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High-performance liquid chromatographic determination of optical purity of planar chiral organometallic compounds resolved by enzymic transformations

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ABSTRACT

The enantiomers of planar chiral organometallic compounds, *i.e.*, 1-hydroxymethyl-2-methyl, 1-acetoxymethyl-2-methyl and 1-methoxycarbonyl-2-methyl derivatives of (η^6-benzene)tricarbonylchromium, tricarbonyl(η^5-cyclopentadienyl)manganese and ferrocene, and their 3-methyl analogues, were separated by high-performance liquid chromatography with a Chiralcel OD column. This method was useful to determine the optical purity for the above compounds formed in enzymic transformations.

INTRODUCTION

Enzymic transformation is one of the most practical methods for preparing optically active organometallics having planar chirality such as homoannularly di- or trisubstituted ferrocene derivatives [1–3] and asymmetrically substituted arenes or alkadienes complexed with tricarbonylmetal clusters [2–8]. These compounds are useful as chiral auxiliaries for asymmetric reagents or catalysts. An important problem in the study on asymmetric bioconversion is to determine the optical purity of the product as easily as possible for screening new biocatalysts or monitoring kinetic resolution.

Optical purities of planar chiral organometallics have heretofore been determined by NMR spectroscopy with a chiral shift reagent [2–6,9], by high performance liquid chromatography (HPLC) on a chiral stationary phase [1,2] or by NMR [7] or HPLC [10] after conversion to diastereomeric derivatives.

The NMR shift reagent method as well as the diastereomeric methods are too complicated to be used in routine work. Chromatographic analysis without pretreatment or derivatization is the best choice in this respect. A β-cyclodextrin-bonded column was successfully used for optical purity determination of planar chiral ferrocene derivatives [1,2], but it did not resolve (±)-tricarbonyl (η^5-1-methoxycarbonyl-2-

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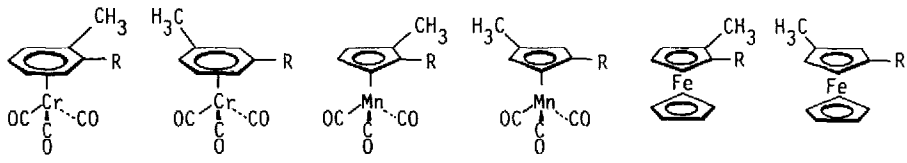

a, R = CH_2OH ; **b**, R = CHO ; **c**, R = $COOCH_3$; **d**, R = CH_2OCOCH_3 ; **e**, R = COOH

Fig. 1. Structures of compounds.

methylcyclopentadienyl)manganese (**3c**) (Fig. 1) in our preliminary experiments, so that the application of this column seems to be limited to some metallocenes [4,11] certainly with regard to the size of the cavity of β-cyclodextrin. Although several stationary phases containing cellulose derivatives [12,13] or a synthetic chiral polymer [14] had been used for the preparative resolution of planar chiral tricarbonylmetal complexes, their utility in analytical HPLC has not been reported. The HPLC analysis of organometallics [15] is still undeveloped from the point of view of treating the stereochemistry. Therefore, we tested seven types of commercially available chiral columns for the optical purity determination of 22 planar chiral organometallic compounds.

EXPERIMENTAL

Chromatographic procedure

The HPLC apparatus was constructed with a Shimadzu LC-6A pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a Shimadzu SPD-6A UV spectrophotometric detector set at 220 nm and a Shimadzu C-R6A data processor. Prepacked Chiralcel OA, OB, OC, OK and OD and Chiralpak OT(+) and OP(+) columns (250 mm × 4.6 mm I.D.) were obtained from Daicel Chemical Industries (Tokyo, Japan). The system was operated at room temperature at a flow-rate of 0.5 ± 0.02 ml/min with 0.65–2.6 *M* 2-propanol or ethanol in hexane as the mobile phase. The solvents were of JIS-guaranteed reagent grade from Wako (Osaka, Japan).

