *endo*-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2-carboxylic acid (HCA) [26] prompted us to compare the acid chlorides of MOA, MTPA and HCA as chiral derivatizing agents for the separation of enantiomers of dihydrodiols and some tetrahydrodiols of the carcinogens 7-methylbenz[c]acridine (7MBAC) and dibenz[a,j]acridine (DBAJAC) (Fig. 1). HCA is a readily prepared reactive bicyclic carboxylic acid and its acid chloride proved to be superior for most compounds tested. A comparison was also made of the ability of some CSP columns to directly separate enantiomeric vicinal diol derivatives of these azaarenes.

EXPERIMENTAL

Derivatives of 7MBAC [27,28] and DBAJAC [29] were available from synthetic studies. (+)-HCA was prepared as described [26] and other reagents were obtained from commercial suppliers. (*R*)-DNBPG-I, (*R*)-DNBPG-C and (*S*)-

DNBLeu-I columns were purchased from Regis (Morton Grove, IL, U.S.A.) and a Brownlee (Santa Clara, CA, U.S.A.) 10-μm silica column (250 mm×4.6 mm I.D.) was used. Eluent composition and flow-rates are presented in Tables I–IV and analytes were detected by their UV absorption at 280 or 254 nm for ethyl acetate–hexane and diethyl ether–cyclohexane eluents, respectively. An Altex 153 detector and an Altex 110B pump were employed.

Small-scale derivatization procedures

Preparation of acylation reagent A for (+)-HCA. *p*-(Dimethylamino)pyridine (25 mg) was dissolved in anhydrous tetrahydrofuran (THF, 0.9 ml) and stored in a Reactivial (1 ml volume, with a PTFE-lined cap).

Preparation of acylation reagent B for (+)-HCA. (+)-HCA (50 mg) was refluxed with thionyl chloride (1 ml) for 2 h. Excess thionyl chloride was removed under reduced pressure and residual thionyl chloride was removed by evaporation of toluene (1 ml). The residue, a colourless gum, was dissolved in anhydrous THF (0.9 ml) and again stored in a 1-ml Reactivial.

Acylation procedure

Equal volumes of reagent A and reagent B were added to the hydroxy compound in a Reactivial and the mixture was vortexed. For quantities of 1–50 μg compound, 25 μl of each reagent were used while 50 μl were used for quantities of 50–100 μg. Reactions were generally found to be complete after a few minutes (checked by thin-layer chromatography, TLC), and for HPLC analysis *p*-(dimethylamino)pyridine and its hydrochloride were removed by adsorption on silica gel. The reaction mixture, dissolved in dichloromethane–ethyl acetate (9:1, v/v, 1 ml) was passed under vacuum through a 30 mm×5 mm I.D. bed of silica gel H (TLC grade) and the bed was washed with fresh solvent (2×1 ml). The residue obtained after evaporation of the combined eluents was chromatographed within three days.

Acylation with (−)-MOA. Reagent A as above and reagent B prepared from (−)-MOA (32.7 mg) as above were used. The acylation procedure was identical.

Acylation with (+)-MTPA. Reagent A contained *p*-(dimethylamino)pyridine (13.8 mg) in THF (0.9 ml) and reagent B was prepared from (+)-MTPA (21.1 mg) with thionyl chloride and a trace of sodium chloride [25]. Acylation of 10–50 μg of hydroxy compound required 50 μl of each reagent and the reaction mixture left for 18 h to facilitate complete reaction. The remainder of the procedure was identical to that for (+)-HCA.

trans-3,4-bis-(−)-Menthoxyacetoxy-1,2,3,4-tetrahydro-7-methylbenz[c]acridine

(−)-MOA (21.1 mg) was refluxed with thionyl chloride for 2 h. Excess reagent was removed under reduced pressure, and toluene (1 ml) was added and removed under reduced pressure. The residue dissolved in THF (1 ml) was added to a THF (1 ml) solution of *trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-7MBAC (4.5 mg) and *p*-(dimethylamino)pyridine (15.5 mg), and the mixture was stirred under nitrogen. TLC examination showed that the reaction was complete after 20 min. The reaction mixture was partitioned between 20% aqueous sodium chloride and

