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Enantiomeric enrichment of partially resolved 4-hydroxy-2-carboxymethylcyclopentanone derivatives by achiral phase chromatography

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Abstract

Enantiomeric purity of the chromatographic peaks of partially resolved (*R*)-4-hydroxy-2-carboxymethyl-2-cyclopentenone (**4**), (*R*)-4-hydroxy-2-methoxycarbonylmethyl-2-cyclopentenone (**2**), (*S*)-4-benzoyloxy-2-carboxymethyl-2-cyclopentenone (**5**), (*R*)-4-methoxy-2-carboxymethyl-2-cyclopentenone (**6**), (*R*)-4-methoxy-2-methoxycarbonylmethyl-2-cyclopentenone (**7**) and (−)-5-oxa-6-oxoprostaglandin E_1 C(15) epimers (**1A**) and (**1B**) in achiral normal-phase chromatography on silica gel is dependent on the fraction which is being examined. The mentioned substances exhibit an enantiomer enrichment at the beginning of the chromatographic peak and a gradual depletion in the following parts. The observed enantiomer enrichment effect can be explained by a mechanistic concept which assumes a preferred association between the antipodes of the solute.

1. Introduction

In spite of being contradictory to the generally accepted concept that for chromatographic methods to produce differences in retention times of enantiomers either the stationary phase or the mobile phase must be optically active, in the last decade experimental data suggesting that this assumption is not generally valid have been obtained [1–9]. This problem has been reviewed recently along with its possible explanations [10].

The most confusing examples concern the resolution of racemates by means of chromatographic methods in a totally achiral system [1,8]. Szczepaniak and Ciszewska [1] explained their results for the resolution of D,L-amino acids by achiral ion-exchange chromatography as a consequence of different free energies of formation of the particular enantiomer complexes and hence the differences in the distribution coefficients. Similarly, complex formation has been held responsible for the resolution of ^{14}C-labelled lactic acid enantiomers by Cecchi and Malaspina [8], who used precoated TLC plates with copper(II) acetate. On the other hand, the above results are contradictory in terms of the common opinion that chiral recognition can be effected only by another chiral molecule, and that with optically inactive reagents all enantiomers will have the same reaction rate and equilibrium constants.

The second group of optical resolution of enantiomers by achiral chromatography pertains to the resolution of enantiomers from mixtures

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in which one of them is already in excess. Actually such a chromatographic system cannot be considered as totally achiral because of the presence of a chiral component, an enantiomerically enriched substrate. Consequently, in this case changes in enantiomeric composition (or enantiomeric enrichment) can be interpreted by an enantiomeric differentiation induced solely by an already existing enantiomeric excess during chromatography [2–7,9]. Enantiomeric enrichment of partially resolved substances by achiral phase chromatography has been observed in the cases of nicotine [2], mono- and dipeptide derivatives [3,5,6], 9-methyl-$\Delta^{5(10)}$-octalin-1,6-dione [4], 1,1′-bi-2-naphthol, 1-anthryl-2,2,2-trifluoroethanol, chloromezanone, benzodiazepine camazepam [6], hydroxycineole [7] and sulfoxides [9]. This effect seems not to be limited by any particular chromatographic method or sorbent; these examples cover traditional column chromatography [3,4], flash chromatography [9] and preparative HPLC [2,5–7] using columns packed with normal- and reversed-phase silica gel [3–5,7,9], a cation exchanger [2], aminopropylsilica gel [6] or alumina [9]. However, in some cases the choice of the sorbent was crucial, e.g., the enantiomeric differentiation during chromatography failed on changing aminopropylsilica gel to an unmodified sorbent [6].

2. Experimental

2.1. General

Optical rotation measurements were conducted on a Perkin-Elmer (Überlingen, Germany) Model 141 polarimeter at 20°C. ^{1}H NMR spectra were recorded at room temperature with WH-90/DS and WM-360 spectrometers (Gilberstreifen, Germany) in deuterochloroform using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a MS-50 mass spectrometer (AEI, Manchester, UK) operating at an ionizing potential of 70 eV. IR spectra were recorded with a Perkin-Elmer (V. Frölunda, Sweden) Model 580 B spectrophotometer.

2.2. Materials

All the solvents were purified before use by routine techniques. Optically enriched (*R*)-4-hydroxy-2-methoxycarbonylmethyl-2-cyclopentenone (**2**), (*R*)-4-hydroxy-2-carboxymethyl-2-cyclopentenone (**4**), (*S*)-4-benzoyloxy-2-carboxymethyl-2-cyclopentenone (**5**), (*R*)-4-methoxy-2-carboxymethyl-2-cyclopentenone (**6**) and (*R*)-4-methoxy-2-methoxycarbonylmethyl-2-cyclopentenone (**7**) were synthesized in our laboratory by published procedures [11,12].

2.3. Chromatography

Analytical and preparative normal-phase HPLC were conducted on a Laboratórni Přístroje (Prague, Czechoslovakia) chromatograph using a 150 × 3 mm I.D. column packed with Separon SGX (5 μm) (Tessek, Prague, Czechoslovakia) and a 250 × 10 mm I.D. column packed by Elsiko (Moscow, Russian Federation) with Silasorb SPH 600 (9 μm) (Lachema, Brno, Czechoslovakia), respectively. A differential refractometer in series with a spectrophotometer were used as detectors. The same mobile phases were used for preparative and analytical HPLC runs (see Tables 2–4). In the optical enrichment experiments by preparative HPLC, 0.3 ml of solution containing 30–50 mg of substrate was introduced into the injector loop and chromatographed at a flow-rate 5 ml/min. A 600 × 25 mm I.D. column (LKB, Bromma, Sweden) packed with Silasorb 600 silica gel (30 μm) was employed for column chromatography. TLC analyses were performed on DC-Alufolien Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany).

