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Enantioselective Synthesis and Antioxidant Activity of 3-(3,4-Dihydroxyphenyl)-Glyceric Acid—Basic Monomeric Moiety of a Biologically Active Polyether from *Symphytum asperum* and *S. caucasicum*

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ABSTRACT The racemic and enantioselective synthesis of a novel glyceric acid derivative, namely, 2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid as well as the antioxidant activities is described. The virtually pure enantiomers, (+)-(2*R*,3*S*)-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid and (−)-(2*S*,3*R*)-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid were synthesized for the first time via Sharpless asymmetric dihydroxylation of trans-caffeic acid derivatives using the enantiocomplementary catalysts, $(DHQD)_2$-PHAL and $(DHQ)_2$-PHAL. The determination of enantiomeric purity of the novel chiral glyceric acid derivatives was performed by high-performance liquid chromatographic techniques on the stage of their alkylated precursors. The novel glyceric acid derivatives show strong antioxidant activity against hypochlorite and *N*,*N*-diphenyl-*N*-picryl-hydrazyl free radical. Their antioxidant activity is about 40-fold higher than that of the corresponding natural polyether and three-fold higher of trans-caffeic acid itself. *Chirality 22:717–725, 2010.*

KEY WORDS: asymmetric sharpless dihydroxylation; trans-caffeic acid; 3-(3,4-dihydroxyphenyl)-glyceric acid; chiral HPLC; enantiomeric analysis; antioxidant activity

INTRODUCTION

Comfrey (*Symphytum* L) extracts have been used in folk and traditional medicine for healing of various diseases.[1] Their most valuable pharmacological properties relate to wound healing and treatment of inflammation through their ability to strengthen regenerative process in injured tissue.[2,3] Generally, these properties are associated with allantoin. Apart from allantoin, several other chemical constituents of comfrey, such as mucilaginous polysaccharides,[4,5] caffeic acid, and its analogues rosmarinic acid[6] and lithospermic acid[7] as well as pyrrolizidine alkaloids[8,9] are considered to possess significant biological activities. Caffeic acid, phenolic acids, and polyphenols occur frequently in fruits, grains, and dietary supplements[10] and exhibit antioxidant, anti-inflammatory, anti-atherosclerotic, antimutagenic, anticancer, immunomodulatory, neuroprotective, or pro-apoptotic properties.[11–13] Pronounced antioxidant activity of caffeic acid derivatives is believed to be associated with their ability to scavenge free radicals, build chelating complexes with metal ions, or inhibit specific free radicals or lipid hydroperoxides forming enzymes. The responsible structural feature of caffeic acid derivatives for such activity is the existence of two adjacent hydroxyl functional groups in their aromatic ring.[13] Recently, high-molecular (>1000 kDa) water-soluble preparations with impressive immunomodulatory (anticomplementary) and antioxidant activity were isolated from the roots and stems of *Symphytum asperum* and *S. caucasicum*.[14,15] According to NMR and IR spectral data, the main constituent of these preparations was found to be a regular caffeic acid-derived polyether, namely, poly-[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)-ethylene] (Fig. 1).[16–18]

This polymer is a representative of a new class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)-glyceric acid as the repeating unit. To our knowledge, 3-(3,4-dihydroxyphenyl)-glyceric acid is not yet described in the literature neither in racemic nor in enantiomerically

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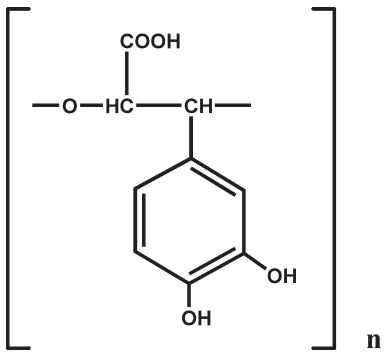


Fig. 1. Poly-[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)-ethylene] isolated from *S. asperum* and *S. caucasicum*.

pure form. Therefore, we have estimated that it would be of interest to elaborate a synthetic method for its preparation. Moreover, as the molecular structures of the novel dihydroxylated compounds contain the characteristic dihydroxyphenyl moiety of caffeic acid with well-known antioxidant activities, we determined their antioxidant activities and compared them with that of trans-caffeic acid itself and that of natural polyether isolated from comfrey species *S. asperum* and *S. caucasicum*. After carefully search in the literature, the most convenient method for the preparation of the novel glyceric acid derivative, and its corresponding enantiomers seems to be the asymmetric Sharpless dihydroxylation reaction (AD) of trans-caffeic acid.

MATERIALS AND METHODS

General Methods

^{1}H- and ^{13}C-NMR spectra were obtained on a Brucker Avance DRX-500 spectrometer (Brucker AG, Karlsruhe, Germany). Chemical shifts are expressed in δ (parts per million) values relative to tetramethylsilane (TMS) as internal reference and coupling constants (*J*) are given in Hertz. Electrospray ionization (ESI) Mass spectra were recorded on a Finnigan spectrometer (Thermo, Manchester, UK) by direct sample injection with a flow rate of 0.1 ml/min using a Harvant Syringe pump. The data are reported as m/z of the most important fragments. Desolvation was performed by hot nitrogen (Dominic-Hunter UHPLC MS-10). IR spectra were obtained using Perkin-Elmer 283 FT-IR spectrometer (Waltham, MA). Elemental analyses (C, H, and N) were carried out on a Perkin-Elmer CHN 2004 instrument (Norwalk, CT). The melting points were recorded on an Electrothermal apparatus and are uncorrected (Engineering Ltd., VT). Absorption spectra were run on a Jasco V-560 spectrophotometer (Jasco Co., Tokyo, Japan). Circular dichroism spectra were performed on a Jasco J-715 instrument (Jasco Co., Tokyo, Japan) equipped with peltier temperature control system. All reactions were monitored by TLC on 0.25 mm precoated silica gel plates Merck 60, GF-254 (Merck, Darmstadt, Germany) and visualized with UV light. Chemiluminescence measurements were made on a 1250 Bio-Orbit luminometer (Turku, Finland). The luminometer (output range 1.0 mV–10 V) is provided with a photomultiplier tube (HAM 105-21) with side window and spectral range from 300 to 620 nm and connected to a potentiometer chart recorder (GOW-MAC Instrument, Model 70-150) or personal computer equipped with a home made software program, which allows the continuous monitoring and analysis of the output signal. HPLC separation of enantiomers was performed with Agilent 1200 HPLC system equipped with auto sampler, column thermostat, and variable wavelength detector. The data collection and treatment were performed by Agilent Chemstation software (Agilent Inc. Waldbronn, Germany). Various commercially available chiral columns of Sepapak (Sepaserve GmbH, Muenster, Germany) series (Sepapak-1, Sepapak-2, Sepapak-3, and Sepapak-4 all of 4.6 × 250 mm size packed with 5 μm particles) were used for the determination of enantiomeric excess on the stage of their benzylated forms. The detection was performed at 254 nm.

