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Stereochemical clarification of the enzyme-catalysed reduction of 2-acetylchromen-4-one

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Abstract—*Lactobacillus kefir* alcohol dehydrogenase catalysed asymmetric reduction of 2-acetylchromen-4-one was achieved with 68% yield and 90% ee. The (*R*)-stereochemistry of the corresponding alcohol was unambiguously assigned by means of single crystal X-ray analysis.

1. Introduction

The chromen-4-one moiety is common to a series of natural products such as flavonoids and thus, it is considered as a privileged structure in the search for new compounds with promising pharmacological profiles.[1] Enantiomerically pure 2-(1-hydroxyethyl)chromen-4-one **2**, an intermediate for the development of such derivatives, has been described in the literature.[2] The key step of the synthesis proceeds from a whole cell microbiological reduction of the corresponding prochiral ketone using several microorganisms. Secondary alcohol **2** was obtained with high enantiomeric excess (ee). Nevertheless, the determination of the absolute stereochemistry proved troublesome. This prompted us to study further this biocatalysed hydrogenation. Herein we report on the successful reduction of 2-acetylchromen-4-one **1** by means of an isolated enzyme with a focus on the elucidation of the absolute stereochemistry of **2**.

2. Results and discussion

The nicotinamide adenine dinucleotide phosphate (NADP)-dependent alcohol dehydrogenase from *Lactobacillus kefir* (LK-ADH) proved a useful tool for the highly enantioselective reduction of methylketones with a bulky second substituent.[3] Thus, we reasoned out that it should be suitable for the chemo- and enantioselective reduction of chromenone **1**. Indeed, it was expected that the vinylogous lactone functionality would be inert to LK-ADH catalysed hydrogenation. Accordingly, a preparative scale reduction of **1** catalysed by LK-ADH in a triethylamine buffer in the presence of $NADP^+$ and 2-propanol gave alcohol **2** in 68% chemical yield and 90% ee. 2-Propanol serves as a hydride donor in the concomitant enzyme-catalysed co-factor regeneration process (Scheme 1).

Since, unlike most of the alcohol dehydrogenases and organisms such as Baker's yeast,[4] LK-ADH preferentially catalyses hydride transfer to the *si*-face of prochiral ketones, it was expected that hydrogenation of the carbonyl function of **1** would yield (*R*)-**2**. However, the secondary alcohol obtained exhibits a specific rotation with a positive value and therefore it has to be enantiomeric to the 2-(1-hydroxyethyl)chromen-4-one obtained from Bakers' yeast catalysed reduction.[2]

Moshers' NMR-based method[5] is still one of the most popular ones for the determination of the absolute

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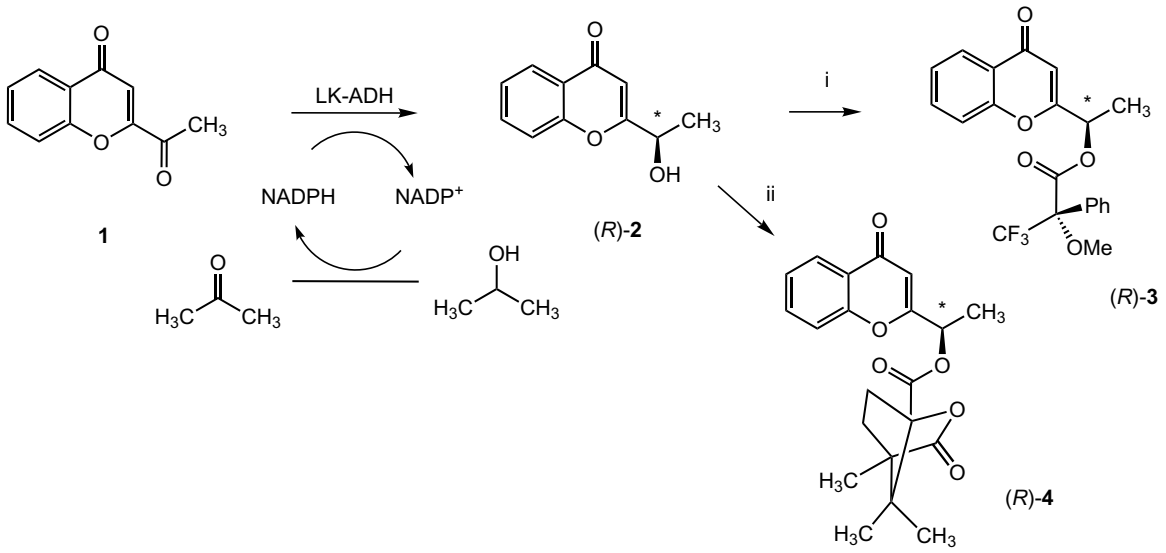