2.4. Syntheses

(R)-4-Hydroxy-2-carboxymethyl-2-cyclopentenone (**4**)

4-Hydroxy-2-methoxycarbonylmethyl-2-cyclopentenone (**2**) (3.5076 g, 20.6 mmol, $[\alpha]_{365}^{20} = -589.8$ ($c = 1.057$, CH_3OH)) and 0.1 *M* HCl (40 ml) were stirred under reflux for 3 h. To the reaction mixture was added pyridine (0.28 ml, 3.5 mmol) and the solvent was evaporated. The

residue was chromatographed on silica gel (120 g) with $CHCl_3$–dioxane–AcOH (65:35:1.5, v/v/v) as eluent to give 2.9623 g (92%) of pure **4**. R_F [$CHCl_3$–dioxane–AcOH (65:35:1, v/v/v)] 0.28. $[\alpha]_{365}^{20} = -488$ ($c = 1.00$, CH_3OH). ^1H NMR (360 MHz), δ: 7.49 (d, $J = 2.6$, 1H), 5.05 (ddd, $J =$ 2.6, 2.2 and 6.0, 1H), 3.34 (s, 2H), 2.88 (dd, $J = 6.0$, 18.8, 1H), 2.39 (dd, $J = 2.2$, 18.8, 1H). MS, m/z (%): 156 (1) $[M]^{+\cdot}$, 138 (60) $[M - H_2O]^{+\cdot}$, 110 (44) $[M - HCOOH]^{+\cdot}$, 96 (100) $[M - CH_3COOH]^{+\cdot}$.

Tetrahydropyranyl 4-iodobutyrate

To a saturated solution of *p*-toluenesulfonic acid in CH_2Cl_2 (50 ml) under an argon atmosphere was added 4-iodobutyric acid (4.87 g, 22.8 mmol) and freshly distilled dihydropyran (12.45 ml, 136.5 mmol) and the resulting solution was stirred at room temperature until the initial compound had disappeared (1.5 h). To the reaction mixture was added triethylamine (0.5 ml, 3.6 mmol), the solvent was evaporated and the residue was dried under vacuum to give 7.5 g of crude product. For a further synthesis, tetrahydropyranyl 4-iodobutyrate was used without an additional purification. R_F [benzene–EtOAc (14:1, v/v)] 0.32. ^1H NMR (90 MHz), δ: 5.96 (m, 1H), 3.72 (m, 2H), 3.24 (t, 2H), 2.48 (t, 2H), 2.13 (m, 2H), 1.70 (m, 6H). IR (film, cm^{-1}): 1742. MS, m/z (%): 197 (36) $[M - C_5H_9O_2]^+$, 171 (2) $[M - I]^+$, 169 (14) $[M - COOC_5H_9O]^+$, 85 (100) $[C_5H_9O]^+$.

(R)-4-Hydroxy-2-(3′-tetrahydropyranyloxycarbonylpropyl)oxycarbonylmethyl-2-cyclopentenone

To a solution of (*R*)-**4** (2.9623 g, 19.0 mmol) with $[\alpha]_{365}^{20} = -488$ ($c = 1.00$, CH_3OH) in dry dimethylformamide (30 ml) under an argon atmosphere was added Cs_2CO_3 (3.25 g, 10.0 mmol) and the reaction mixture was stirred at 50°C for 1 h. To the reaction medium was added a solution of crude tetrahydropyranyl 4-iodobutyrate [prepared from 4.87 g (22.8 mmol) of 4-iodobutyric acid as described above] in dimethylformamide (25 ml) and the resulting mixture was stirred at 50°C for 6.5 h. The dark mixture was diluted with EtOAc (500 ml), washed with 1% $NaHCO_3$ (3×150 ml) and saturated NaCl (100 ml) and dried (Na_2SO_4). Before drying, to the EtOAc extract was added triethylamine (TEA) (0.5 ml, 3.6 mmol). The solvent was removed and the residue (12.4 g) was chromatographed on silica gel (150 g) with hexane–EtOAc–*iso*–PrOH–TEA (50:50:1:0.1, v/v) (900 ml) and (20:80:1:0.1, v/v) (800 ml) as eluents to give 4.55 g (73.5%) of pure product. $[\alpha]_{365}^{20} = -203$ ($c = 1.06$, CH_3OH), -210 ($c = 1.03$, $CHCl_3$). R_F [hexane–EtOAc–dioxane (7:5:3, v/v/v)] 0.20. ^1H NMR (90 MHz), δ: 7.43 (m, 1H), 5.98 (m, 1H), 4.98 (m, 1H), 4.18 (t, 2H), 3.78 (m, 2H), 3.27 (m, 2H), 3.09 (s, 1H), 2.84 (dd, 1H), 2.47 (t, 2H), 2.41 (dd, 1H), 2.01 (m, 2H), 1.73 (m, 6H). MS, m/z (%): 139 (51) $[M - O(CH_2)_3COOC_5H_9O)]^+$, 138 (51) $[M - CH_2 = CHCH_2COOC_5H_9O - H_2O]^{+\cdot}$, 110 (100) $[M - HCOO(CH_2)_3COOC_5H_9O]^{+\cdot}$, 84 (9) $[C_5H_8O]^{+\cdot}$. IR (CH_2Cl_2, cm^{-1}): 3605, 3458, 1740, 1719, 1646.

(R)-4-Trimethylsilyloxy-2-(3′-tetrahydropyranyloxycarbonylpropyl)-oxycarbonylmethyl-2-cyclopentenone (**3**)

To a solution of the above-prepared (*R*)-4-hydroxy-2-(3′-tetrahydropyranyloxycarbonylpropyl)oxycarbonylmethyl-2-cyclopentenone (4.55 g, 13.9 mmol) with $[\alpha]_{365}^{20} = -210$ ($c = 1.03$, $CHCl_3$) in dry benzene (17 ml) under an argon atmosphere were successively added hexamethyldisilazane (3.00 ml, 14.2 mmol) and chlorotrimethylsilane (0.92 ml, 7.2 mmol), and the reaction mixture was stirred at room temperature for 3 h. The solvent and volatiles were evaporated and the residue was dissolved in Et_2O–hexane (1:1, v/v) (200 ml) and stored at -18°C overnight. The solution was filtered, washed with Et_2O–hexane (1:1, v/v) (30 ml) and evaporated to give 5.00 g (90.0%) of pure **3**. $[\alpha]_{365}^{20} = -258$ ($c = 0.96$, $CHCl_3$). R_F [hexane–EtOAc–dioxane (7:5:3, v/v/v) 0.65. ^1H NMR (90 MHz), δ: 7.31 (m, 1H), 5.96 (m, 1H), 4.92 (m, 1H), 4.15 (t, 2H), 3.73 (m, 2H), 3.23 (m, 2H), 2.76 (dd, 1H), 2.44 (t, 2H), 2.33 (dd, 1H), 1.99 (m, 2H), 1.69 (m, 6H), 0.18 (s, 9H). MS,

m/z (%): 314 (3) $[M-C_5H_8O]^{+\cdot}$, 211 (35) $[M-O(CH_2)_3COOC_5H_9O]^+$, 182 (39) $[M-HCOO(CH_2)_3COOC_5H_9O]^{+\cdot}$, 85 (87) $[C_5H_9O]^+$, 75 (100) $[(CH_3)_2SiOH]^+$. IR (CH_2Cl_2, cm^{-1}): 1739, 1717, 1646.