All commercially available reagents, trans-caffeic acid, dimethylsulphate, benzyl bromide, citric acid, *N*-methylmorpholine-*N*-oxide (NMO), potassium osmate, palladium on carbon (10% Pd/C), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), 1,4-bis(9-O-dihydroquinine)-phthalazine [$(DHQ)_2PHAL$], 1,4-bis(9-O-dihydroquinidine)-phthalazine [$(DHQD)_2PHAL$] were of analytical grade and used without further purification (all from Sigma-Aldrich, Schnelldorf, Germany).

Synthetic Procedures

Alkylation of trans-caffeic acid. The alkylation of trans-caffeic acid proceeded according to a known procedure.[19] In brief, a mixture containing trans-caffeic acid **1** (1.8 g, 10 mmol), 50 ml acetone, powdered potassium carbonate (4.55 g), dimethylsulfate (3.12 ml) or benzyl bromide (3.91 ml) was refluxed overnight under stirring. The solid residues were filtered and washed three times with 50-ml portions of acetone. The solvent was evaporated, and the resulted viscous yellowish residues were purified by crystallization in diethyl ether—petroleum ether.

Methyl 3-(3, 4-dimethoxyphenyl)-propenoate (2a). Yield: 2.0 g (90%), mp. 69–70°C. ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): δ 7.61 (1H, d, J = 15.9, olefinic proton), 7.08 (1H d, J = 1.9), 7.02 (1H, dd, J = 8.02, 1.8), 6.84 (1H, d, J = 8.4), 6.28 (1H, d, J = 15.9, olefinic proton), 3.88 (6H, s, 2 OCH_3), 3.77 (3H, s, OCH_3,); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.57 (carbonyl group), 151.15, 149.23, 144.71 (olefinic carbon), 127.38, 122.53, 115.50, 111.09 (olefinic carbon), 109.75, 55.93 (OCH_3), 55.87 (OCH_3), 51.50 (OCH_3). IR (KBr), ν_{max} (cm^{-1}): 2944, 2857, 1708 (carbonyl group), 1620, 1596, 1520, 1440, 1260, 1150, 980. MS (ESI): m/z 191 ($M^+ - OCH_3$), 163 ($M^+ - COOCH_3$), 148 ($M^{+1} - CH_2COOCH_3$).

Benzyl 3-(3,4-dibenzyloxyphenyl)-propenoate (2b). Yield: 4.05 g (90%), mp. 80–82°C. ^{1}H NMR (500 MHz, $CDCl_3$, 30°C): δ = 7.59 (1H, d, J = 15.9), 7.30–7.45 (15H, m, aromatic protons), 7.11 (1H, d, J = 1.8 Hz), 7.06 (1H, dd, J = 8.4, 1.8), 6.92 (1H, d, J = 8.2), 6.26 (1H, d, J = 15.9), 5.22 (2H, s, benzylic protons), 5.19 (2H, s, benzylic protons), 5.16 (2H, s, benzylic protons,). ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 167.1 (C=O, ester group), 151.5, 149.3, 145.3

(olefinic carbon), 136.8, 136.7, 128.6–127.27 (complex area, aromatic carbons), 127.14, 123.3, 116.1, 114.6, 114.1, 71.7 (benzylic—CH_2), 71.3 (benzylic—CH_2), 66.6 (benzylic—CH_2). IR (KBr), ν_{max} (cm^{-1}): 3068, 3025, 2907, 2857, 1683 (carbonyl group), 1595, 1516, 1445, 1390, 1272, 1130, 1021, 946, 871, 840, 816.

Typical Procedure for Dihydroxylation

a) Racemic dihydroxylation of trans-caffeic acid derivatives. The protected trans-caffeic acid derivatives **2** were dihydroxylated according to a Sharpless procedure.[20] Briefly, the alkylated caffeic acid derivatives **2a** (2.22 g, 10 mmol) or **2b** (4.14 g, 10 mmol) and citric acid (3 g, 7.5 mmol) were dissolved in 10 ml of a 3:3:1 mixture of acetonitrile–acetone–water in a 100 ml Erlenmeyer flask. Potassium osmate (3.7 mg, 0.1 mol %) was then added, followed by 50% water solution of NMO (2.28 ml, 1.1 mmol). The reaction mixture turned bright green. After stirring at room temperature for 4 hours the reaction mixture became nearly colorless. The organic solvents were removed on a rotary evaporator and the aqueous residue was then acidified with hydrochloric acid (1M, 12 ml) and extracted with ethyl acetate (2 × 50 ml). The combined organic extracts were dried with sodium sulfate and concentrated giving colorless oils. The oils solidified at room temperature as white solids and recrystallized from ether-petroleum ether.

Methyl syn-*2,3-dihydroxy-3-(3,4-dimethoxyphenyl)-propionate (3a)*. Yield: 2.35 g, 92%, mp. 76–78°C (lit.,[21] mp. 75–78°C). ^{1}H NMR (500 MHz, acetone-d_6, 30°C): δ = 7.07 (1H, d, *J* = 1.9), 6.93 (1H, dd, *J* = 8.2 and 1.9), 6.88 (1H, d, *J* = 8.2), 4.91 (1H, d, *J* = 3.4, β-C*H*OH), 4.50 (1H, brd s, OH), 4.23 (1H, d, *J* = 3.4, α-C*H*OH), 4.14 (1H, s, OH), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.67 (3H, s, OCH_3). ^{13}C NMR (125 MHz, acetone-d_6): δ = 173.11 (C=O, ester), 148.72, 148.53, 132.50, 118.54, 110.78, 109.55, 75.15 (β-*C*HOH), 74.31 (α-*C*HOH), 55.78 (OCH_3), 55.74 (OCH_3), 52.51 (OCH_3). IR (KBr), ν_{max} (cm^{-1}): 3456, 3001, 2954, 2839, 1736, 1597, 1512, 1450, 1265, 1142, 1026, 910, 864, 810.