ethyl acetate, and the ethyl acetate extract was dried and concentrated to give a residue which was purified by short-column vacuum chromatography [30] (silica gel H bed, 30 mm×45 mm I.D., solvent hexane–ethyl acetate, 9:1) to give the mixed diastereoisomers as a colourless syrup (9.5 mg, 88%). ^{1}H NMR (8.4 mg per 0.25 ml, C^2HCl_3) δ 0.68–1.74 (m, 30 H), 1.89–2.47 (m, 8H), 2.98–3.38 (m, 2H), 3.10 (s, 3H), 3.66 (m, 2H), 5.42 (q, H_3), 6.34 (d, H_4), 7.37 (d, H_5), 7.48–7.85 (m, H_9 and H_{10}), 8.12 (d, H_6), 8.18–8.29 (H_8 and H_{11}); $J_{2,3}=J_{2',3}=J_{3,4}=5.5$ Hz, $J_{5,6}=9.6$ Hz. Chemical ionization mass spectrometry (CIMS) *m/e* (relative intensity) 672 (M+1, 1.9), 534 (0.7), 458 (39), 320 (4), 246 (13), 245 (6), 244 (8), 139 (100), 137 (16), 123 (9), 105 (8).

Preparation of diasteoisomeric esters from trans-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[a,j]acridine and (−)-menthoxyacetic acid

Diastereoisomeric esters (37.8 mg, 100%) were prepared from *trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-DBAJAC (16.6 mg) and (−)-MOA (56.4 mg) using the method described previously and partially separated by preparative HPLC (LiChrosorb 5-μm silica, 50 cm×0.9 cm I.D., cyclohexane–diethyl ether, 7:1, 10 ml/min) to give samples of the less polar diastereoisomer (13.3 mg, 95% diastereoisomeric purity) and the more polar diastereoisomer (13 mg, 90% diastereoisomeric purity). The less polar diastereoisomer was identified as *trans*-3*R*,4*R*-bis-(−)-menthoxyacetoxy-1,2,3,4-tetrahydrodibenz[a,j]acridine. ^{1}H NMR (13 mg per 0.2 ml, C^2HCl_3) δ 0.66–2.72 (m, 38 H), 3.12 (m, 2H), 3.52 (m, 2H), 4.16 (m, 4H), 5.43 (m, H_3), 6.35 (d, H_4), 7.54–8.29 (m, 5H), 8.00 (s, H_8 and H_9), 8.78 (m, H_{13}), 9.59 (s, H_{14}); $J_{3,4}=5.1$ Hz. The four-proton multiplet at δ 4.16 was resolved at 400 MHz to two overlapping AB quartets at δ_A 4.056, δ_B 4.146, $\delta_{A'}$ 4.169, $\delta_{B'}$ 4.235; $J_{AB}=16.5$ Hz, $J_{A'B'}=16.6$ Hz. The more polar diastereoisomer was identified as *trans*-3*S*,4*S*-bis-(−)-menthoxyacetoxy-1,2,3,4-tetrahydrodibenz[a,j]acridine. ^{1}H NMR (13 mg per 0.2 ml C^2HCl_3) δ 0.64–2.72 (m, 38 H), 3.12 (m, 2H), 3.52 (m, 2H), 4.16 (m, 4H), 5.43 (m, H_3), 6.35 (d, H_4), 7.52–8.29 (m, 5H), 8.00 (s, H_8 and H_9), 8.78 (m, H_{13}), 9.59 (s, H_{14}); $J_{3,4}=5.1$ Hz. The four-proton multiplet at δ 4.16 was resolved into two overlapping AB quartets at 400 MHz, δ_A 4.063, δ_B 4.147, $\delta_{A'}$ 4.186, $\delta_{B'}$ 4.220; $J_{AB}=16.4$ Hz, $J_{A'B'}=16.6$ Hz.

Preparation of diastereoisomeric esters from trans-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[a,j]acridine and (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid

(+)-MTPA (30 mg was refluxed with thionyl chloride (3 ml) and a trace of sodium chloride under nitrogen for 50 h. Excess thionyl chloride was removed under reduced pressure, and toluene (1 ml) was added and removed under reduced pressure. The residue dissolved in THF (1 ml) was added to a THF (1.5 ml) solution of *trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-DBAJAC (8.1 mg) and *p*-(dimethylamino)pyridine (21.9 mg) and the mixture was stirred under nitrogen. TLC examination after 5 min showed complete reaction. The reaction mixture was partitioned between 20% aqueous sodium chloride and ethyl acetate, and the ethyl acetate extract was dried and concentrated to give a colourless gum (21.1 mg). Purification by short-column vacuum chromatography (silica gel H bed, 25 mm×45 mm I.D., solvent gradient hexane–ethyl acetate, 4:1 to 2:1) gave the