(−)-5-Oxa-6-oxoprostaglandin E_1 (**1A**) *and (−)-5-oxa-6-oxo-15-epi-prostaglandin E_1* (**1B**)

The (*RR* + *SS*)-diastereomer of 1-iodo-3-terahydrofuranyloxy-1-octene [13] (4.27 g, 13.2 mmol) under an argon atmosphere was dissolved in dry Et_2O (70 ml) and to this solution at −30°C was slowly (ca. 5 min) added 3.31 *M* *n*-BuLi hexane solution (4.18 ml, 13.8 mmol). The reaction mixture was stirred at −30 to −25°C for 15 min, then powdered pentynylcopper (1.46 g, 11.2 mmol) was added in one portion. The yellow suspension was stirred at −25 to −20°C for 15 min, cooled to −70°C and (*R*)-**3** (5.00 g, 12.5 mmol, $[\alpha]_{365}^{20} = -258$ (c = 0.96, $CHCl_3$)) solution in Et_2O (35 ml) was slowly (ca. 20 min) added at −70 to −65°C. Stirring was continued at −70°C for 10 min, then the mixture was allowed gradually to warm to −40°C during 30 min. To the yellowish brown suspension was added glacial AcOH (1.5 ml, 26.2 mmol), the cooling bath was removed and the mixture was stirred for 15 min. The reaction mixture was diluted with EtOAc (100 ml), filtered and the precipitate was washed with EtOAc (2 × 50 ml). The green filtrate was evaporated, the residue was dissolved in dioxane (100 ml), and to the solution was added 0.1 *M* HCl (100 ml). The reaction mixture was stirred at room temperature for 3 h, saturated with crystalline NaCl, extracted with EtOAc (3 × 150 ml), washed with saturated NaCl (50 ml) and dried (Na_2SO_4). Before drying, to the extract was added triethylamine (0.2 ml, 1.4 mmol). The solvent was evaporated and the residue (9.49 g) was chromatographed on silica gel (100 g) with hexane–dioxane–AcOH (58:42:1, v/v/v) (1000 ml) and (20:80:1, v/v/v) (300 ml) as eluents to give 3.948 g of product containing mainly the isomers **1A** and **1B**. Spatial orientation of the C(15) OH group was ascribed on the basis of the observations that usually the 15α-isomer of prostaglandins and their derivatives in normal-phase chromatography is more polar than the corresponding 15β-isomer [14]. A 0.9333-g amount of the isomer mixture **1A** + **1B** was chromatographed on silica gel (110 g) with $CHCl_3$–acetone–AcOH (40:60:1, v/v/v) (1000 ml) as eluent. Each of the less polar isomer **1B** and more polar **1A** were divided into five consecutive fractions, which after evaporation and exhaustive drying afforded 0.3351 g (0.03276, 0.10181, 0.08790, 0.07577 and 0.03685 g) of **1B** (30.5%) and 0.3298 g (0.03946, 0.04593, 0.06661, 0.09981 and 0.07797 g) of **1A** (30.0%). **1A:** $[\alpha]_D^{20}$, see Table 3, entry 6. R_F [$CHCl_3$–acetone–AcOH (50:50:1, v/v/v)] 0.18. ^1H NMR (360 MHz), δ: 5.78 (dd, 1H), 5.57 (dd, 1H), 4.18 (dt, 1H), 4.13 (q, 1H), 4.10 (q, 1H), 4.09 (dt, 1H), 2.79 (dd, 1H), 2.60 (dd, 1H), 2.55 (dd, 1H), 2.50 (m, 1H), 2.43 (t, 2H), 2.39 (m, 1H), 2.36 (dd, 1H), 1.98 (q, 2H), 1.53 (m, 2H), 1.30 (m, 6H), 0.89 (t, 3H), *OH* and *COOH* are not indicated. IR (THF, cm^{-1}): 3439, 3058, 1748, 1740. MS was recorded for methyl ester of **1A:** R_F [$CHCl_3$–acetone–AcOH (50:50:1, v/v/v)] 0.31. MS, *m/z* (%): 348 (0.2) $[M-2H_2O]^{+\cdot}$, 295 (0.6) $[M-H_2O-C_5H_{11}]^+$, 202 (7) $[M-2H_2O-HCOO(CH_2)_3COOCH_3]^{+\cdot}$, 177 (17) $[M-H_2O-C_5H_{11}-HO(CH_2)_3COOCH_3]^+$, 149 (48) $[M-H_2O-C_5H_{11}-HCOO(CH_2)_3COOCH_3]^+$, 101 (100) $[(CH_2)_3COOCH_3]^+$. Isomer **1B:** $[\alpha]_D^{20}$, see Table 3, entry 6. R_F [$CHCl_3$–acetone–AcOH (50:50:1, v/v/v)] 0.27. ^1H NMR (360 MHz), δ: 5.73 (dd, 1H), 5.60 (dd, 1H), 4.17 (dt, 1H), 4.15 (q, 1H), 4.13 (q, 1H); 4.12 (dt, 1H), 2.79 (dd, 1H), 2.61 (dd, 1H), 2.55 (dd, 1H), 2.50 (m, 1H), 2.43 (t, 2H), 2.40 (m, 1H), 2.35 (dd, 1H), 1.98 (q, 2H), 1.53 (m, 2H), 1.30 (m, 6H), 0.89 (t, 3H), *OH* and *COOH* are not indicated. IR (CH_2Cl_2, cm^{-1}): 3600, 1748, 1736, 1718. MS was recorded for methyl ester of **1B:** R_F [$CHCl_3$–acetone–AcOH (50:50:1, v/v/v) 0.36. MS, *m/z* (%): 348 (0.5) $[M-2H_2O]^{+\cdot}$, 295 (2) $[M-H_2O-C_5H_{11}]^+$, 202 (5) $[M-2H_2O-HCOO(CH_2)_3COOCH_3]^{+\cdot}$, 177 (16) $[M-H_2O-C_5H_{11}-HO(CH_2)_3COOCH_3]^+$, 149 (24) $[M-H_2O-C_5H_{11}-HCOO(CH_2)_3COOCH_3]^+$, 101 (100) $[(CH_2)_3COOCH_3]^+$.