Benzyl syn-*2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate (3b)*. Yield: 4.45 g, 92%, mp. 126–128°C. ^{1}H NMR (500 MHz, acetone-d_6): δ = 7.5–7.30 (15H, m, aromatic protons), 7.25 (d, *J* = 1.8, 1H), 7.01 (dd, *J* = 8.4, 1.8, 1H), 6.97 (d, *J* = 8.2, 1H), 5.22 (2H, s, benzylic protons), 5.16 (2H, s, benzylic protons), 5.14 (2H, s, benzylic protons), 4.90 (1H, d, *J* = 3.4, β-C*H*OH), 4.50 (1H, broad s, OH), 4.34 (1H, d, *J* = 3.4, α-C*H*OH), 4.21 (1H, broad s, OH). ^{13}C-NMR (125 MHz, acetone-d_6): δ = 172.48 (C=O, ester), 151.65, 149.36, 133.47, 129.06–127.86 (complex area, aromatic carbons), 127.67, 119.93, 115.25, 113.90, 75.11 (β-*C*HOH), 74.58 (α-*C*HOH), 71.71 (2 benzylic—CH_2), 68.11 (benzylic—CH_2). IR (KBr), ν_{max} (cm^{-1}): 3508, 3063, 3030, 1720 (carbonyl group), 1595, 1504, 1445, 1384, 1344,1250, 1132, 1120, 1008, 1090, 941, 909, 890, 852, 818. MS (ESI): m/z = 507 (M^+ + Na). Elem. Analysis, calculated for $C_{30}H_{28}O_6$ (484.548), theoretical C = 74.36, H = 5.82, found C = 74.26, H = 5.80.

b) Asymmetric sharpless dihydroxylation of trans-caffeic acid derivatives. Trans-caffeic acid derivatives 2 were dihydroxylated according to a known procedure.[21] Briefly, in a 50 ml Erlenmeyer flask containing 20 ml solution of compound **2b** (2.25 g, 5 mmol; 3:3:1 acetone–acetonitrile–water mixture) were added, successively, $(DHQ)_2$PHAL or $(DHQD)_2$PHAL (0.0194 g, 0.025 mmol), hydrated potassium osmate ($K_2OsO_4 \cdot 2H_2O$, 0.0038 g, 0.01 mmol) and 1.5 ml NMO (50%). The reaction mixture was stirred at room temperature (23°C) for 2 hours, then solution of sodium sulfite (0.74 g, 5.9 mmol) in 3 ml water was charged in, the mixture was stirred for 30 min and allowed to stand for 15 min. The organic phase was concentrated into half volume under reduced vacuum and extracted three times with ethyl acetate. The combined organic phases were washed with 10% NaCl (2 × 45 ml). The aqueous phase was extracted with ethyl acetate twice and the combined organic phases were concentrated. The obtained crude product was purified by column chromatography on silica gel, chloroform–methanol (10:1).

*Benzyl (2*R*,3*S*)-2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate (5)*. Yield: 2.17 g, 90%, mp. 97–100°C. ^{1}H NMR (500 MHz, acetone-d_6): δ = 7.45–7.37 (15H, m, aromatic protons), 7.25 (d, *J* = 1.8, 1H), 7.06 (dd, *J* = 8.4, 1.8, 1H), 6.90 (d, *J* = 8.2, 1H), 5.22 (2H, s, benzylic protons), 5.19 (2H, s, benzylic protons), 5.15 (2H, s, benzylic protons), 4.92 (1H, d, *J* = 3.4, β-C*H*OH), 4.34 (1H, d, *J* = 3.4, α-C*H*OH). ^{13}C-NMR (125 MHz, acetone-d_6): δ = 172.56 (C=O, ester), 149.4, 148.91, 137.2, 134.9, 133.1, 128.65–127.26 (complex area, aromatic carbons), 119.51, 114.8, 113.47, 74.8 (β-*C*HOH), 74.31 (α-*C*HOH), 71.29 (2 benzylic—CH_2), 67.72 (benzylic—CH_2). IR (KBr), ν_{max} (cm^{-1}): 3506, 3060, 3032, 1722 (carbonyl group), 1593, 1502, 1443, 1382, 1342,1253, 1134, 1124, 1006, 1092, 940, 906, 888, 850, 819. ee: 97.18% (HPLC).

*Benzyl (2*S*,3*R*)-2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate (6)*. Yield: 2.05 g, 85%, mp. 88–91°C. ^{1}H NMR (500 MHz, acetone-d_6): δ = 7.47–7.37 (15H, m, aromatic protons), 7.32 (d, *J* = 1.8, 1H), 7.06 (dd, *J* = 8.4, 1.8, 1H), 6.91 (d, *J* = 8.2, 1H), 5.22 (2H, s, benzylic protons), 5.19 (2H, s, benzylic protons), 5.163 (2H, s, benzylic protons), 4.92 (1H, d, *J* = 3.4, β-C*H*OH), 4.35 (1H, d, *J* = 3.4, α-C*H*OH). ^{13}C-NMR (125 MHz, acetone-d_6): δ = 172.48 (C=O, ester), 149.4, 148.9, 137.1, 134.8, 133.0, 128.66–127.25 (complex area, aromatic carbons), 119.49, 114.8, 113.46, 74.72 (β-*C*HOH), 74.29 (α-*C*HOH), 71.29 (2 benzylic—CH_2), 67.75 (benzylic—CH_2). IR (KBr), ν_{max} (cm^{-1}): 3510, 3066, 3034, 1724 (carbonyl group), 1596, 1507, 1447, 1386, 1343,1252, 1133, 1123, 1007, 1094, 943, 909, 892, 854, 812. ee: 98.2% (HPLC).

Typical Procedure for Removing Benzyl Groups

The best yields for products **4, 7,** and **8** were obtained by using the catalytic hydrogenation procedure described by Spencer and coworkers.[22] In brief, a solution of products **3b, 5** or **6** (2.0 mmol) in a 1:1 mixture of ethanol–tetrahydrofuran (30 ml) was added to a stirred suspension of palladium/carbon (10 mol %) in the same solvent system (20 ml) that had previously been evacuated, purged with hydrogen, and stirred for 30 min under a hydrogen atmosphere. The reaction mixture was stirred overnight and then filtered through celite. The organic extracts were

concentrated under vacuum, and the resulting residue was purified by crystallization in ethanol. Products **4, 7,** or **8** were obtained as white-yellowish solids.