diastereoisomers (19.2 mg, 93%). CIMS *m/e* (relative abundance) 748 (M+1, 1.5), 542 (0.5), 514 (13), 282 (10), 231 (13), 203 (100), 189 (20), 179 (10). Samples of the less polar diastereoisomer (8.7 mg, 90% diastereoisomeric purity) and the more polar diastereoisomer (5.8 mg, approximately 100% diastereoisomeric purity) were isolated after preparative HPLC (LiChrosorb 5-μm silica, 50 cm$\times$0.9 cm, hexane–ethyl acetate, 4:1, 10 ml min).

trans-3*R*,4*R*-bis[(+)-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-1,2,3,4-tetrahydrodibenz[a,j]acridine, the less polar diastereoisomer, was recrystallised from hexane–dichloromethane to give colourless needles (5.5 mg), m.p. 114–117°C. ^{1}H NMR (1.3 mg per 0.2 ml, C^2HCl_3) δ 2.24–2.50 (m, 2H), 3.21–3.60 (m, 2H), 3.46 (q, 3H), 3.56 (q, 3H), 5.65 (q, H_3), 6.47 (d, H_4), 7.17–8.19 (m, 17H), 8.75 (m, H_{13}), 9.50 (s, H_{14}); $J_{H,F}$=1.1 Hz, $J_{2,3}=J_{2',3}=J_{3,4}$=4.6 Hz.

trans-3*S*,4*S*-bis[(+)-α-Methoxy-α-(trifluoromethyl)phenylacetoxy]-1,2,3,4-tetrahydrodibenz[a,j]acridine, the more polar diastereoisomer, was a colourless syrup. ^{1}H NMR (4.6 mg per 0.2 ml, C^2HCl_3) δ 2.24–2.50 (m, 2H), 3.23–3.79 (m, 2H), 3.40 (q, 3H), 3.51 (q, 3H), 5.51 (q, H_3), 6.42 (d, H_4), 7.17–8.19 (m, 17 H), 8.75 (m, H_{13}), 9.56 (s, H_{14}); $J_{H,F}$=1.1 Hz, $J_{2,3}=J_{2',3}=J_{3,4}$=4.2 Hz.

Hydrolysis of diastereoisomeric esters

Each diastereomeric ester (1–5 mg) was dissolved in THF (2 ml), 10% aqueous sodium hydroxide (0.5 ml) and methanol (1 ml) were added and the solution was stirred under nitrogen for 30 min. Then each reaction mixture was partitioned between 20% aqueous sodium chloride and ethyl acetate, and the ethyl acetate extract was dried and concentrated to give a crude product that was purified by short-column vacuum chromatography (silica gel H bed, 15 mm$\times$45 mm I.D., solvent gradient chloroform to chloroform–ethanol, 9:1) to give a pale yellow crystalline solid. Hydrolysis of *trans*-3*R*,4*R*-bis-[(−)-menthoxyacetoxy)-1,2,3,4-tetrahydro-DBAJAC (3.3 mg) gave *trans*-3*R*,4*R*-dihydroxy-1,2,3,4-tetrahydro-DBAJAC [2.1. mg, 86% enantiomeric excess of purity (ee) by (+)-HCA method]. Hydrolysis of the 3*S*,4*S*-diastereoisomer (2.9 mg) gave *trans*-3*S*,4*S*-dihydroxy-1,2,3,4-tetrahydro-DBAJAC [1.7 mg, 75% ee by (+)-HCA method]. Hydrolysis of *trans*-3*R*,4*R*-bis-[(+)-α-methoxy-α-(trifluoromethyl)phenylacetoxy]-1,2,3,4-tetrahydro-DBAJAC (1 mg) gave *trans*-3*R*,4*R*-dihydroxy-1,2,3,4-tetrahydro-DBAJAC [0.4 mg, 99.7% ee by (+)-HCA method]. Hydrolysis of the 3*S*,4*S*-diastereoisomer (4.0 mg) gave *trans*-3*S*,4*S*-dihydroxy-1,2,3,4-tetrahydro-DBAJAC [2.5 mg, 96% ee by (+)-HCA method].