Table 1
Crystal data and experimental conditions for X-ray analysis of **4**

Formula	$C_9H_8O_4$
Molecular mass	156.65
Crystal dimensions (mm)	0.10 × 0.35 × 0.45
Crystal system	Triclinic
Space group	$P\bar{1}$
a (Å)	4.691 (1)
b (Å)	5.351 (1)
c (Å)	14.345 (2)
α (°)	97.23 (1)
β (°)	95.64 (1)
γ (°)	96.48 (1)
V (Å^3)	354.8 (1)
Z	2
Calc. density (g/cm^3)	1.46
μ (Mo Kα) (cm^{-1})	0.8
Radiation	Mo Kα
λ (Å) (graphite monochromator)	0.7107
Diffractometer	Syntex $P2_1$
Maximum value (sin θ/λ)	0.596
Measured reflections	1424
Unique reflections	1255
Observed reflections	922
$R(F)$	0.0481
Max. residual electron density (e/Å^3)	0.20

2.5. X-ray diffraction studies

A racemic crystal for X-ray measurements of compound **4** was grown from a mixture of hexane and ethyl acetate. The reflection intensities were collected on a Syntex (Cupertino, CA, USA) $P2_1$ single-crystal diffractometer using graphite-monochromated Mo Kα radiation ($\lambda = 0.71069$ Å). The structure was solved by a direct method using the program SHELXS 86 [15] and refined by SHELXS 76 implemented on an IBM-PC/AT [16]. During refinement, absorption correction (program DIFABS) was applied [17]. Crystallographic data for **4** are listed in Table 1.

Results and discussion

We studied the phenomenon of enantiomeric enrichment of a partially resolved material by achiral-normal phase chromatography on silica gel working with cyclopentanone derivatives.

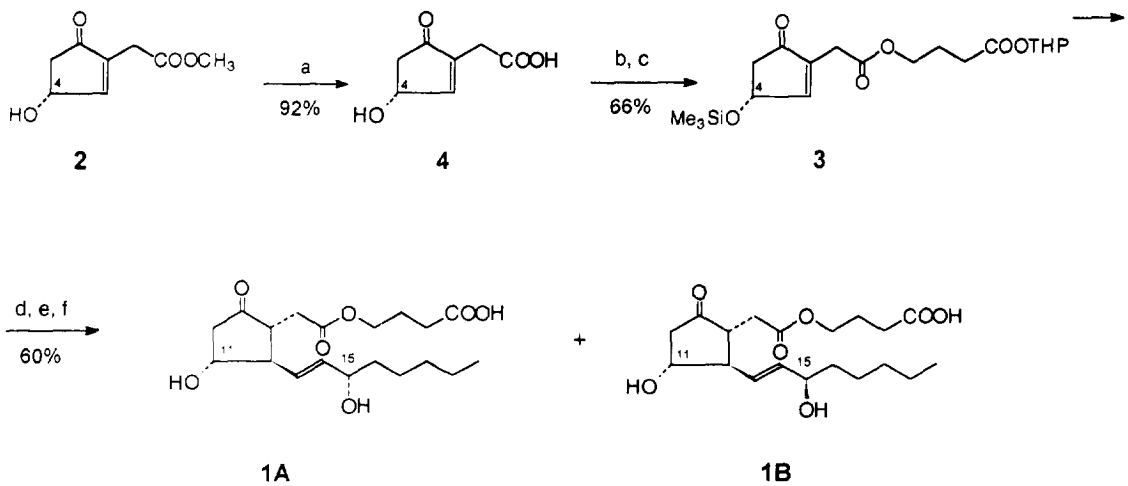
In connection with our recent efforts to prepare 5-hetero-6-oxoprostaglandins E_1 [18–20] we targeted the synthesis of optically active 5-oxa-6-oxoprostaglandin E_1 (**1A**) (Fig. 1). 4-Hydroxy-2-methoxycarbonylmethyl-2-cyclopentenone (**2**) partially resolved [enantiomeric excess (*ee*) ca. 40%] by enzymatic methods [11] served as a starting compound for this synthesis. Cuprate addition of a racemic prostaglandin ω-chain to an appropriately modified synthon **3** followed by a hydrolysis of protective groups afforded the desired prostanoid **1A** as a mixture along with its C(15) epimer **1B**.

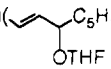
It was envisaged that the *ee* of each of the isomers **1A** and **1B** should be the same as for the starting synthon **2** provided that the chiral centre on a C(4) atom [or C(11) atom according to prostaglandin nomenclature] would not suffer racemization under the reaction conditions outlined in Fig. 1.

The epimer mixture **1A** + **1B** was purified from by-products using routine column chromatography on silica gel, but a further isolation of the individual C(15) isomers **1A** and **1B** was performed by preparative HPLC. Each of the baseline-separated chromatographic peaks of 5-oxa-6-oxoprostaglandin E_1 C(15) epimers **1A** and **1B** were divided into two parts in their maxima (Fig. 2).

Optical rotation measurements (at five wavelengths) of the samples obtained revealed apparent enantiomeric enrichment (changes in the optical purity really reflect changes in *ee*, as will be discussed later) of both C(15) epimers in the beginning of the chromatographic peak (a, c) and depletion in the last part (b, d) (Fig. 2 and Table 2).

The observed changes in optical purity of eluate were not due to undetected impurities or unexpected racemization during the chromatographic process because of the following considerations. (1) The ratios of optical rotation of the samples $a:b$ or $c:d$ (see Fig. 2 and Table 2) at the five wavelengths were constant, indicating an identical shape of the optical dispersion curve of the material obtained from both parts of the chromatographic peak. (2) Optical rotation values of the isomers **1A** and **1B** obtained by



a) 0.1N HCl, reflux. b) Cs_2CO_3 / $I(CH_2)_3COOTHP$ / DMF. c) Me_3SiCl / $(Me_3Si)_2NH$.
d) LiCu()($-C\equiv C-C_3H_7$). e) O.1N HCl-dioxane, 1:1. f) Chromatography.

Abbreviations: THF - tetrahydrofyranyl, THP - tetrahydropyranyl.