Organometallics

The following compounds were obtained in our previous work: (±)-**1a** [4], (1*S*)-(+)-**1a** [6], (±)-**1b** [4], (1*R*)-(−)-**1b** [4], (1*R*)-(+)-**2a** [6], (1*R*)-(−)-**3a** [2], (±)-**3b** [4], (±)-**4b** [5], (±)- and (1*R*)-**5a** [1], (±)-**5b** [1], (±)- and (1*R*)-**5c** [1], (±)-**6a** [1], (±)-**6b** [1] and (±)-**6c** [1]. Three $Cr(CO)_3$ complexes, (±)-**1c**, (±)-**2a** and (±)-**2c**, were prepared by complexation of methyl 2-methylbenzoate, 3-methylbenzyl alcohol and methyl 3-methylbenzoate with $Cr(CO)_6$ [16], respectively. $Mn(CO)_3$-complexed esters (±)-**3c** and (±)-**4c** were synthesized according to the literature [17]. The enantiomerically enriched methyl esters (1*R*)-**2c**, (1*S*)-**3c**, (1*S*)-**4c** and (1*R*)-**6c** were prepared by methylation of (1*R*)-(+)-**2e** [3], (1*S*)-(−)-**3e** [4], (1*S*)-(−)-**4e** [3] and (1*R*)-(−)-**6e** [3], respectively, with diazomethane. Dimethyl sulphoxide oxidation [5] of (±)-**2a** gave (±)-**2b** and $LiAlH_4$ reduction of (±)-**3b**, (±)-**4b**, (1*R*)-(+)-**4b** and

(1*R*)-**6c** gave (±)-**3a**, (±)-**4a**, (1*R*)-**4a** and (1*R*)-**6a**, respectively. The optically active aldehyde (1*R*)-(+)-**4b** was obtained as the less reactive enantiomer in horse liver alcohol dehydrogenase (HLADH)-catalysed reduction of (±)-**4b** [5] in 34% yield, $[\alpha]_D^{22}$ + 27° (*c* = 2.1, benzene). The absolute configuration of (+)-**4b** was determined by Ag_2O oxidation [5] to (1*R*)-(+)-**4e**, m.p. 182–183°C, $[\alpha]_D^{23}$ + 8.3° (*c* = 1, ethanol). Acetates (±)-**1d**, (1*S*)-**1d**, (±)-**2d**, (1*R*)-**2d**, (±)-**3d**, (1*R*)-**3d**, (±)-**4d** and (1*R*)-**4d** were prepared by treatment of the corresponding alcohols (1–2 mg) with acetic anhydride (20 μl) and pyridine (20 μl) in 100 μl of toluene and purified by preparative thin-layer chromatography (TLC) on silica gel F_{254} (0.25 mm thick) (Merck) developed with 0–10% ethyl acetate in benzene. All compounds thus prepared showed the molecular ion peak corresponding to each structure in the mass spectrum.

Optical purity determination of the products of lipase-catalysed acetylation

Lipase P (dry powder; 23 U/mg, as assayed with PVA-emulsified olive oil and reciprocal shaking [18]) was supplied by Amano Pharmaceutical (Nagoya, Japan). To 4.9 ml of toluene were added 0.1 ml of vinyl acetate, 50 mg of (±)-**1a** or (±)-**2a** and 0.15 g of lipase P. The mixtures were stirred at 4°C. Aliquots (0.3 ml) were withdrawn at time intervals and then immediately centrifuged to remove the enzyme. A 1-μl volume of each supernatant was dried under vacuum and the residue was dissolved in 1 ml of 10% ethanol in hexane for HPLC analysis (Figs. 3 and 4).