RESULTS AND DISCUSSION

7MBAC and DBAJAC are carcinogenic polycyclic azaaromatic hydrocarbons found in tobacco smoke condensate or urban air [31–33]. Analogously to polycyclic aromatic hydrocarbons, biological activation was expected to proceed through arene oxides and dihydrodiols, and metabolic studies in vitro with rat liver microsomal preparations have demonstrated the presence of some of these pathways [34,35]. CSP columns giving the best separations with polycyclic aromatic hydrocarbon derivatives were (*R*)-DNBPG-I, (*R*)-DNBPG-C and

TABLE I

CHIRAL STATIONARY PHASE HPLC OF DIBENZ[a,j]ACRIDINE DERIVATIVES

Chemical names are abbreviated by showing the names, positions and stereochemistry of substitution as illustrated by the following examples: 1-OH-H_4-DBAJAC = 1-hydroxy-1,2,3,4-tetrahydrodibenz[a,j]acridine; *t*-5,6-H_2-5-MeO-6-OH-DBAJAC = *trans*-6-hydroxy-5-methoxy-5,6-dihydrodibenz[a,j]acridine; *c*-5,6-H_2-diol-DBAJAC = *cis*-5,6-dihydroxy-5,6-dihydrodibenz[a,j]acridine; *t*-3,4-H_4-diol-7MBAC = *trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-7-methylbenz[c]acridine.

Compound	CSP[a]	Percent A[b]	Retention time (min)		RV[c]
			Peak a	Peak b	
t-1,2-H_2 diol	(*R*)-DNBPG-I	10	61.8	62.5	0.1
	(*R*)-DNBPG-C	10	38	38	0
	(*S*)-DNBLeu-I	10	49.6	51.2	0.5
t-3,4-H_2 diol	(*R*)-DNBPG-I	10	51	51	0
	(*R*)-DNBPG-C	10	31	31	0
	(*S*)-DNBLeu-I	10	37	37	0
t-3,4-H_4 diol	(*R*)-DNBPG-I	10	63.2	65.2	0.3
	(*R*)-DNBPG-C	5	147	152	0.6
	(*S*)-DNBLeu-I	10	41	41	0
t-5,6-H_2 diol	(*R*)-DNBPG-I	10	37	37	0
	(*R*)-DNBPG-C	10	29[d]	29[d]	0
	(*S*)-DNBLeu-I	10	23	23	0
c-5,6-H_2 diol	(*R*)-DNBPG-I	10	36	36	0
	(*R*)-DNBPG-C	10	26[d]	26[d]	0
	(*S*)-DNBLeu-I	10	32	32	0
t-5,6-H_2-5-MeO-6-OH	(*R*)-DNBPG-I	2	17	17	0
	(*R*)-DNBPG-C	10	8[d]	8[d]	0
	(*S*)-DNBLeu-I	2	12	12	0
t-5,6-H_2-5-OH-6-MeO	(*R*)-DNBPG-I	2	60.0 (5*R*,6*R*)	66.6 (5*S*,6*S*)	1.4
	(*R*)-DNBPG-C	2	59.5	62.1	0.6
	(*S*)-DNBLeu-I	2	50.0	50.5	0.1
1-OH H_4	(*R*)-DNBPG-I	5	18	18	0
	(*R*)-DNBPG-C	2	63	63	0
	(*S*)-DNBLeu-I	2	33.3	35.2	0.7
4-OH H_4	(*R*)-DNBPG-I	5	32	32	0
	(*R*)-DNBPG-C	2	131	131	0
	(*S*)-DNBLeu-I	2	79.8	83.6	0.9
5,6-Oxide	(*R*)-DNBPG-I	1	22.0 (5*S*,6*R*)	23.1 (5*R*,6*S*)	0.9
	(*S*)-DNBLeu-I	0.5	30	30	0

[a]CSP (chiral stationary phase): (*R*)-DNBPG-I, (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine ionically bonded; (*R*)-DNBPG-C covalently bonded; (*S*)-DNBLeu-I, (*S*)-N-(3,5-dinitrobenzoyl)leucine ionically bonded to 3-aminopropylsilanised silica.
[b]Percent (v/v) solvent A (ethanol-acetonitrile, 2:1) in hexane at 2.0 ml/min.
[c]RV (resolution value) = $2(V_2 - V_1)/(W_2 + W_1)$.
[d]Broad peak.

(*S*)DNBLeu-I [3–10], but when they were used with the DBAJAC (Table I) and 7MBAC (Table II) derivatives in this work, only limited success was achieved. No separation of the enantiomers of the metabolites, *trans*-3,4-dihydroxy-3,4-

TABLE II

CHIRAL STATIONARY PHASE HPLC OF 7-METHYLBENZ[c]ACRIDINE DERIVATIVES

See Table I for column headings.