Fig. 1. Synthesis of **1A** and **1B**.

collecting all the corresponding chromatographic peak were nearly the same as calculated from the divided samples (Table 2), i.e., an overall depletion of one antipode in the part of eluate was

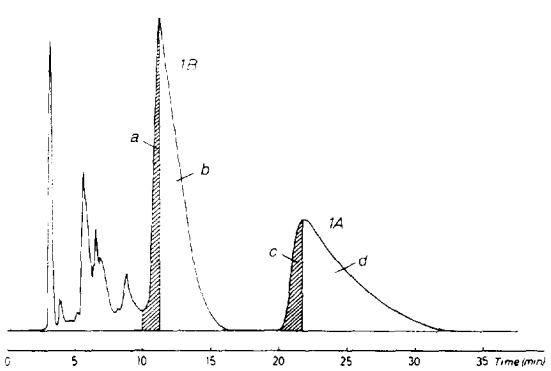


Fig. 2. HPLC separation of (−)-5-oxa-6-oxoprostaglandin E_1 C(15) epimers **1A** and **1B**. Conditions: mobile phase, hexane–ethyl acetate–acetic acid (20:80:1, v/v/v); column, 250 × 10 mm I.D., Silasorb SPH 600 (9 μm); injection volume, 0.3 ml; flow-rate, 5 ml/min; detector, refractometer.

equivalent to its enrichment in the other as required by the mass balance. (3) HPLC analysis of the samples *a–d* (Fig. 2) did not reveal impurity peaks; further, both *a* and *b* or *c* and *d* were chromatographically (HPLC and TLC) and spectroscopically (NMR) non-distinctive. (4) The observed changes of the optical rotation were not due to racemization because it is impossible to imagine a reasonable process changing simultaneously to the same extent all four different stereogenic centres of a prostanoid molecule, but epimerization of a few of them should be easily detectable by HPLC and NMR.

Several experiments were carried out revealing that the optical enrichment of prostanoids **1A** and **1B** during chromatography was not accidental but really was an intrinsic feature persistent to the chromatographic system under consideration.

First, it was established that each of the preliminary separated single isomers **1A** and **1B** on passing through a new preparative HPLC

Table 2
Optical rotation data ($[\alpha]_\lambda^{20}$, in CH_3OH) for samples of prostaglandin E_1 analogues **1A** and **1B** obtained by dividing the corresponding preparative HPLC peaks into two parts in their maxima

λ (nm)	Compound **1B**					Compound **1A**				
	$[\alpha]_\lambda^{20}$ of divided[a] chrom. peak		Ratio of $[\alpha]_\lambda^{20}$ for	$[\alpha]_\lambda^{20}$ calculated[b] for full peak	$[\alpha]_\lambda^{20}$ of full peak	$[\alpha]_\lambda^{20}$ of divided[a] chrom. peak		Ratio of $[\alpha]_\lambda^{20}$ for	$[\alpha]_\lambda^{20}$ calculated[b] for full peak	$[\alpha]_\lambda^{20}$ of full peak
	a	*b*	*a* : *b*			*c*	*d*	*c* : *d*		
589	30.3	−11.3	2.67	15.3	−16.5	−24.3	−11.2	2.17	−14.0	−14.3
578	−31.9	11.9	2.68	−16.2	17.4	25.1	11.7	2.20	14.7	−15.1
546	−37.7	−14.1	2.67	−19.1	−20.3	30.4	−13.9	2.19	−17.4	17.9
436	−82.8	−31.3	2.65	42.3	−45.1	−68.6	−31.2	2.20	−39.2	−40.6
365	201.3	79.9	2.52	−105.8	−112.3	−173.5	79.2	2.19	99.4	−103.5
Mass (%)[c]	21.3	78.7			100	21.4	78.6			100
c	1.63	1.62			1.90	1.67	1.65			1.87

Eluent, hexane–ethyl acetate–acetic acid (20:80:1), v/v/v); for other conditions of HPLC separation, see Experimental.
[a] For designations *a*, *b*, *c* and *d*, see Fig. 2.
[b] Optical activity $[\alpha]$ of full chromatographic peak was calculated by the equation $[\alpha] = \Sigma[\alpha]_n m_n$ where $[\alpha]_n$ is the optical activity of part *n* of the peak and m_n is the relative mass of part *n* with respect to the full peak.
[c] With respect to the full peak.

column gave an optically enriched material in the first portion of the chromatographic peak and gradually depleted in the following portions (Table 3; entries 1 and 2).

The same was true using the isomer **1A** of different optical purity obtained in the previous optical enrichment experiments (Table 3; entries 3 and 4). These results indicated that the enrichment was not due to a column contaminated with an unknown chiral compound and further strengthened our confidence that there were no undetected impurities in the sample responsible for the observed effect. Changing the eluent from hexane–ethyl acetate–acetic acid (20:80:1, v/v/v) to chloroform–acetone–acetic acid (60:40:1, v/v/v) diminished the observed effect (Table 3; entry 5). The enantiomer enrichment effect remained after changing the HPLC column to the usual LC column, although the changes in optical purity were not so expressive (Table 3, entry 6).

Being interested in this unexpected phenomenon, we also examined the chromatographic behaviour of 4-hydroxy-2-carboxymethyl-2-cyclopentenone (**4**) and several its derivatives, **2**, **5**, **6** and **7** (see Table 4).

Surprisingly, virtually all of the compounds tested showed a similar effect: an optical enrichment in the first part of the chromatographic peak and gradual depletion in the following parts of the peak (Table 4). On the basis of the optical rotation measurements depicted in Table 4, one can easily confirm that the arguments (1) and (2) proposed to prove the reliability of an enantiomeric enrichment phenomenon for prostanoids **1A** and **1B** still remain valid. The same applies to HPLC, TLC and NMR analysis; samples obtained from divided chromatographic peaks by preparative HPLC of the corresponding cyclopentenone derivatives were non-distinctive. Since the cyclopentenone molecules **2** and **4–7** possess only one stereogenic centre, excluding racemization of the compound during chromatography is more difficult. However, in our case this was not a cause because the values of the total optical activity of fractionated samples were equal to those before chromatography (Table 4). In addition, it has been shown [12,21] that protonation of 4-hydroxy-2-carboxymethyl-2-cyclopentenone (**4**) and its methyl ester **2** in moderately acidic media and chromatographic purification do not affect the stereogenic centre of the molecule.