Scheme 1. Reagents and conditions: (i) (*R*)-(−)-2-methoxy-2-phenyl-3,3,3-trifluoropropanoic acid chloride, pyridine, 1.5 h, rt; (ii) (−)-camphanic acid chloride, DMAP, pyridine, 48 h, rt.

stereochemistry of secondary alcohols. Thus, **2** was converted quantitatively to the corresponding ester **3** with (*R*)-(−)-2-methoxy-2-phenyl-3,3,3-trifluoropropanoic acid chloride in pyridine. Subsequent ^{1}H NMR analysis revealed two discrete doublets for the methyl group and two individual multiplets for the methoxy group arising from the two diastereomeric Mosher esters. According to Moshers' conformational hypothesis the downfield shifted major signal groups can tentatively be assigned to the diastereomeric ester (*R*)-**3** giving a strong hint for favoured hydride delivery from the *si*-face. Among the enantiomerically pure derivatisation reagents suitable for secondary alcohols, (−)-camphanic acid chloride gives rise to esters, which are prone to crystallisation. Hence, in order to finally prove its absolute stereochemistry, (+)-**2** was reacted to the corresponding camphanic ester **4** in the presence of 4-(dimethylamino)pyridine in pyridine (81%). Upon single crystal X-ray analysis (Fig. 1), the expected (*R*)-configuration could be unambiguously assigned to (+)-**2**, thus confirming the anti-Prelog[6] selectivity of LK-ADH. This configuration is in accordance with the conclusions obtained by means of vibrational circular dichroism from the acetate derivative of (−)-**2**.[7]

3. Experimental

3.1. General methods

All reagents used were of analytical grade. Solvents were dessicated by standard methods if necessary. Melting points were determined with a Büchi B-540 apparatus and are uncorrected. Thin-layer chromatography (TLC) was carried out on aluminium sheets precoated with silica gel 60F_{254} (Merck). Detection was performed by UV-light ($\lambda = 254$ nm). Preparative column chromatography was carried out on silica gel 60 (Merck) (particle size: 40–63 μm). ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, on a AMX-300 (Bruker Physik AG, Germany). Chemical shifts δ are reported in parts per

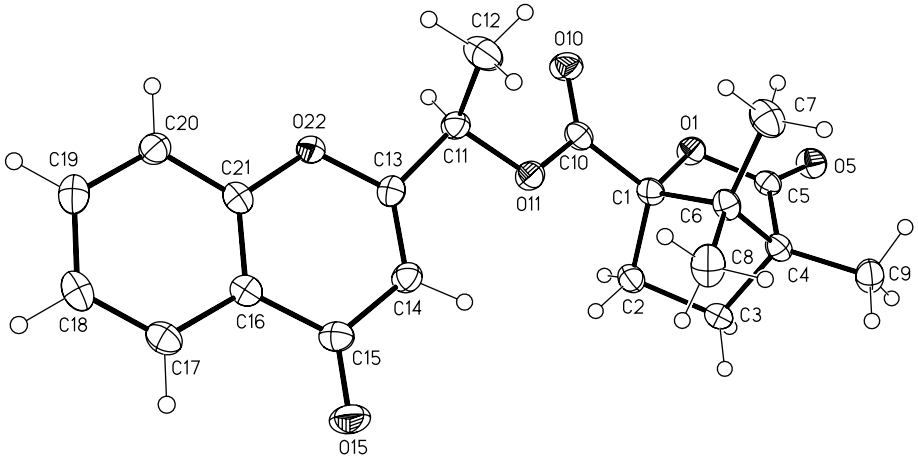

Figure 1. Molecular structure of (*R*)-**4** with displacement parameters at 50% probability level.

million relative to $CHCl_3$ (^1H, $\delta = 7.25$) and $CDCl_3$ (^{13}C, $\delta = 77.00$) as the internal standard. Chiral phase HPLC was performed with an LC Series 1100 (Agilent) equipment using a Chiralcel-OB (Daicel) column. The elution rate was 1 ml/min with ethylacetate/hexane 5:95 as the mobile phase. Optical rotations were measured on a polarimeter P-1020 (Jasco).