Optical purity determination of the product of HLADH-catalysed reduction

To a solution of (±)-**4b** (0.7 mg) in 50 μl of ethanol were added 0.43 ml of 0.1 *M* phosphate buffer (pH 7.5) containing 0.5 mg of NADH. The turbid mixture was cooled with ice and the reaction was started by addition of 0.04 U of HLADH (Sigma, 2 U/mg, as assayed with ethanol [1]) dissolved in 20 μl of the phosphate buffer. The mixture was stirred at 4°C for 45 min and then at room temperature for 1 h. The reaction progress was monitored by TLC on silica gel F_{254} developed with 20% ethyl acetate in benzene (R_F 0.82 for **4b** and 0.50 for **4a**). Aliquots (0.1 ml) were withdrawn at time intervals and then immediately extracted with hexane (0.5 ml each) in the presence of 30–40 mg of sodium chloride. After appropriate dilution with hexane, the organic layers were analysed by HPLC (Fig. 5).

RESULTS AND DISCUSSION

First, the resolution power of seven columns was investigated with $Cr(CO)_3$-complexed 2-methylbenzyl alcohol (**1a**) and its derivatives of different functionality (**1b–1d**). The Chiralcel OB, OC and OD and Chiralpak OT(+) columns resolved at least one of the four racemic compounds (Table I). The Chiralcel OA and OK and Chiralpak OP(+) columns did not show any clear resolution, although they retained the compounds ($4 < k' < 15$).

The remarkably high resolution given by the OD column is interesting in terms of the structure of the stationary phase. According to the maker's catalogue, the packing materials of the Chiralcel OA, OB, OK, OC and OD columns are coated with cellulose triacetate, tribenzoate, tricinnamate, tris(phenylcarbamate) and tris(3,5-dimethylphenylcarbamate), respectively. It may be reasonable to consider that the solutes are adsorbed on the OD stationary phase by hydrogen bonding to the carbamyl

TABLE I

OPTICAL RESOLUTION OF PLANAR CHIRAL ORGANOMETALLIC COMPOUNDS BY HPLC WITH FOUR CHIRAL COLUMNS[a]

Compound	Column					
	OC			OT(+)		
	k'[b]	α[c]	R_s[d]	k'	α	R_s
1a	*8.86*, 9.33	1.05	0.69	*1.39*, 1.50	1.08	0.51
1b	8.85			*1.85*, 2.07	1.12	0.82
1c	3.46			1.39		
1d	7.00			1.63		
	OB			OD		
	k'	α	R_s	k'	α	R_s
1a	5.31			9.30, *10.02*	1.08	1.20
1b	7.83			-[e]		
1c	3.04, *3.39*	1.12	0.56	*2.06*, 2.28	1.11	1.38
1d	*6.44*, 7.13	1.11	0.68	3.86, *4.39*	1.14	2.08
2a	4.77			*5.86*, 14.77	2.52	15.63
2b	6.43			-[e]		
2c	3.27			*2.39*, 3.48	1.46	5.81
2d	6.16, *6.73*	1.09	0.64	*2.82*, 3.01	1.07	1.08
3a	0.86			3.66, *3.88*	1.06	0.91
3b	1.80			-[e]		
3c	0.68			*0.72*, 0.96	1.33	2.31
3d	0.98			0.99, *1.10*	1.10	1.21
4a	0.83			*2.48*, 3.71	1.50	5.90
4b	1.83			-[e]		
4c	0.78			*1.27*, 1.50	1.18	2.03
4d	0.95			*0.83*, 0.88	1.06	0.70
5a	1.01			2.27, *2.58*	1.14	1.81
5b	1.45			-[e]		
5c	0.65			*0.63*, 0.88	1.39	2.94
6a	0.96			*1.77*, 1.99	1.12	1.46
6b	1.46			-[e]		
6c	0.80			*0.99*, 1.35	1.35	3.41

[a] Chiralcel OB, OC and OD and Chiralpak OT(+) columns. Mobile phase, 1.3 *M* 2-propanol in hexane; flow-rate, 0.5 ml/min.