***rac syn*-2,3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid (4).** Yield: 402.3 mg (94%), mp. 152–158°C. UV (H_2O), λ_{max} nm (ϵ): 230 (1470), 278 (5170). ^{1}H NMR (500 MHz, D_2O): δ = 6.86 (1H, d, *J* = 1.9 Hz), 6.80 (1H, dd, *J* = 8.2, 1.9 Hz), 6.75 (1H, d, *J* = 8.2 Hz), 4.95 (1H, d, *J* = 3.4 Hz, β-C*H*OH), 4.31 (1H, d, *J* = 3.4 Hz, α-C*H*OH). ^{13}C NMR (125 MHz, D_2O): δ = 176.23 (C=O, carboxylic carbon), 144.15, 143.96, 132.99, 119.30, 116.40, 114.68, 75.58 (β-*C*HOH), 74.25 (α-*C*HOH). IR (KBr), ν_{max} (cm^{-1}): 3445, 3374, 3337 (hydroxyl groups), 1748 (C=O, carboxylic group), 1700, 1602, 1523, 1443, 1406, 1289, 1206, 1106, 1039, 985, 937, 879, 864, 826, 810, 769. MS (CI), m/*z*: 153 (M^{+1} $-CO_2$, $-H_2O$), 135 ($M^{+1}-CO_2$, $-2H_2O$), 123 ($M^{+1}-CO_2$, $-2H_2O$, $-CH_2O$). Elem. Analysis, calculated for $C_9H_{10}O_6 \times 2H_2O$ (248.187), theor. C = 43.55, H = 4.87, found C = 43.52, H = 4.42.

(+)-(2*R*,3*S*)- 2,3-Dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid (7). Yield: 407 mg (95%), mp. 157–160°C. ^{1}H NMR (500 MHz, D_2O): δ = 6.90 (1H, d, J = 1.9 Hz), 6.85 (1H, dd, *J* = 8.2, 1.9 Hz), 6.81 (1H, d, *J* = 8.2 Hz), 4.92 (1H, d, *J* = 3.4 Hz, β-C*H*OH), 4.32 (1H, d, *J* = 3.4 Hz, α-C*H*OH). ^{13}C NMR (125 MHz, D_2O): δ = 175.6 (C=O, carboxylic carbon), 144.0, 143.77, 132.58, 119.08, 116.16, 114.46, 75.14 (β-*C*HOH), 73.96 (α-*C*HOH). IR (KBr), ν_{max} (cm^{-1}): 3333 (hydroxyl groups), 1750 (C=O, carboxylic group), 1702, 1605, 1522, 1446, 1410, 1299, 1210, 1105, 1037, 980, 935, 877, 862, 822, 811, 764. $[\alpha]_D$ = +33.3° (C = 0.48, H_2O), CD-spectrum (C = 0.56 × 10^{-3} M, H_2O): $\Delta\varepsilon_{275}$ = −0.4, $\Delta\varepsilon_{228}$ = +2.5, $\Delta\varepsilon_{209}$ = +4.8.

(−)-(2*S*,3*R*)-2,3-Dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid (8). Yield: 364 mg (85%), mp. 155–158°C. ^{1}H NMR (500 MHz, D_2O): δ = 6.88 (1H, d, *J* = 1.9 Hz), 6.82 (1H, dd, *J* = 8.2, 1.9 Hz), 6.79 (1H, d, *J* = 8.2 Hz), 4.90 (1H, d, *J* = 3.4 Hz, β-C*H*OH), 4.30 (1H, d, *J* = 3.4 Hz, α-C*H*OH). ^{13}C NMR (125 MHz, D_2O): δ = 176.0 (C=O, carboxylic carbon), 144.1, 143.89, 132.52, 119.04, 116.12, 114.40. IR (KBr), ν_{max} (cm^{-1}): 3332 (hydroxyl groups), 1753 (C=O, carboxylic group), 1704, 1607, 1523, 1448, 1412, 1297, 1213, 1103, 1036, 957, 937, 880, 864, 826, 810, 769. $[\alpha]_D$ = −32.5° (C = 0.4, H_2O), CD-spectrum (C = 0.5 × 10^{-3} M, H_2O): $\Delta\varepsilon_{275}$ = −1, $\Delta\varepsilon_{228}$ = −5, $\Delta\varepsilon_{209}$ = −10.5.

Typical Procedures of Antioxidant Activities Measurements

The antioxidant activity (AA%) of novel compounds was determined spectrophotometrically (DPPH-method) or with chemiluminescent techniques (luminol-hypochlorite system).

a) DPPH-method. Briefly, the absorption of a methanolic solution of 2.0 ml DPPH (500 μM) and 2.0 ml methanol was measured at 515 nm (blank) and compared with the absorbance of samples containing 2.0 ml DPPH and 2.0 ml methanolic solutions of sample compounds in concentrations ranging from 0.1—250 μg/ml.[12] The absorbance of the samples was measured after the reaction reached a plateau (about 30 min). All measurements were done at room temperature (23°C) and repeated four times. The radical scavenging activity of the samples was expressed in terms of IC_{50} (concentration in μM required for a 50% decrease in absorbance of DPPH radical) and calculated using the equation $[(A_{blank} - A_{sample})/A_{blank})] \times 100$), where A_{blank} is the absorbance of the control (DPPH solution without sample) and A_{sample} the absorbance of the test compound (DPPH solution plus antioxidant). A plot of absorbance vs. concentration was made to establish the standard curve, and the linear regression equations from which the IC_{50} values were calculated.