Unfortunately, we were not able to find an alternative direct method to detect the enantiomeric purity of the samples; the only criterion to judge changes in enantiomeric ratios were the optical rotation measurements. Nevertheless, optical rotation data obtained at five wavelengths combined with chemical purity control (analytical HPLC and TLC) provide a sufficiently reasonable basis to consider that our results really reflect the changes in enantiomeric excess during chromatography of the investigated compounds, although in a relative sense; however, this does not change the point of the matter.

The experimental data obtained fit a mechanistic concept for the explanation of an enantiomeric enrichment effect which is based on thermodynamically non-ideal behaviour of molecules, i.e., on molecular interactions [3,4].

Retention in liquid chromatography is a complex process involving solute interactions in both the mobile and stationary phases that are difficult to describe exactly [22]; nevertheless, a simplified working hypothesis assuming bilayer formation (solvent interaction model) on the surface of adsorbent would be the following.

It is evident that the molecular surrounding of, e.g., the (−)-isomer in the enantiomerically enriched [(−)] > [(+)] mixture (or a solution of their mixture) is different from the molecular surrounding of the corresponding (+)-isomer in the same mixture. Statistically, *homo*-associations (−)···(−) in this case numerically exceed *cross*-associations (−)···(+), which in turn exceed *homo*-associations (+)···(+). The thermodynamics of these interactions may or may not be the same, thus affecting these statistical ratios. On the other hand, interactions of the both (−)- and (+)-antipodes with a sorbent should be identical (provided that distortions induced by thermodynamically non-ideal behaviour of molecules are ignored) forming the first layer of adsorbed molecules which contains both enantiomers in amounts close to their initial

Table 3
Optical purity changes across the chromatographic peaks of prostaglandin E_1 analogues **1A** and **1B** in achiral normal-phase chromatography

Expt. No.	Substrate	Method	Eluent composition (v/v)	$[\alpha]_D^{20}$ [a] (mass-% of peak)	$[\alpha]_D^{20}$ calculated[b] for full peak	$[\alpha]_D^{20}$ of full peak
1	**1A**	HPLC	Hexane–EtOAc–AcOH (10:90:1)	−32.8 (25.9); −13.8 (36.6); −9.7 (37.5)	−17.2	−16.2[c]
2	**1B**	HPLC	Hexane–EtOAc–AcOH (25:75:1)	−40.8 (17.8); −22.8 (34.9); −12.0 (47.1)	−20.9	−19.8[c]
3	**1A**	HPLC	Hexane–EtOAc–AcOH (10:90:1)	−26.3 (23.3); −8.8 (76.7)	−12.8	−12.0[c]
4	**1A**	HPLC	Hexane–EtOAc AcOH (10:90:1)	−33.8 (20.8); −30.4 (42.0); −17.0 (37.2)	−26.1	−27.1[c]
5	**1A** + **1B**	HPLC	$CHCl_3$–acetone–AcOH (60:40:1)	**1B:** −25.0 (21.8); −15.1 (78.2) **1A:** −24.9 (25.2); −15.1 (74.8)	−17.3 −17.6	−16.5[d] −14.3[d]
6	**1A** + **1B**	LC	$CHCl_3$–acetone–AcOH (60:40:1)	**1B:** −16.4 (9.8); −19.0 (30.4); −16.2 (26.2); −15.4 (22.6); −11.5 (11.6) **1A:** −16.9 (12.0); −18.2 (13.9); −16.7 (20.2); −15.1 (30.3); −13.2 (23.6)	−16.4 −15.6	−16.5[d] −14.3[d]

For conditions of HPLC and LC separations, see Experimental.

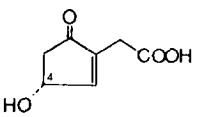
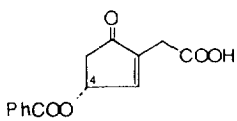
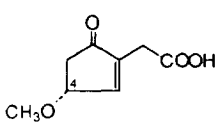
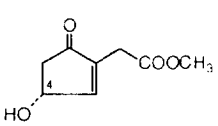
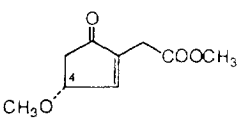
[a] $[\alpha]_D^{20}$ values of chromatographic fractions (c = 1.00–1.15, CH_3OH) are given in order of their elution from the column; the first fraction in the experiments 1–5 covers the chromatographic peak from the beginning up to its maximum point. Optical rotation was measured at 589 (D line), 578, 546, 436, and 365 nm; analysis of the data obtained allowed the same conclusions to be drawn as from the measurements depicted in Table 2.

[b] Footnote b, Table 2.

[c] Before chromatography.

[d] Purified with hexane–EtOAc–AcOH (20:80:1, v/v/v) as eluent. Value taken from Table 2.

Table 4
Optical purity changes across the chromatographic peaks of cyclopentenones **2** and **4**–**7** in achiral normal-phase HPLC

Compound and eluent composition (v/v)	$[\alpha]_\lambda^{20}$ ($c = 0.58$–1.45, CH_3OH)						Ratio of $[\alpha]_\lambda^{20}$ for $a:b:c$
	λ (nm)	Divided peak[a]			Before chrom.	Calculated[b] for full peak	
		a	*b*	*c*			
 **4**	589	37.9	29.5	21.4	26.9	27.5	1.77 : 1.38 : 1
	578	37.6	29.1	21.2	26.5	27.2	1.77 : 1.37 : 1
	546	36.0	28.0	20.3	25.4	26.1	1.77 : 1.38 : 1
	436	−53.8	−41.0	−31.7	−39.2	−39.2	1.70 : 1.29 : 1
	365	−1215.0	−938.8	−706.5	−874.0	−887.7	1.72 : 1.33 : 1
Hexane–EtOAc–AcOH (20:80:1)	Mass (%)[c]	16.9	41.0	42.1			
 **5**	589	−97.7	−95.1	−65.5	−78.8	−79.1	1.49 : 1.45 : 1
	578	−101.0	−99.1	−73.4	−82.1	−85.1	1.38 : 1.35 : 1
	546	−114.9	−112.0	−77.1	−92.6	−93.1	1.49 : 1.45 : 1
	436	−171.3	−167.5	−115.4	−137.9	−139.1	1.48 : 1.45 : 1
	365	157.5	152.4	86.0	129.9	116.3	1.83 : 1.77 : 1
Hexane–EtOAc (70:30)	Mass (%)[c]	21.0	23.0	56.0			
 **6**	589	3.8	2.0		2.4	2.4	1.90 : 1
	578	5.5	3.3		3.1	3.8	1.67 : 1
	546	2.8	1.6		1.1	1.9	1.75 : 1
	436	−54.2	34.9		−37.2	−39.3	1.55 : 1
	365	−651.2	−414.4		−435.3	−468.8	1.57 : 1
Hexane–EtOAc (40:60)	Mass (%)[c]	23.0	77.0				
 **2**	589	19.4	14.8	12.6	15.4	15.0	1.54 : 1.17 : 1
	578	19.9	15.3	13.1	15.6	15.5	1.52 : 1.17 : 1
	546	19.2	14.6	12.7	15.2	14.9	1.51 : 1.15 : 1
	436	−24.6	−19.5	−16.1	−19.0	−19.4	1.52 : 1.21 : 1
	365	−609.6	473.8	−396.8	−475.8	−476.6	1.54 : 1.19 : 1
Hexane–EtOAc (30:70)	Mass (%)[c]	22.0	42.8	35.2			
 **7**	589	7.6	5.4			5.8	1.41 : 1
	578	9.4	6.7			7.2	1.40 : 1
	546	4.7	3.0			3.3	1.51 : 1
	436	−88.9	−72.7			−75.6	1.22 : 1
	365	−1090.8	−879.7			−917.9	1.24 : 1
Hexane–EtOAc (40:60)	Mass (%)[c]	18.1	81.9				