Compound	CSP	Percent A	Retention time (min)		RV
			Peak a	Peak b	
t-1,2-H_2 diol	(*R*)-DNBPG-I	10	20.4	21.6	0.6
	(*R*)-DNBPG-C	5	32.0	33.9	0.9
	(*S*)-DNBLeu-I	10	22	22	0
t-3,4-H_2 diol	(*R*)-DNBPG-I	10	36.2	37.5	0.3
	(*R*)-DNBPG-C	10	18	18	0
	(*S*)-DNBLeu-I	10	25	25	0
t-3,4-H_4 diol	(*R*)-DNBPG-I	10	31.0 (3*S*,4*S*)	33.8 (3*R*,4*R*)	0.9
	(*R*)-DNBPG-C	10	17.5	17.5	0
	(*S*)-DNBLeu-I	10	26	26	0
t-5,6-H_2 diol	(*R*)-DNBPG-I	6.7	48	48	0
	(*R*)-DNBPG-C	5	63.0	66.7	1.0
	(*S*)-DNBLeu-I	5	53.0	55.8	0.7
c-5,6-H_2 diol	(*R*)-DNBPG-I	10	22.4	23.6	0.5
	(*R*)-DNBPG-C	10	15.9	16.4	0.2
	(*S*)-DNBLeu-I	10	18.4	19.9	0.7
t-8,9-H_2 diol	(*R*)-DNBPG-I	10	41	41	0
	(*R*)-DNBPG-C	5	85.8	88.6	0.5
	(*S*)-DNBLeu-I	10	29.3	30.5	0.5
t-10,11-H_2 diol	(*R*)-DNBPG-I	10	21	21	0
	(*R*)-DNBPG-C	5	30	30	0
	(*S*)-DNBLeu-I	10	19.2	20.4	0.7
5,6-Oxide	(*R*)-DNBPG-I	0.5	18	18	0
	(*S*)-DNBLeu-I	0.25	18	18	0
anti-1,2-epoxy-*t*-	(*R*)-DNBPG-I	10	83	83	0
3,4-diol-H_4	(*S*)-DNBLeu-I	10	55.8	59.8	0.9

dihydro- and *trans*-5,6-dihydroxy-5,6-dihydro-DBAJAC and 7MBAC-5,6-oxide, was observed. The synthetically useful *trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-7MBAC gave a moderate separation on (*R*)-DNBPG-I [resolution value (RV) = 0.9] but the synthetic standard for the proximate carcinogen of 7MBAC, *trans*-3,4-dihydroxy-3,4-dihydro-7MBAC, gave a very poor separation with (*R*)-DNBPG-I (RV = 0.3). In general, separations showing an RV < 0.9 are not of practical use. *trans*-1,2-Dihydroxy-1,2-dihydro- and *trans*-5,6-dihydroxy-5,6-dihydro-7MBAC gave moderate separations on (*R*)-DNBPG-C (RV = 0.9 and RV = 1.0, respectively). *anti*-1,2-Epoxy-*trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-7MBAC, a proposed ultimate carcinogen, gave a moderate separation on (*S*)-DNBLeu-I (RV = 0.9) and the enantiomers were recovered from the HPLC column unchanged as shown by TLC. The only potential metabolite showing a practical separation was DBAJAC-5,6-oxide [RV = 0.9 on (*R*)-DNBPG-I]. Good separation was observed for the minor methanolysis product of DBAJAC-5,6-oxide, *trans*-5-hydroxy-6-methoxy-5,6-dihydro-DBAJAC, on (*R*)-DNBPG-I