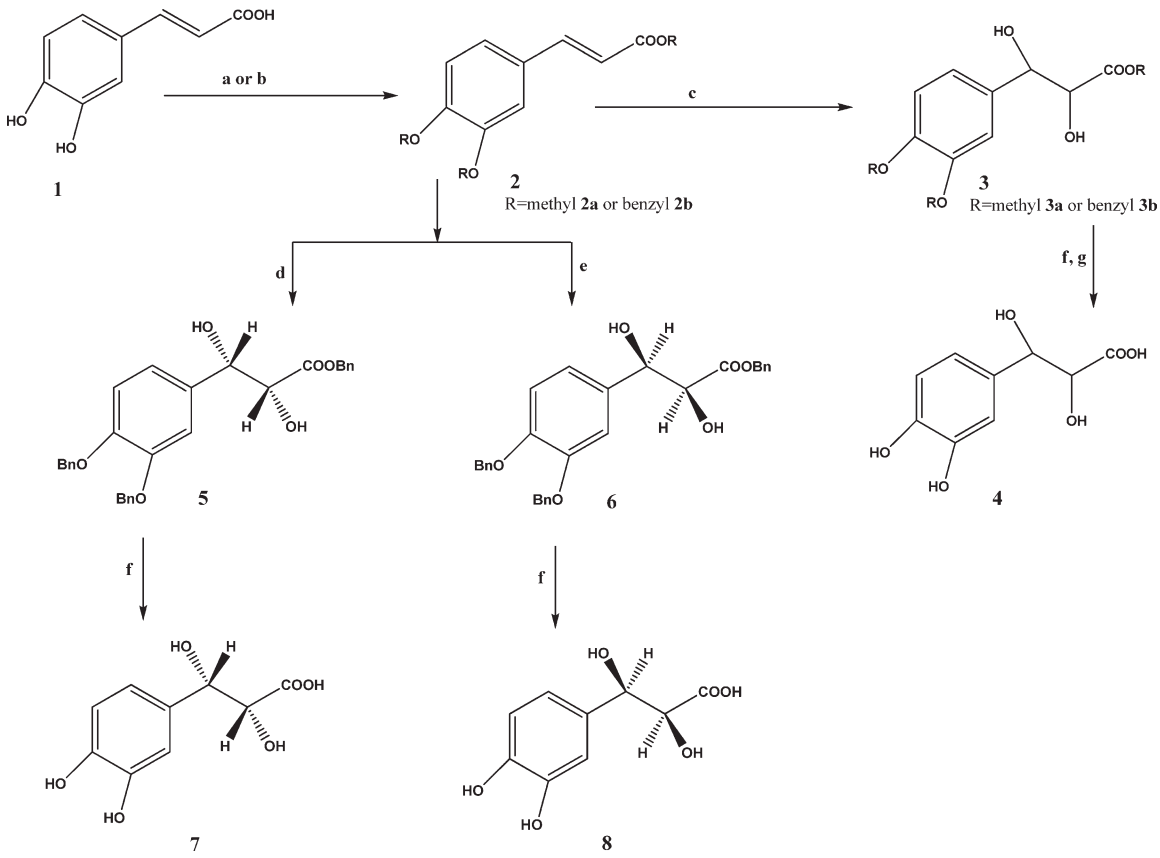
b) Chemiluminescence technique. The light reaction of the blank was started by adding sodium hypochlorite (100 μl, 1.0%) into a mixture of alkaline luminol (100 μl, 50 μM) and sodium hydroxide (100 μl, 0.1 M).[23] The obtained light intensities were compared with the light intensities of samples containing alkaline luminol solutions (100 μL, 50 μM), test compound solutions of various concentrations (100 μL, 1.0–250 μg/ml diluted in 0.1M NaOH) and sodium hypochlorite (100 μl, 1.0%). The solutions were injected into the reactor cell with a Hamilton syringe through a septum. The antioxidant activity of the samples was calculated from the equation: AA (%) = $(I_0 - I)/I_0) \times 100$, where I_0 and I, are the relative light intensities of the blank and sample solutions, respectively. All measurements were repeated four times, at room temperature (23°C). The light signals reached their intensity maximum in less than 0.7 seconds and disappeared after 10 seconds. A plot of CL intensities vs. concentration was made to establish the standard curve and the linear regression equations from which the IC_{50} values were calculated.

RESULTS AND DISCUSSION

Synthesis and Structure Elucidation of Synthesized Compounds

A systematic search in the literature and preliminary test experiments revealed that the most economic way for the preparation of the monomer, 3-(3,4-dihydroxyphenyl)-glyceric acid **4,** and its corresponding antipodes **7** and **8** is the asymmetric Sharpless dihydroxylation of alkylated trans-caffeic acid derivatives (Scheme 1).

The hydroxyl groups as well as the carboxylic group of trans-caffeic acid **1** are appropriately protected by benzyl or methyl groups[19] and the double bond of caffeic acid derivative **2** dihydroxylated according to a modified Sharpless asymmetric dihydroxylation (AD) procedure.[20,21] At this point, it must be mentioned that it is of paramount importance the right choice of chiral auxiliaries for the synthesis of both enantiomers of 3-(3.4-dihydroxyphenyl)-glyceric acid **4**. Taking into account the mechanistic rules of Sharpless[24] and using the cinchona alkaloid derivatives $(DHQ)_2$-PHAL and $(DHQD)_2$-PHAL as chiral auxiliaries, the dihydroxylated products **5** and **6,** respectively, could be produced in virtually optically pure forms by Sharpless asymmetric dihydroxylation reaction. All dihydroxylated



Scheme 1. Synthetic route for the preparation of 3-(3,4-dihydroxyphenyl)-glyceric acid **(4)** and its antipodes **(7)** and **(8)**. **(a)** Dimethyl sulfate, potassium carbonate, acetone, reflux **(b)** Benzyl bromide, potassium carbonate, acetone, reflux **(c)** citric acid, potassium osmate, NMO acetonitrile/acetone/water (3:3:1), room temperature **(d)** [$(DHQ)_2$PHAL], potassium osmate, NMO, acetonitrile/acetone/water (3:3:1), room temperature **(e)** [$(DHQD)_2$PHAL], potassium osmate, NMO, acetonitrile/acetone/water (3:3:1), room temperature **(f)** 10% Pd/C, H_2, ethanol–tetrahydrofuran, room temperature **(g)** HBr/acetic acid, reflux; trimethylsilyl chloride–potassium iodide, acetonitrile, room temperature.

products show the relative *syn*-configuration. This has been explained by comparison of the measured coupling constants (J = 3.4 Hz) of protons attached to the α and β-carbons on the glyceric moiety with those of cinnamic and caffeic acid derivatives obtained under similar reaction conditions.[20] The absolute configurations of dihydroxylated products **5, 6, 7,** and **8** were determined assuming the same mechanistic approach proposed by Sharpless[25] for the enantioselective dihydroxylation of trans-cinnamic acid derivatives, where the chiral auxiliary $(DHQ)_2$-PHAL leads to the (2*R*,3*S*) configuration, while $(DHQD)_2$-PHAL to the opposite configured enantiomer. So to products **5** and **7** configuration was assigned (2*R*, 3*S*) [chiral auxiliary $(DHQ)_2$-PHAL] and (2*S*,3*R*) to products **6** and **8** [chiral auxiliary $(DHQD)_2$-PHAL]. The opposite configuration of both enantiomers is also confirmed by measurements of the optical rotation (+)/(−)-values and circular dichroism spectra of products **7** and **8** as well as by HPLC analysis of products **5** and **6** using chiral columns (Sepapak) (retention times of the enanantiomers). In optical rotation measurements, the (+)-sign is assigned to the stereoisomer (2*R*,3*S*) while the (−)-sign to the opposite enantiomer (2*S*,3*R*). The same results found in their corresponding CD spectra. As given in experimental part, the (2R,3S)-isomer of **7** shows negative signs (−) at wavelengths 228 and 209 nm, while the (2*S*,3*R*)-isomer of **8** shows positive signs at the same wavelengths.