For conditions of HPLC separations, see Experimental.
[a] Fractions from divided chromatographic peak are given in the order of their elution from the column; fraction *a* covers the chromatographic peak from the beginning up to the maximum point.
[b] Footnote b, Table 2.
[c] With respect to the full peak.

statistical ratio. If the (−)-isomer energetically prefers *cross*-associations (−)···(+) to *homo*-associations (−)···(−) the second layer of more weakly associated molecules should contain both enantiomers in a ratio different from the initial ratio or, in our example, increased in the (+)-isomer compared with the initial ratio. As a result, the partition coefficient of the (+)-isomer between the stationary and the mobile phases will be larger than that of the (−)-isomer {provided that all the time [(−)] > [(+)]} and hence the first fractions of the eluate will be enriched in the (−)-isomer.

If this mechanistic concept is applicable to interpret the enantiomeric enrichment phenomenon, there should be the following consequences: (1) if *cross*-associations of solutes are preferred to *homo*-associations, the first fractions of eluate will be enriched in the enantiomer present in excess; (2) if *homo*-associations of solutes are preferred to *cross*-associations, the outcome will be the opposite, i.e., the last fractions will be enriched in the enantiomer present in excess; (3) apparently, one might under the most favourable circumstances be able to separate not more than the excess enantiomer from the residual racemic mixture by this procedure. It is noteworthy that the character of enantiomeric enrichment (enriched at the beginning of the chromatographic peak or vice versa) should not be susceptible to the retention mechanism (type of adsorbent).

All our optical resolution experiments by achiral normal-phase chromatography gave an eluate enriched in the first fractions with the enantiomer present in excess {an example of a reversed enantiomeric enrichment (i.e., the eluate enriched in the last fractions of the chromatographic peak) can be found in the Ref. [3]}. Consequently, according to the above-discussed mechanistic concept, in our case *cross*-associations of enantiomers should be favoured over *homo*-associations. Trying to find evidence for such a behaviour, we found that crystalline enantiomerically enriched cyclopentanone derivatives **1A**, **4** and **5** all tend to crystallize as racemates. This observation indicates that enantiomeric crystal packing forces of **1A**, **4** and **5** are stronger than homomeric ones. Moreover, in the case of racemic **4**, X-ray structural investigations were performed. A perspective view of the molecular packing of **4** in the unit cell with crystallographic labelling of atoms is shown in Fig. 3.

Two kinds of hydrogen bonds of an intermolecular nature were found in the crystal structure of **4** (Table 5). The shortest of them includes two hydrogen bonds (e.g., O3–H103···O2) which are generated by an inversion centre; they bond enantiomeric molecules (antipodes) in the crystal via carboxyl groups. The second kind of hydrogen bonds is formed by hydroxy and oxo functions of the cyclopentenone **4** molecule, and they are responsible for bonding of infinite chains parallel to shorter diagonals of the *ab* planes of the crystal.

Another explanation of the enantiomeric enrichment phenomenon is based on an assumption

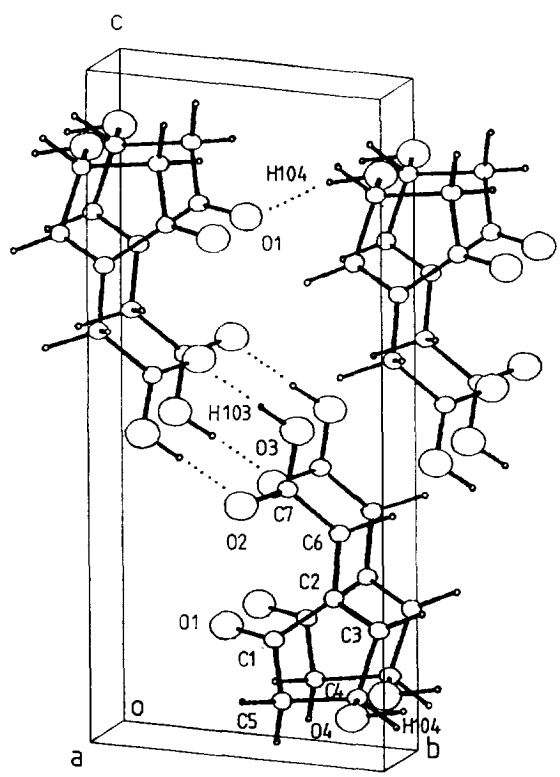


Fig. 3. Perspective view of the molecular packing of (±)-**4** in the unit cell.

