Absolute configuration and anti-hypercholesterolemic activity of (+)- and (−)-4-(2,6-dimethylheptyl)benzoic acids

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The biologically inactive (+)-enantiomer of 4-(2,6-dimethylheptyl)benzoic acid was converted into the known (*R*)-3,7-dimethyloctanol with *ee* ≥ 96% by ozonolysis followed by a two-step reduction of the ozonide. This confirmed the *R* configuration of the starting acid, the *S* configuration of its biologically active levorotatory antipode, and the absolute configurations assigned to their synthetic precursors.

Key words: (*R*)-4-(2,6-dimethylheptyl)benzoic acid, ozonolysis; stereochemical correlation; anti-hypercholesterolemic activity.

Racemic 4-(2,6-dimethylheptyl)benzoic acid ((±)-**1**) efficiently inhibits *in vivo* the accumulation of excess cholesterol in the blood of rats[1]; in experiments *in vitro*, it suppresses the accumulation of cholesterol in the culture of sclerotized human aortic cells.[2] Biomedical properties of the enantiomers of this acid, (*S*)-**1** and (*R*)-**1**, have not been studied previously because both compounds were unknown.

Recently,[3] both enantiomers of acid **1** were prepared from α,β-enal **2** [2] *via* a stereodivergent route involving enzymatic kinetic resolution of racemic alcohol (±)-**3** or its acetate (±)-**3a** as well as their chromiumtricarbonyl complexes using porcine pancreatic lipase (PPL) to give dextrorotatory ((+)-**3**) and levorotatory ((−)-**3**) chiral building blocks.[4,5] Alcohol (−)-**3** was also isolated upon the reduction of its allylic congener prepared from the corresponding enal **2** with *Saccharomyces cerevisiae* yeast (Scheme 1).[4] The configuration of alcohol (−)-**3** was tentatively assigned the *S*-configuration because of the known *S*-selectivity of biohydrogenation of C=C bonds in allylic alcohols of this type with *S. Cerevisiae*[6] cultures; the assignment was also confirmed by the direction of the Eu(fod)$_3$-induced shifts of the signals of the MeO and CF_3 groups in the ^1H and ^{19}F NMR spectra of diastereomeric MTPA esters prepared from alcohol (−)-**3** and (*R*)- and (*S*)-Mosher acids.[4] Correspondingly, *R* configuration was attributed to alcohol (+)-**3**,[4] and the *R* and *S* configurations were ascribed to acids (+)-**1** and (−)-**1**, respectively, relying on the routes of their synthesis.[3]

The arguments supporting the *S* configuration of alcohol (−)-**3** were nevertheless conjectural. So, for reliable assignment of all configurations, it was necessary to make direct stereochemical correlation of one of the above chiral compounds with a compound with firmly established absolute configuration. The solution of this problem and evaluation of the biological potency of acids (+)-**1** and (−)-**1** constitute the subject of this communication.

The anti-hypercholesterolemic activity of acids (+)-**1** and (−)-**1** was estimated from their effect on the content of total cholesterol in human aortic cells cultivated *in vitro* from the adipose layer.* Acid (−)-**1** in concentrations of 10^{-4}—10^{-5} mol L^{-1} produced a statistically significant cholesterol level; in the case of (+)-**1**, the results were almost indistinguishable from the control.

The biologically inactive acid (+)-**1** was subjected to ozonization at 18—20 °C in AcOH up to complete conversion. The reductive decomposition of the ozonide with zinc dust in moist Et_2O yielded 3,7-dimethyloctanal **4**, which was isolated from the reaction mixture and identified by comparison (TLC) with an authentic[8] sample of **4**. Without further purification, the resulting aldehyde was treated with $NaBH_4$ in MeOH at 0 °C to give individual (*R*)-3,7-dimethyloctanol (**5**) with $[\alpha]_D^{20}$ +5.9° (MeOH) in 52% overall yield (based on (+)-**1**). Since a specimen of **5** with *ee* ≈ 100% displays $[\alpha]_D^{20}$ +6.1° (MeOH)[8] and its enantiomer with *ee* ≈ 100% is characterized by $[\alpha]_D^{20}$ −6.1° (MeOH),[9] the alcohol **5** prepared from acid (+)-**1** should have *ee* ≥ 96% (Scheme 2).