The enantiomeric excess of enantiomeric novel glyceric acid derivatives was determined by high performance liquid chromatography on the stage of their benzylated derivatives **3b**, **5** and **6** using sepapak chiral columns (Figs. 2, 3 and 4). In Fig. 2 the chiral resolution of **3b** is reported. The enantiomeric excess of the corresponding enantiomers **5** and **6** was 97.18 and 98.2%, respectively (Figs. 3 and 4).

At this point it must be noted, that polysaccharide phenylcarbamate derivatives (filling material of sepapak columns) represent the most powerful group of chiral stationary phases for analytical and preparative scale separations of enantiomers.[26,27] The phenylcarbamate derivatives of cellulose and amylose containing electron-donating and electron-withdrawing substituents on their phenyl moiety are characterized with quite universal chiral recognition ability and can be used for separation of enantiomers in normal-, polar organic-, and reversed-phase mode.[28–30] Several of these new derivatives of polysaccharides have been commercialized recently and used for solving of various separation problems of pharmaceutical and biomedical relevance.[31–33]

In frame of this work, five different chiral sepapak columns (Fig. 5) were tested for the evaluation of enantio-

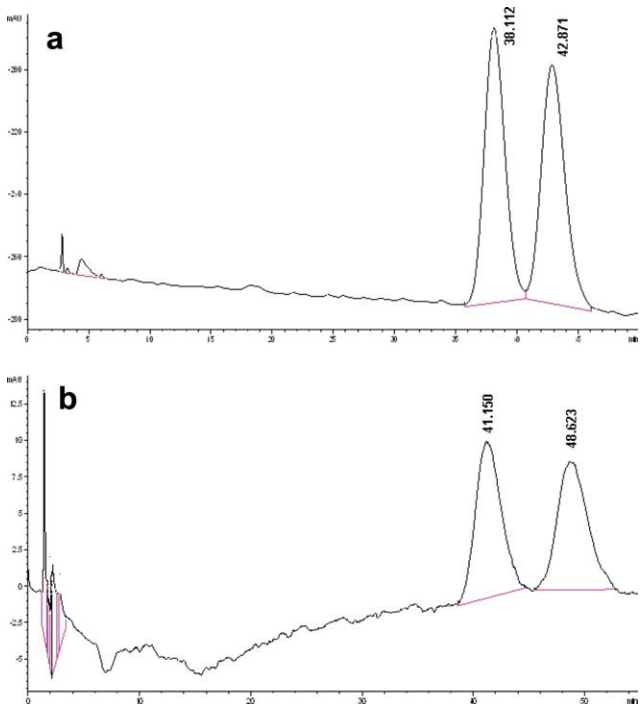


Fig. 2. a. Chromatogram of racemic benzyl-[2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)]-propionate on Sepapak-4 column. Mobile phase: methanol/water, 80/20 (v/v). Flow rate 2.0 ml/min. **b.** Chromatogram of racemic benzyl-[2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)]-propionate on Sepapak-4 column. Mobile phase: *n*-hexane/2-propanol, 85/15 (v/v). Flow rate 2.0 ml/min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

meric excess of enantiopure glyceric acid derivatives **5** and **6** using initially methanol as a mobile phase.

Partial separation of the enantiomers **5** and **6** was observed using Sepapak-1, Sepapak-3, and Sepapak-4 columns. Sepapak-2 and Sepapak-5 did not provide any measurable separation of the enantiomers (data not shown). Since Sepapak-4 provided the most promising separation in pure methanol further optimization of the mobile phase was performed with this material. Water additives were considered at first to convert/transform the pure polar organic mobile phase separation to reversed phase enantioseparation. This allowed longer retention and eventually better separation of enantiomers. The longer retention was actually observed with increasing content of water in the mobile phase. Nearly/almost baseline enantioseparation was observed with the mobile phase containing 20% of water in methanol (v/v) with analysis time of 38.1 and 42.8, respectively. Typical normal phase eluent such as *n*-hexane/2-propanol (85/15, v/v) was used as the next in combination with Sepapak-4 column. A good baseline separation of two enantiomers was observed, therefore, this system was used for the enantiomeric excess determination of benzylated derivatives (Figs. 3 and 4). According to HPLC analysis data, the enantiomeric excess (ee) for products **5** and **6** was 97.18 and 98.2%, respectively.

At this point, it must also be noted that preliminary studies on unprotected acidic (free acid) monomers did not interact/retain with chiral stationary phases sufficiently and so could not be separated. Therefore, benzyl derivatives were used for the enantiomeric excess determination.

The chemical yields of products **3, 5,** and **6** obtained in acetone–acetonitrile–water mixture (3:1:1) were in the range of 85–92% and much lower in case of t-butanol–water (1:1). On the last step the protective (benzyl) groups were removed successfully by hydrogenation using 10% Pd/C in tetrahydrofuran–ethanol.[22] Using concentrated hydrogen bromide–acetic acid or trimethylsylilchloride–potassium iodide in acetonitrile, the reaction proceeded well but the purification of product **4** was not successful. The desired 3-(3,4-dihydroxyphenyl)-glyceric acid, **4** was obtained in good yield only by hydrogenation. The structures of all products were elucidated by UV-Visible, ^{1}H NMR-, ^{13}C NMR-, FTIR-, and MS-spectral data.

All spectra of monomer **4** were compared with that of polyether obtained from *S. asperum* and *S. caucasicum*. In the ultra-violet spectrum of glyceric acid derivative **4** the two characteristic bands at 230 and 278 nm (Fig. 6) are similar to those of the natural polyether but shifted about 7–8 nm toward lower wavelengths. The absorption bands of polyether appear at 237 and 286 nm (Fig. 6).