[b] Capacity factor. Values in italics are for the 1*R*-enantiomer. The enantiomeric assignment is based on the chromatography of the optically active or enantiomerically enriched specimen, except for **1c**. The assignment for **1c** was done by HPLC of the alcohol obtained by $LiAlH_4$ reduction of the ester resolved by the OD column.

[c] Separation factor.

[d] Resolution. Calculated with the width at the peak bottom by the equation $R_s = 2$ (difference between two retention times)/(sum of two peak widths).

[e] Not eluted. The aldehydes were also tested on the OT(+) column (1.3 *M* 2-propanol in hexane) but no resolution was observed for any aldehyde except **1b**.

group, as suggested by Okamoto *et al.* [19] in a study with other chiral compounds and various triphenylcarbamyl cellulose adsorbents. However, the chiral recognition on this phase must be mainly due to a steric effect from the 3,5-dimethyl groups of the phenyl moiety, as the unsubstituted triphenylcarbamate column (OC) was less effective than the OD column for the optical resolution of the present compounds. The dimethyl groups may depress the adsorption by steric hindrance of the adsorbate molecule [20] or modify the size and shape of the spaces in the adsorbent [21] so as to increase the slight difference between the adsorption modes of two enantiomers.

One unsatisfactory result with the OD column was the irreversible binding of aldehyde **1b**. The adsorption was so strong that **1b** was not eluted even with 100% 2-propanol.

The resolving power of the OD column was further studied with other planar chiral organometallic compounds (**2a**–**6c**). The results are summarized in Table I together with those given by the OB column for comparison. The OD column resolved, partly or completely, all compounds except for the aldehydes (**2b**, **3b**, **4b**, **5b** and **6b**). The resolution was considerably influenced by the difference in the metal atom and/or the structure around it. However, the elution order of enantiomers was definite in each of three series of compounds. The 1*R*-enantiomers of all 2-methyl alcohols and acetates (**1a**, **1d**, **3a**, **3d** and **5a**) were eluted after the corresponding 1*S*-enantiomer, and the reverse was found for all 3-methyl alcohols and acetates (**2a**, **2d**, **4a**, **4d** and **6a**). The enantiomers of all methyl esters (**1c**, **2c**, **3c**, **4c**, **5c** and **6c**) were eluted in the order $1R > 1S$. These regularities suggest that the spatial arrangement of two ring substituents (*i.e.*, planar chirality) is the predominant factor in controlling the binding mode, probably through steric interactions with the substituents of the adsorbent.

The separation factor (α) for (±)-**1a**, -**1d**, -**2a** and -**2d** on the OD column was hardly affected by changing the concentration of 2-propanol or ethanol in the mobile phase, whereas the capacity factor (k') and resolution (R_s) increased with decreasing concentration of the alcohols. A typical result for (±)-**1a** is shown in Fig. 2. The

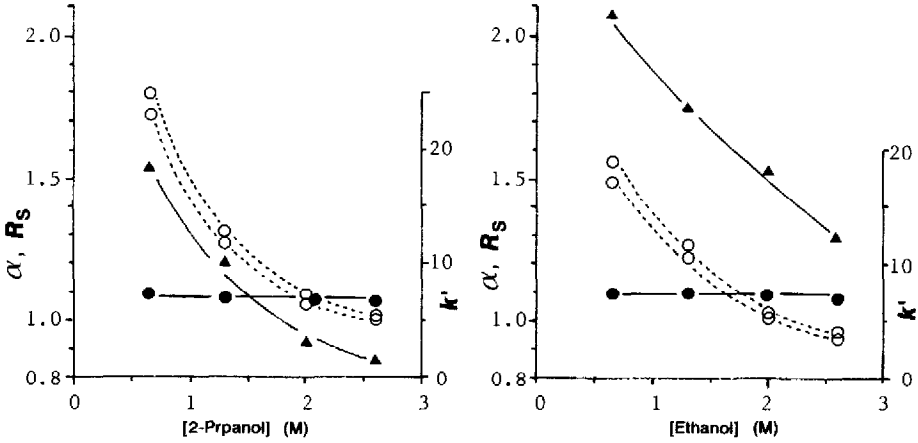

Fig. 2. Effect of the solvent on HPLC of (±)-**1a**. Column, Chiralcel OD (250 mm × 4.6 mm I.D.); flow-rate, 0.5 ml/min; solvent, hexane containing 2-propanol or ethanol. (○) Capacity factor (k'); (●) separation factor (α); (▲) resolution (R_s).