TABLE III

NORMAL-PHASE SILICA HPLC OF DIASTEREOISOMERIC ESTERS PREPARED FROM DIBENZ[a,j]ACRIDINE DERIVATIVES

Compound	Chiral ester derivative[a]	Percent diethyl ether[b]	Retention time (min)		RV[c]
			Peak a	Peak b	
t-1,2-H_2 diol	(+)-HCA	20	33.7	45.6	3.3(4.7)
	(−)-MOA	15	28.0	31.5	1.2(1.5)
t-3,4-H_2 diol	(+)-HCA	15	25.1 (3*S*,4*S*)	30.7 (3*R*,4*R*)	2.1(3.2)
	(−)-MOA	7.5	29	29	0 (0)
	(+)-MTPA	20	23	23	0 (0[d])
t-3,4-H_4 diol	(+)-HCA	15	34.4 (3*S*,4*S*)	44.2 (3*R*,4*R*)	2.5(3.8)
	(−)-MOA	7.5	36.6 (3*R*,4*R*)	40.0 (3*S*,4*S*)	0.9(0)
	(+)-MTPA	20	27.6	29.3	0.6(0.9[d])
t-5,6-H_2 diol	(+)-HCA	15	7.2 (5*R*,6*R*)	26.9 (5*S*,6*S*)	10.0(5.2)
	(−)-MOA	10	26.8	31.0	1.6(0)
	(+)-MTPA[e]				
c-5,6-H_2 diol	(+)-HCA	10	10.4	12.1	0.7(0.9)
	(−)-MOA	10	23.8	30.4	1.6(0.9)
t-5,6-H_2-5-MeO-6-OH	(+)-HCA	15	13.0 (5*R*,6*R*)	36.6 (5*S*,6*S*)	9.6(6.7)
	(−)-MOA	15	20.2	22.3	1.0(0.8)
	(+)-MTPA[e]				
t-5,6-H_2-5-OH-6-MeO	(+)-HCA	15	23.0 (5*R*,6*R*)	67.4 (5*S*,6*S*)	11.1(9.7)
	(−)-MOA	15	24.7	30.8	2.3(1.6)
	(+)-MTPA	20	13.6	17.2	2.2(1.8[d])
1-OH H_4	(+)-HCA	15	38.9	51.6	3.0(2.8)
	(−)-MOA	15	28	28	0 (0)
	(+)-MTPA[e]				
4-OH H_4	(+)-HCA	15	37.2	65.4	5.8(5.5)
	(−)-MOA	15	35.0	39.3	1.2(0.9)
	(+)-MTPA	20	23	23	0 (0[d])

[a]Chiral ester derivatives prepared from acid chloride of: (+)-HCA, (+)-*endo*-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene-2-carboxylic acid; (−)-MOA, (−)-menthoxyacetic acid; (+)-MTPA (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid.

[b]Percent (v/v) diethyl ether in cyclohexane at 0.7 ml/min.

[c]RV (resolution value) $= 2(V_2 - V_1)/(W_2 + W_1)$ for diethyl ether–cyclohexane or, in brackets, 10% (v/v) ethyl acetate–hexane.

[d]12.5% (v/v) Ethyl acetate–hexane.

[e]Derivatisation unsuccessful.

(RV = 1.4). It was observed that the three DBAJAC derivatives with a 6-hydroxyl group that are capable of intramolecular H-bonding with the ring nitrogen, *cis*- and *trans*-5,6-dihydroxy-5,6-dihydro-DBAJAC and *trans*-6-hydroxy-5-methoxy-5,6-dihydro-DBAJAC, gave very broad peaks on (*R*)-DNBPG-I.

As many potential metabolites gave no or poor enantiomeric separations on the three CSP columns used in this work, other methods of resolution were investigated. The optically active acids commonly used to prepare separable diastereoisomers, (−)-MOA and (+)-MTPA, gave bis-esters of *trans*-3,4-dihydroxy-1,2,3,4-tetrahydro-DBAJAC. These were separated by HPLC and