The IR spectrum of monomer **4** is also similar to that of natural polyether and contains all the characteristic bands corresponding to the hydroxyl groups attached to the aro-

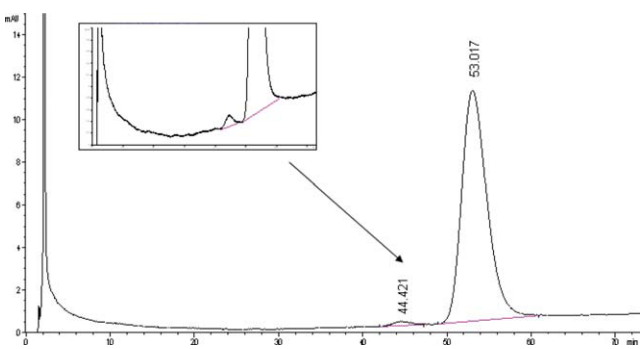


Fig. 3. Chromatogram of virtually enantiopure benzyl (*2R,3S)*-2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate. Chiral column: Sepapak-4; mobile phase: *n*-hexane/2-propanol, 85/15 (v/v); flow rate 2.0 ml/min. The major product is obtained using the chiral auxiliary $(DHQ)_2$-PHAL. (ee = 97.18%). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

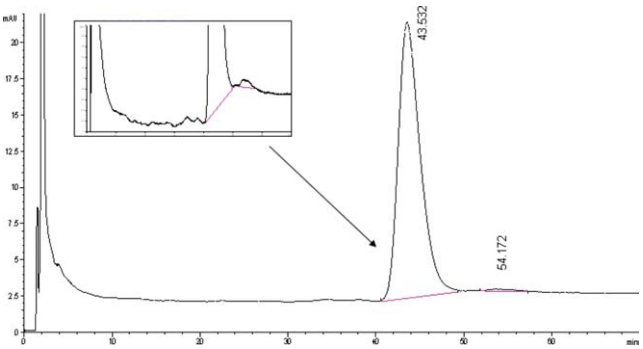


Fig. 4. Chromatogram of virtually enantiopure benzyl (*2S,3R)*-2,3-dihydroxy-3-(3,4-dibenzyloxyphenyl)-propionate. Chiral column: Sepapak-4; mobile phase: *n*-hexane/2-propanol (85/15 v/v); flow rate 2.0 ml/min. The major product is produced using the chiral auxiliary $(DHQD)_2$-PHAL. (ee = 98.2%). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

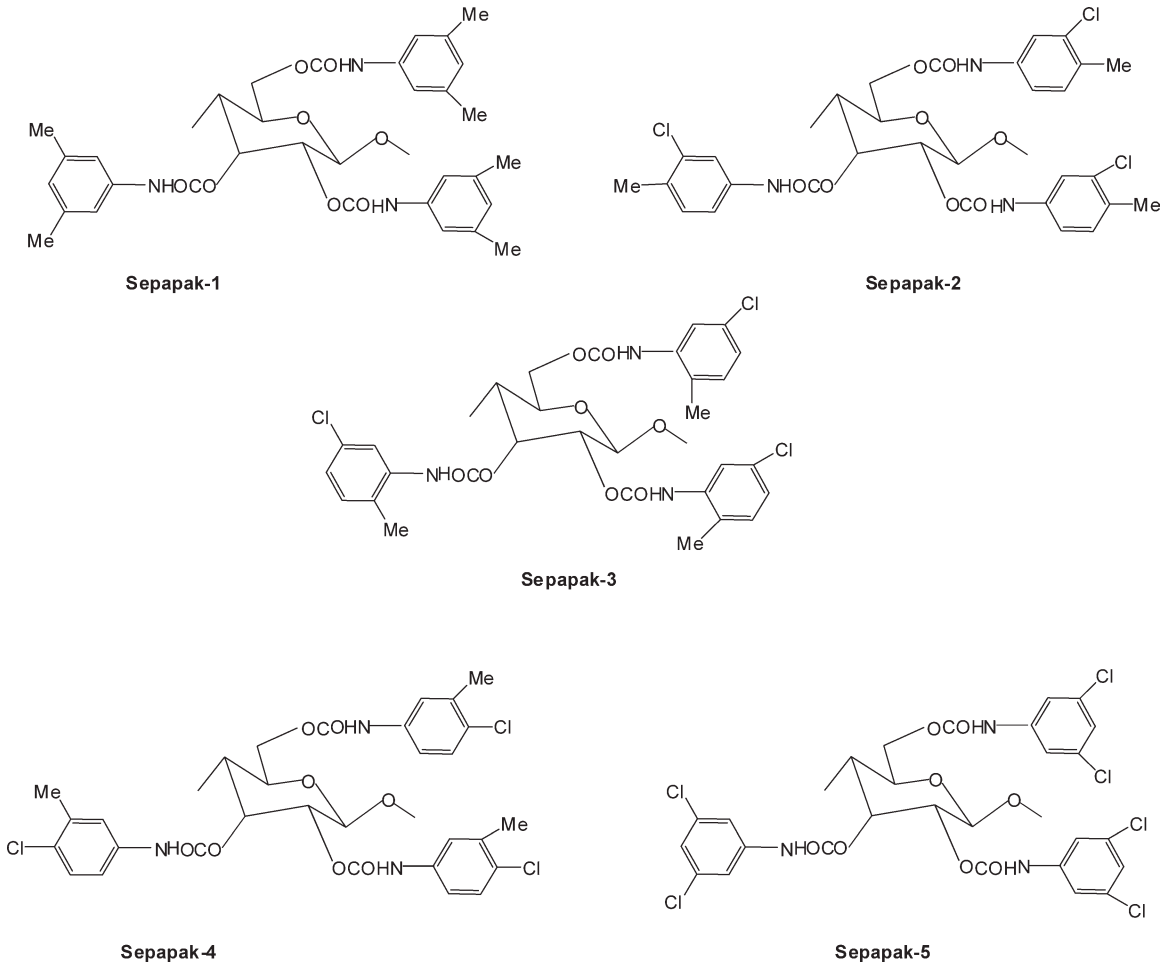


Fig. 5. Structures of filling materials in Sepapak columns. Sepapak-1: cellulose tris-(3,5-dimethylphenylcarbamate); Sepapak-2: cellulose tris-(3-chloro-4-methylpenylcarbamate); Sepapak-3: amylose tris-(5-chloro-2-methylphenylcarbamate); Sepapak-4: cellulose tris-(4-chloro-3-methylphenylcarbamate); Sepapak-5: cellulose tris-(3,5-dichlorophenylcarbamate).