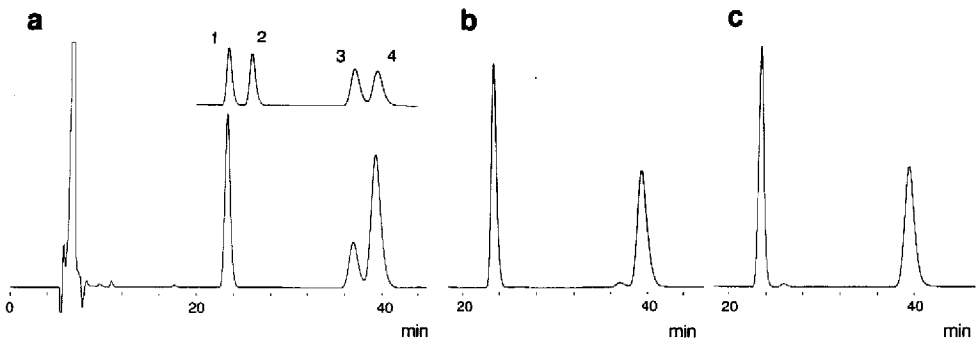

Fig. 3. HPLC of reaction mixtures of (±)-**1a** with lipase P and vinyl acetate after (a) 1 h, (b) 2 h and (c) 3 h. Column, Chiralcel OD; mobile phase, 2.0 *M* 2-propanol in hexane; flow-rate, 0.5 ml/min. The inset is the chromatogram (20–44 min) for an authentic mixture of (1*S*)-**1d** (peak 1), (1*R*)-**1d** (peak 2), (1*S*)-**1a** (peak 3) and (1*R*)-**1a** (peak 4).

alcohols in the mobile phase must work as hydrogen bonding modifiers. The aforementioned effect of the alcohol concentration on the chromatographic parameters supports the idea that the solutes are adsorbed on the OD phase via hydrogen bonding to the carbamyl group, but that this bonding would not be essential to the chiral recognition represented by α. In addition, the analysis with (±)-**1a** showed that ethanol was better than 2-propanol in obtaining high R_s (Fig. 2). This relationship was the reverse, however, for (±)-**1d**, -**2a** and -**2d** (data not shown).

HPLC with the OD column was applied to study the stereoselectivity of two enzymic transformations of planar chiral organometallic compounds. The chromatograms in Fig. 3 show that the (1*S*)-acetate (**1d**) formed by the lipase-catalysed acetylation of (±)-**1a** was optically very pure after 1 h of reaction (Fig. 3a), but the purity decreased to 98.2% e.e. after 2 h (Fig. 3b) and the remaining (1*R*)-**1a** became optically pure after 3 h (Fig. 3c). This result indicates that the optimum chemical and optical yields for both enantiomers are achieved after 2–3 h under the reaction condi-