TABLE IV

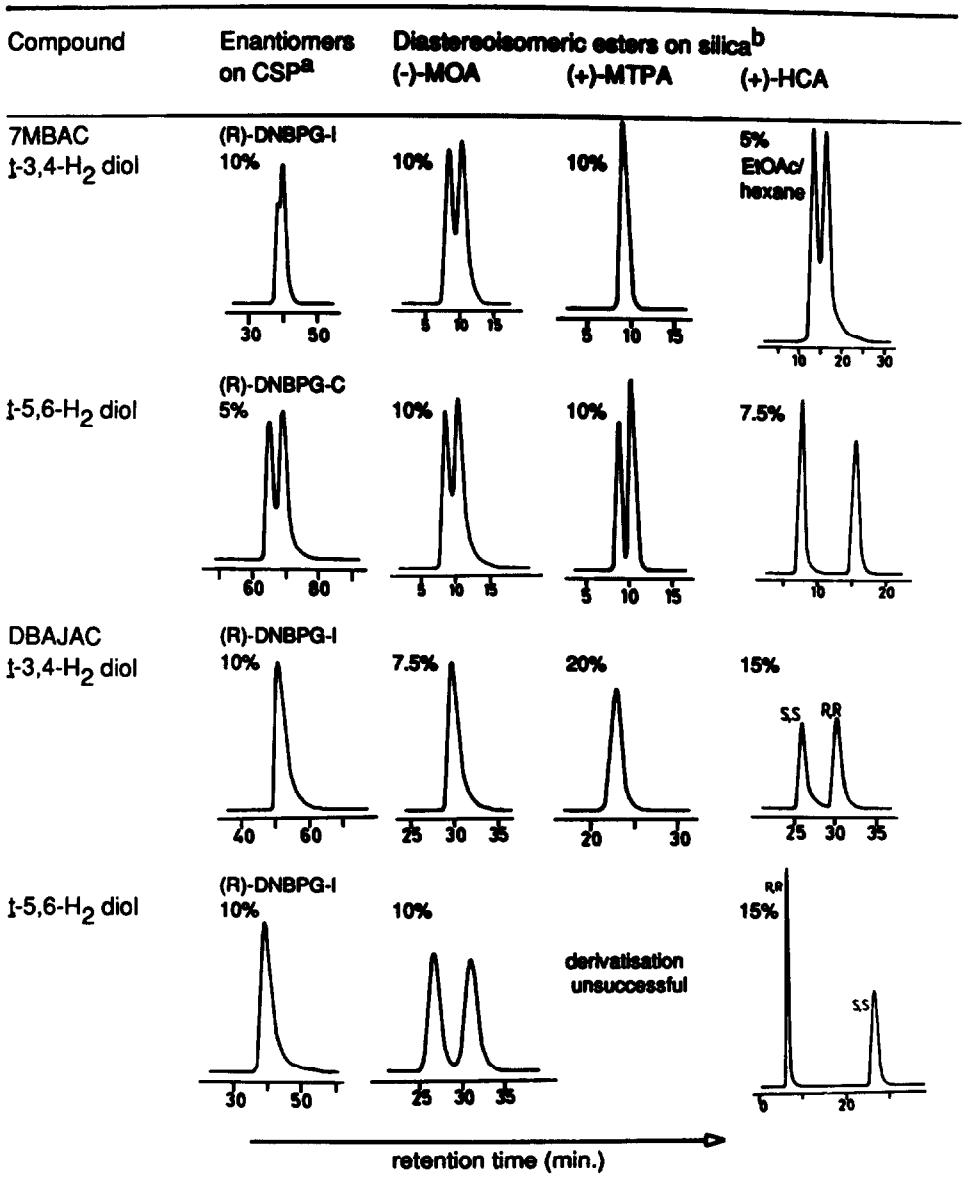
NORMAL-PHASE SILICA HPLC OF DIASTEREOISOMERIC ESTERS PREPARED FROM 7-METHYLBENZ[c]ACRIDINE DERIVATIVES

Compound	Chiral ester derivative[a]	Percent diethyl ether[b]	Retention time (min)		RV[c]
			Peak a	Peak b	
t-1,2-H_2 diol	(+)-HCA	7.5	9.4	11.5	1.5(3.0)
	(−)-MOA	10	14.7	18.0	1.7(1.5)
t-3,4-H_2 diol	(+)-HCA	7.5	7.3	8.0	0.5(0.9)
	(−)-MOA	10	8.6	10.0	0.9(0.6)
	(+)-MTPA	10	10	10	0 (0)
t-3,4-H_4 diol	(+)-HCA	15	5.5 (3*S*,4*S*)	7.3 (3*R*,4*R*)	1.3(1.4)
	(−)-MOA	10	14.8 (3*R*,4*R*)	16.1 (3*S*,4*S*)	0.5(0)
	(+)-MTPA	10	9	9	0 (0)
t-5,6-H_2 diol	(+)-HCA	7.5	7.4	15.3	4.2(4.1)
	(−)-MOA	10	8.8	10.2	1.1(0.3)
	(+)-MTPA	10	8.8	10.3	1.2(1.4)
c-5,6-H_2 diol	(+)-HCA	7.5	7.7	23.6	7.0(6.3)
	(−)-MOA	10	9.7	11.1	0.9(0.8)
t-8,9-H_2 diol	(+)-HCA	7.5	6.7	13.6	4.8(4.2)
	(−)-MOA	10	8.5	11.1	2.2(1.1)
t-10,11-H_2 diol	(+)-HCA	7.5	9.5	13.9	3.5(5.1)
	(−)-MOA	10	14	14	0 (0.6)

[a]See Table III.
[b]See Table III.
[c]RV (resolution value) as defined in Table III for diethyl ether–cyclohexane or, in brackets, 5% (v/v) ethyl acetate–hexane.

characterised by their NMR and mass spectra; however, no chromatographic separation of the corresponding bis-esters of *trans*-3,4-dihydroxy-3,4-dihydro-DBAJAC was observed (Table III).