matic ring as well as the carboxylic group. No significant differences are observed in the ^{13}C NMR spectra of the monomer and isolated natural polyether. Nine distinct signals of carbon atoms were observed in the spectra of both compounds.[16–18] The signals at 74.25 and 75.58 ppm are assigned to the aliphatic carbon atoms, whereas the six signals at 114.68, 116.40, 119.30 (protonated carbons) and 132.99, 143.96, 144.15 ppm (nonprotonated carbons) belong to the aromatic ones. The signal at 176.23 ppm is assigned to the carboxylic group. Small differences were observed also in the ^{1}H NMR spectra of monomer and its corresponding polymer. In particular, the peaks corresponding to the aromatic protons at 6.89, 6.84, and 6.79 ppm and those to the aliphatic ones at 4.88 and 4.27 ppm are sharp signals, while those of the polyether are broadened and shifted. Namely, the aliphatic protons appear at 4.88 and 5.33 ppm, while the aromatic ones at 7.13 and 7.24.[16–18]

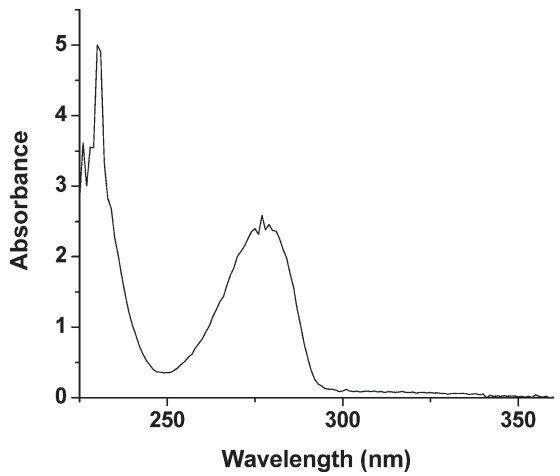


Fig. 6. UV-Vis spectrum of 3-(3,4-dihydroxyphenyl)-glyceric acid, **4**.

Antioxidant Activities of Racemic Derivative 4 and its Enantiomers 7 and 8

The ability of 3-(3,4-dihydroxyphenyl)-glyceric acid to scavenge free radicals or neutral reactive oxygen species was tested against the relatively stable DPPH-radical and

TABLE 1. Measured antioxidant activities of racemic 4 and virtually enantiopure products 7 and 8 as well as those of natural polyether isolated from the roots of *S. asperum* and trans-caffeic acid against hypochlorite and DPPH-radicals

Compound	Antioxidant activity (IC_{50}[a], μg/ml)	
	DPPH-method	CL-method
rac 2,3-Dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid	3.8 ± 0.3	2.8 ± 0.2
(+)-(2*R*,3*S*)-2,3-Dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid	4.1 ± 0.4	3.2 ± 0.3
(−)-(2*S*,3*R*)-2,3-Dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid	3.9 ± 0.2	3.0 ± 0.2
Poly-[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)-ethylene]	146.5 ± 7.6	120.4 ± 9.8
trans-Caffeic acid	12.2 ± 0.7	9.4 ± 0.5

[a]IC_{50} ± standard deviation ($n = 4$)

hypochlorite. Sodium hypochlorite was chosen as representative for reactive oxygen species because of its importance in oxidizing reactions in biological systems.[34] In living organisms hypochlorous acid is produced by certain oxidizers from chloride anions and hydrogen peroxide.[35] The in vitro antioxidant activity estimation of 3-(3, 4-dihydroxyphenyl)-glyceric acid derivatives against hypochlorite ions, could also be used for the estimation of this reactive oxygen species in vivo experiments, too. The antioxidant activities of racemic and vitually enantiopure products **4, 7,** and **8** as well as those of natural polyether isolated from the roots of *S. asperum* and trans-caffeic acid are given in Table 1.

As shown in Table 1, all phenolic compounds show strong antioxidant activities against hypochlorite ions and DPPH free radical. The inhibitory effect of racemic product **4** as well as this of the corresponding enantiomers appeared to be about 40-fold and three-fold higher than that of polyether or trans-caffeic acid, respectively. The unexpected lowest antioxidant activity of polyether may be explained with the less stabilizing effects of phenoxide radicals via intra- or intermolecular hydrogen bonds observed in monomeric products. Such stabilizing effects of phenoxide radicals in phenolic compounds via intra- or intermolecular hydrogen bonds are reported in reference.[36] This hypothesis is strengthened by the similarity of structural features of novel 3-(3,4-dihydroxyphenyl)-glyceric acid derivatives **4, 7,** and **8** and trans-caffeic acid. In case of compounds **4, 7,** and **8,** the phenoxide radicals produced during the reaction can be stabilized intramolecularly via hydrogen bonds, while in case of trans-caffeic acid, over the extended conjugated system. As shown in Table 1 no significant differences have been noted regarding the antioxidant activities of pure enantiomers and the racemic monomer.

CONCLUSIONS

In this work, it is shown that the racemic as well as the pure enantiomers of the novel 3-(3,4-dihydroxyphenyl)-glyceric acid can be synthesized in high yields and with high enantioselectivities via Sharpless asymmetric dihydroxylation reaction of trans-caffeic acid using the enantiocomplementary chiral auxiliaries $(DHQ)_2$-PHAL and $(DHQD)_2$-PHAL. The enantiomeric excess can be easily determined by high performance liquid chromatography using chiral sepapak columns. The absolute and relative configurations of the novel enantiopure glyceric acid derivatives **7** and **8** were assigned on the stage of their alkylated forms and determined by assuming the same mechanistic approach suggested by Sharpless for dihydroxylated products obtained from trans-cinnamic acid derivatives.

Moreover, it has been shown that the novel racemic glyceric acid derivative **4** as well as its enantiomeric pure derivatives **7** and **8** possess strong antioxidant activities against reactive oxygen species, such as hypochlorite or free radicals such as DPPH. Such pronounced antioxidant properties give appropriate background for further deep investigation of the biological activity of this phenolic compound. The use either as food additive or in medicinal preparations for treatment of oxidative disorder-related diseases can be considered.

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