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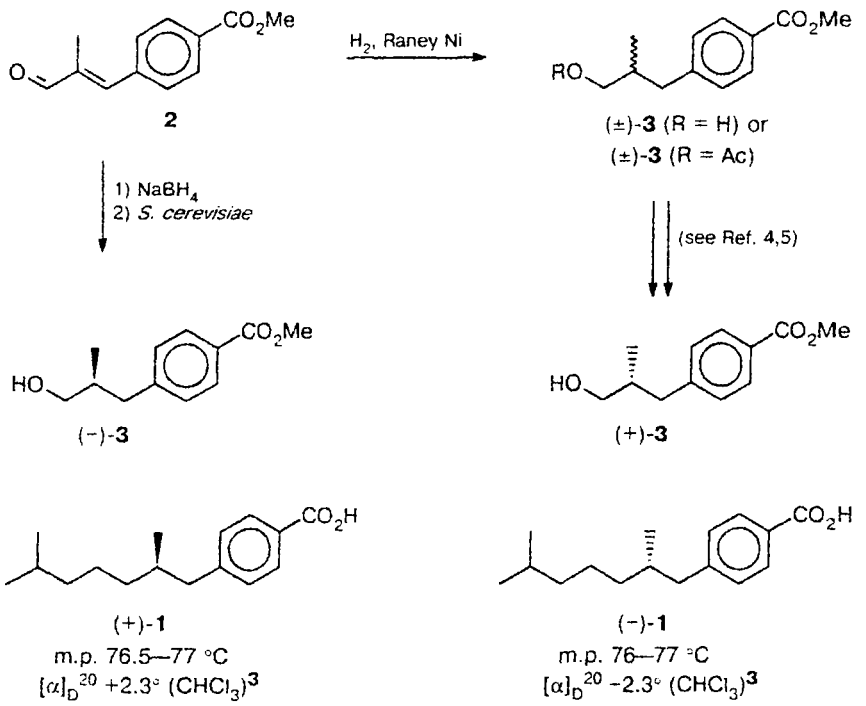
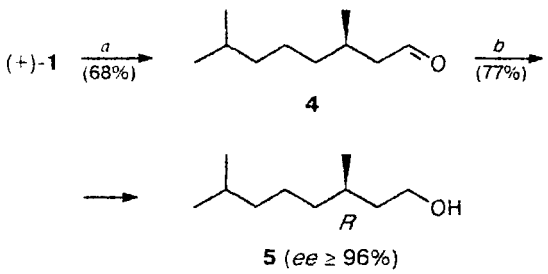
Scheme 1



Thus, the results of direct stereochemical correlation confirm both the *R*-configuration assigned previously to acid (+)-**1** and high enantiomeric purity of acid (+)-**1**.

Scheme 2



Reagents and conditions: *a*. 1) O_3/AcOH, 20 °C; 2) Zn—H_2O/AcOH—Et_2O, 0 °C; *b*. $NaBH_4$/MeOH, 0 °C.

Hence, the biologically active acid (−)-**1** actually has *S* configuration, as was assumed previously.[3] This also confirms the validity of the previous assignment[4] of *R*-configuration to alcohol (+)-**3** and the "abnormal" *R*-enantioselectivity of PPL in the enzymatic acylation of alcohol (±)-**3**[3] and enzymatic hydrolysis of its acetate (±)-**3a**.[4]

Experimental

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker WM-250 instrument. The $[\alpha]_D$ values were measured using a JASCO DIP 360 polarimeter. Column chromatography was carried out on Woelm silica gel (32—63 μm); TLC analyses were performed on Silufol® plates with a fixed SiO_2 layer. Acid (+)-**1** with m.p. 76.5—77 °C (from MeOH—H_2O) and $[\alpha]_D$ +2.3° (*c* 1.0, $CHCl_3$) was prepared by a method described previously.[3] Ozone was generated from O_2 using a high-frequency flow-type ozonizing setup[10] (frequency 3000 Hz, voltage 8 kV, maximum flow rate of O_2 30 L h^{-1}, the concentration of O_3 in the mixture was 5% (v/v)).

Ozonolysis of acid (+)-1. A solution of acid (+)-**1** (50 mg, 0.201 mmol) in glacial AcOH (1 mL) was placed in a flask equipped with a capillary for the inlet of the gas mixture and a reflux condenser; the outlet of the condenser was connected to two successive traps containing 1 mL of glacial AcOH each. A mixture of ozone with oxygen was passed through the solution (5 : 95, v/v, flow rate 5 L h^{-1}). After 1 h, the original substrate completely disappeared from the reaction mixture (TLC data, hexane—AcOEt, 2 : 1). The content of two traps was added to the reaction mixture cooled to 0 °C; then a double volume of Et_2O, two drops of distilled water, and zinc dust on the tip of a spatula were added successively. The mixture was vigorously stirred at 0 °C for 5 min. The alternating addition of water and Zn dust was continued until the evolution of H_2 bubbles became visible. When testing of the reaction mixture for oxidants (iodide—starch paper) gave a negative result, the mixture was diluted with water (2 mL), the organic layer was separated,

and the aqueous phase was extracted with Et_2O (4×3 mL). The combined organic extract was washed with water (4×2 mL), aqueous Na_2CO_3 (3×2 mL), and again with water, dried (Na_2SO_4), and concentrated at 35—40 °C in a vacuum of a water aspirator to give 21.5 mg (68%) of chromatographically homogeneous (*R*)-3,7-dimethyloctanal **4** with R_f 0.53 (hexane—AcOEt, 5 : 1); for an authentic[8] levorotatory sample of **4**, R_f = 0.53 in the same system.

(*R*)-3,7-Dimethyloctanol (5). Solid $NaBH_4$ (2.5 mg, 0.07 mmol) was added to a cooled (0 °C) solution of aldehyde **4** from the previous step (21 mg, 0.134 mmol) in anhydrous MeOH (1 mL). The reaction mixture was stirred for 1 h at 20 °C, methanol was evaporated at a reduced pressure at 35—40 °C (bath), and the residue was diluted with water (0.2 mL). The reaction product was extracted with ether (3×0.2 mL), and the extract was dried (Na_2SO_4) and concentrated at 35—40 °C (bath) and 75 Torr. The residue was chromatographed on a column using gradient elution, pentane—Et_2O (0→3% Et_2O). This gave 16 mg (77%) of alcohol **5** with R_f 0.44 (hexane—AcOEt, 5 : 1), n_D^{20} 1.4355, and $[\alpha]_D^{20}$ +5.9° (*c* 1.0, MeOH). According to IR and ^1H NMR spectra, this product was identical to the sample prepared previously[8] (*cf.* Ref. 8: $[\alpha]_D^{20}$ +6.1° (*c* 1.0, MeOH)).

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