For the preparation of HCA esters the use of *p*-(dimethylamino)pyridine as base [36] rather than pyridine in anhydrous THF ensured rapid and complete esterification with the acid chloride of (+)-HCA. Complete separation of the diastereoisomers of *trans*-3,4-dihydroxy-3,4-dihydro-DBAJAC derivatives was achieved, and no additional products resulting from a change in configuration of the carboxy group from *endo* to *exo* were observed by HPLC analysis or by ^{1}H NMR spectra. The characterisation of (+)-HCA diastereoisomers will appear elsewhere. A complete comparison of the diastereoisomeric derivatives of alcohols from DBAJAC and 7MBAC with (−)-MOA, (+)-HCA and (+)-MTPA was hampered by the difficulty of achieving complete derivatization on an analytical scale using the acid chloride of (+)-MTPA [24], and by occasional decomposition of MTPA esters [17]. Seven out of ten derivatizations with (+)-MTPA were successful (Tables III and IV) and of these only three showed an RV > 0.8. The comparison of the three chiral acids is shown in Table III for DBAJAC derivatives and in Table IV for 7MBAC derivatives and in Fig. 2. Using (−)-MOA all sixteen compounds were successfully derivatized, and twelve out of six-



a chiral stationary phase;
% v/v Solvent A (EtOH/MeCN 2:1) in hexane at 2.0mL/min.
b normal phase;
% v/v ether/cyclohexane at 0.7ml/min (unless otherwise specified)

Fig. 2. HPLC separation of DBAJAC and 7MBAC derivatives.

teen gave an RV > 0.8. In eleven out of thirteen cases using cyclohexane–diethyl ether as solvent higher RV values were obtained than with hexane–ethyl acetate. For (+)-HCA all compounds were successfully derivatized and all gave an

RV > 0.8. In eight out of sixteen cases cyclohexane–diethyl ether was a superior solvent to hexane–ethyl acetate. (+)-HCA was the best resolving agent in fourteen out of sixteen examples, (−)-MOA was better for *cis*-5,6-dihydroxy-5,6-dihydro-DBAJAC and (+)-HCA and (−)-MOA were similar for *trans*-3,4-dihydroxy-3,4-dihydro-7MBAC.

Three *trans*-K-region derivatives of DBAJAC gave extremely good separations with (+)-HCA (RV values of 10.0, 9.6 and 11.1). Interestingly, separation with the (+)-HCA derivative of *cis*-5,6-dihydroxy-5,6-dihydro-DBAJAC was much less (RV = 0.9, ethyl acetate–hexane). Products were stable over a period of days but K-region derivatives were decomposed after storage for two to three months. Analytical derivatizations of microgram quantities of metabolites were possible using (+)-HCA chloride. Derivatizations with (+)-HCA chloride and (−)-MOA chloride were rapid and complete with *p*-(dimethylamino)pyridine as base and avoided the long reaction times reported using pyridine as base. Quantitative uncertainty associated with incomplete derivatization was not a problem under the conditions described. Alkaline hydrolysis of the preparatively separated diastereoisomeric esters gave optically pure alcohols (> 99% enantiomeric excess).

It appears from these results, and from previous work [26], that the enantiomers of HCA should provide the first reagent of choice for separation of alcohols of polycyclic aromatic compounds. This is illustrated in the metabolism of DBAJAC by rodent liver microsomes which affords relatively large amounts of *trans*-3,4-dihydrodiol-DBAJAC [37]. The enantiomeric compositions of this metabolite were determined by esterification with (+)-HCA and the diastereoisomers separated by HPLC (Fig. 2) and quantified radiochemically. Metabolism with microsomes prepared from livers of animals which were untreated or treated with phenobarbital or 3-methylcholanthrene all showed an enantiomeric composition for the *trans*-3,4-dihydrodiol-DBAJAC of 30–37% for the 3*S*,4*S*-enantiomer and 70–63% for the 3*R*,4*R*-enantiomer.

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