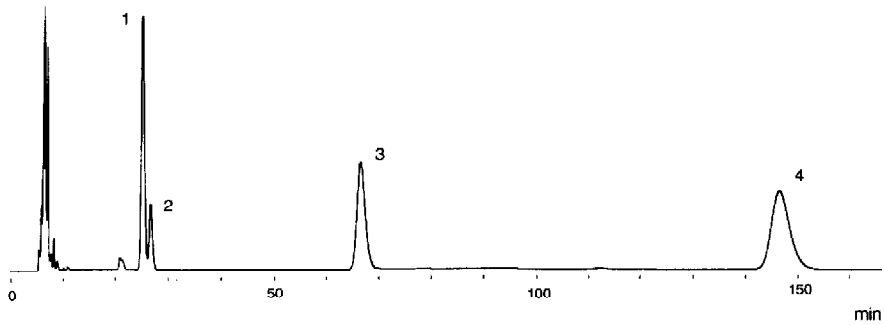

Fig. 4. HPLC of the reaction mixture of (±)-**2a** with lipase P and vinyl acetate after 4 h. Column, Chiralcel OD; mobile phase, 0.65 *M* ethanol in hexane; flow-rate, 0.5 ml/min. Peaks 1, 2, 3 and 4 were assigned as (1*R*)-**2d**, (1*S*)-**2d**, (1*R*)-**2a** and (1*S*)-**2a**, respectively. The ratio of peak areas is 24.0:7.2:26.2:42.5 in the above order.

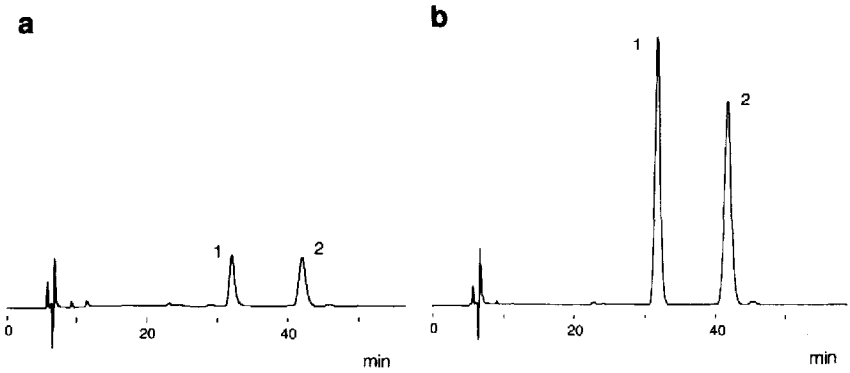

Fig. 5. HPLC of the reaction mixture of (±)-**4b** with HLADH, NADH and ethanol in an ice-bath (a) after 30 min and (b) after standing at room temperature for 1 h. Chromatographic conditions as in Fig. 4. Peaks 1 and 2 are assigned as (1*R*)-**4a** and (1*S*)-**4a**, respectively, and the ratio of the peak areas [(1*S*)-**4a**/(1*R*)-**4a**] is 1.235 ± 0.029 (n = 6) for (a), obtained in three separate runs, and 0.999 ± 0.003 (n = 6) for (b).

tions used. In any case, the 2-methylbenzyl alcohol complex (**1a**) is efficiently resolved by the lipase reaction. This reaction was, however, less enantioselective for the 3-methylbenzyl alcohol complex (**2a**). The chromatogram in Fig. 4 shows that the enantiomeric purity of the product [(1*R*)-**2d**] was 54% e.e. at a conversion rate of about 30%.

The enantioselectivity of HLADH-catalysed reduction was found to be low for the $Mn(CO)_3$-complexed 3-methyl aldehyde (**4b**) [5]. Determination of the small E value (an index of enantioselectivity [22]) was tried using HPLC (Fig. 5). E is given by the equation $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$, where c and ee_p are conversion rate and optical purity (e.e.) of the product, respectively. It was evidenced by TLC that the reaction had been completed before sampling for Fig. 5b. The conversion rate for the sample in Fig. 5a was calculated to be 22.5% by comparison with Fig. 5b. The enantiomeric purity of the alcohol [(1*S*)-**4a**] found in Fig. 5a was 11.5% e.e., and hence E was determined to be 1.30.

In conclusion, HPLC with the Chiralcel OD column is a convenient method for determining the optical purity of planar chiral organometallic alcohols and esters, and will serve for studies of the bioconversion of this special class of compounds.

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