Table 5
Intermolecular hydrogen bonds in **4**

D–H···A	D–H (Å)	D···A (Å)	H···A (Å)	Angle at H (°)	Acceptor symmetry
O3–H103···O2	0.93 (4)	2.674 (3)	1.75 (4)	175 (3)	$-x, -y, -z$
O4–H104···O1	1.01 (4)	2.813 (3)	1.83 (4)	164 (4)	$1+x, 1+y, z$

that differences in retention times of the racemate and an individual antipode could be due to different mobilities of more or less stable *homo*- and *hetero*-associates during chromatography [2,5,6]. On the other hand, it is well known [23] that carboxylic acids tend to form dimeric associates via carboxyl groups, especially in an aprotic medium. We tested the analytical HPLC mobilities of compounds **1A** and **4** [the amount of samples chromatographed was 15 μg in 3 μl and the eluent was hexane–EtOAc–AcOH (10:90:1)]. Unfortunately, we were not able to find any statistically credible retention time differences for racemic and optically active forms of these substances. The obtained retention times (retention time values are given in the form $x \pm$ S.D., where x is the mean value and S.D. is the standard deviation) were as follows: (±)-**1A**, 12.74 ± 0.23 (five injections); (−)-**1A** {$[\alpha]_D^{20} = -33.8$ ($c = 1.02$, CH_3OH)}, 12.73 ± 0.30 (five injections); (±)-**4**, 7.69 ± 0.32 (six injections); and (*R*)-**4** {$[\alpha]_D^{20} = +37.9$ ($c = 0.96$, CH_3OH), *ee* ca. 100%}, 7.72 ± 0.33 min (six injections). Moreover, according to the structures of cyclopentenones given in Table 4, carboxylic acids **4**, **5** and **6** should be able to form the strongest H-bonds. H-bonding of the secondary alcohol **2** should be less strong than in the case of the above carboxylic acids but the cyclopentenone **7** is not capable of forming H-bonds at all. Nevertheless, the data given in Table 4 indicate that there are no substantial enantiomer enrichment differences among the compounds of these groups. Apparently, in the case of cyclopentenones **2** and **4**–**7** the capability to form H-bonds and hence more stable associates does not play a crucial role in ensuring the enantiomeric enrichment during chromatography. It is likely that other kinds of associates, e.g., formed by Van der Waals forces, could also be of great importance.

Summing up the observations made by other investigators [2–7,9] and guided by our experience, it seems reasonable to assume that the enantiomeric differentiation proceeds in a relatively dense medium where the formation of more organized structures than in solution is possible. Such a medium could be, e.g., on the surface of the absorbent [3,4]. Moreover, the observations [5–7] that enantiomer differentiation increases up to a point on increasing the amount of sample loaded and experiments performed by Diter et al. [9] showing that changes in the length of chromatographic column (six-fold!) only slightly affected the enantiomeric enrichment strongly suggest that the main part of the optical resolution proceeds directly after loading the column, maybe even under overloaded conditions [5].

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References

[1] W. Szczepaniak and W. Ciszewska, Chromatographia, 15 (1982) 38.
[2] K.C. Cundy and P.A. Crooks, J. Chromatogr., 281 (1983) 17.

[3] R. Charles and E. Gil-Av, J. Chromatogr., 298 (1984) 516.
[4] W.L. Tsai, K. Hermann, E. Hug, B. Rohde and A.S. Dreiding, Helv. Chim. Acta, 68 (1985) 2238.
[5] A. Dobashi, Y. Motoyama, K. Kinoshita and S. Hara, Anal. Chem., 59 (1987) 2209.
[6] R. Matusch and C. Coors, Angew. Chem., Int. Ed. Engl., 28 (1989) 626.
[7] R.M. Carman and K.D. Klika, Aust. J. Chem., 44 (1991) 895.
[8] L. Cecchi and P. Malaspina, Anal. Biochem., 192 (1991) 219.
[9] P. Diter, S. Taudien, O. Samuel and H.B. Kagan, J. Org. Chem., 59 (1994) 370.
[10] J. Martens and R. Bhushan, J. Liq. Chromatogr., 15 (1992) 1.
[11] I.G. Veinberga, J.F. Freimanis and H.A. Kažoka, Bioorg. Khim., 17 (1991) 760.
[12] D.O. Loļa, J.F. Freimanis, I.V. Vosekalna and A.J. Liepiņa, Zh. Org. Khim., 24 (1988) 1422.
[13] V.R. Korics, G.P. Sokolovs, J.F. Freimanis, O.V. Sahartova, I.A. Mīlmanis, M.B. Fleišers, V.T. Glēzers, A.F. Mišņevs and J.J. Bleidelis, Zh. Org. Khim., 21 (1985) 305.
[14] A.K. Banerjee, B.J. Broughton, T.S. Burton, M.P.L. Caton, A.J. Christmas, E.C.J. Coffee, Crowshaw, C.J. Hardy, M.A. Heazell, M.N. Palfreyman, T. Parker, L.C. Saunders and K.A.J. Stuttle, Prostaglandins, 22 (1981) 167.
[15] G.M. Sheldrick, in G.M. Sheldrick, C. Krüger and R. Goddard (Editors), SHELXS-86. Crystallographic Computing 3, Oxford University Press, Oxford, 1985, pp. 175–189.
[16] G.M. Sheldrick, SHELXS-76. Program for Crystal Structure Determination and Refinement, University of Cambridge, UK, 1976; 400 Atomic Version, Ch. Weizmann Institute of Science, Rehovot, Israel, 1979; Implemented on IBM PC/AT, 1987.
[17] N. Walker and D. Stuart, Acta Crystallogr., Sect. A, 39 (1983) 158.
[18] E.V. Loža, D.O. Loļa, J.F. Freimanis, I.V. Turovskis and S.H. Rozīte, Izv. Akad. Nauk Latv. SSR, Ser. Khim., 2 (1989) 234.
[19] E.V. Loža, D.O. Loļa, J.F. Freimanis, I.V. Turovskis, S.H. Rozīte and I.V. Dipāns, Izv. Akad. Nauk Latv. SSR, Ser. Khim., 4 (1989) 472.
[20] E.V. Loža, D.O. Loļa, J.F. Freimanis, I.V. Turovskis, E.E. Liepiņš, S.H. Rozīte and O.V. Sahartova, Zh. Org. Khim., 26 (1990) 1024.
[21] D.O. Loļa, J.F. Freimanis, I.V. Turovskis and M.P. Gavars, Zh. Org. Khim., 26 (1990) 1919.
[22] C.F. Poole and S.K. Poole, Chromatography Today, Elsevier, Amsterdam, 1991, pp. 375–453.
[23] D. Hadži, in S. Patai (Editor), The Chemistry of Acid Derivatives, Part 1, Wiley, Chichester, 1979, pp. 213–266.