3.1.1. 2-[(*R*)-1-Hydroxyethyl]chromen-4-one (*R*)-2. Triethylamine buffer pH 6.5 (80 mL, 100 nM) was implemented with $NADP^+$ (40 mg), LK-ADH (50 U) and 2-propanol (10 mL). A solution of 2-acetylchromen-4-one **1** (165 mg, 0.88 mmol in 10 mL 2-propanol) was added in portions of 1 mL every hour. After completion of the addition, a further 20 U LK-ADH was added and the reaction mixture stirred at rt for 48 h, after which it was extracted twice with ethyl acetate (100 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified on a silica gel column eluted with hexane/ethyl acetate 2:1 to provide **2** (114 mg, 0.60 mmol, 69%) as a white powder. Alcohol **2** was submitted to chiral phase HPLC and an ee of 90% was determined. Mp = 105 °C; $[\alpha]_D^{22} = +57.55$ (*c* 0.9, $CHCl_3$); t_R (min) 30 (*S*), 58 (*R*); ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.59$ (d, $J = 6.6$ Hz, 3H), 3.8 (br s, 1H), 4.75 (q, $J = 6.6$ Hz, 1H), 6.52 (s, 1H), 7.37 (dd, $J = 8.1$, 8.1 Hz; 1H), 7.33 (d, $J = 8.0$ Hz, 1H), 7.62 (ddd, $J = 8.6$, 8.6, 1.6 Hz, 1H), 8.12 (dd, $J = 8.0$, 1.6 Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) $\delta = 21.5$, 67.2, 107.3, 118.2, 123.9, 125.4, 125.8, 134.0, 156.4, 172.1, 179.2.

3.1.2. (2*S*)-[(1*S*)-(Chromen-4-on-2-yl)ethyl] 2-methoxy-2-phenyl-3,3,3-trifluoropropanoate (*S*)-3 and (2*S*)-[(1*R*)-(chromen-4-on-2-yl)ethyl] 2-methoxy-2-phenyl-3,3,3-trifluoropropanoate (*R*)-3. Alcohol **2** (10 mg, 0.526 mmol) was taken up in pyridine (1 mL) and (*R*)-Moshers' acid chloride (33.4 μL, 0.179 mmol) was added at rt and stirring was maintained for 1.5 h. A saturated solution of $NaHCO_3$ was added and stirring was continued for another 0.5 h. The reaction mixture was extracted with ethylacetate (10 mL), and the organics were washed with H_2O, NH_4Cl satd, brine, dried over Na_2SO_4, filtered and evaporated to dryness. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 1.66$ (d, $J = 6.7$ Hz, 3H, $-CH_3$ (*S*)-**3**), 1.73 (d, $J = 6.7$ Hz, 3H, $-CH_3$ (*R*)-**3**), 3.55–3.57 (m, 3H, $-OCH_3$ (*S*)-**3**), 3.57–3.60 (m, 3H, $-OCH_3$ (*R*)-**4**).

3.1.3. (1*S*,4*R*)-[(1*R*)-Chromen-4-on-2-yl] camphanoate (*R*)-4. Alcohol **2** (50 mg, 0.26 mmol) was taken up in pyridine (5 mL), dimethylaminopyridine (11.3 mg, 0.09 mmol) and (−)-camphanic acid chloride (142.5 mg, 0.66 mmol) were added at rt. Stirring was maintained for 1 h, the reaction mixture was acidified with 2 M HCl and extracted with CH_2Cl_2 (10 mL). The organic layer was washed with 2 M HCl, $NaHCO_3$ satd and H_2O, dried over Na_2SO_4, filtered and evaporated to dryness to yield ester **4** (76.3 mg, 0.21 mmol, 81%). Recrystallisation from hexane/ethyl acetate gave needles (70 mg), which were submitted to single crystal X-ray analysis. ^1H NMR (300 MHz, $CDCl_3$) $\delta = 0.96$ (s, 3H), 1.06 (s, 3H), 1.12 (s, 3H), 1.65–1.75 (m, 1H), 1.69 (d, $J = 6.7$ Hz, 3H), 1.94 (ddd, $J = 13.1$, 10.7, 4.5 Hz, 1H), 2.09 (ddd, $J = 13.7$, 9.3, 4.5 Hz, 1H), 2.47 (ddd, $J = 13.4$, 10.7, 4.3 Hz, 1H), 5.86 (q, $J = 6.7$ Hz, 1H), 6.36 (s, 1H), 7.3–7.45 (m, 2H), 7.69 (ddd, $J = 10.3$, 7.6, 3.3 Hz, 1H), 8.17 (dd, $J = 8.0$, 1.6 Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) $\delta = 9.3$, 16.9, 17.0, 18.8, 29.1, 30.9, 54.7, 55.0, 66.8, 90.9, 109.0, 118.1, 124.1, 125.7, 126.0, 134.2, 136.7, 156.2, 165.4, 166.7, 177.9.

3.1.4. Single crystal X-ray analysis of (*R*)-4. Compound (*R*)-**4:** colourless crystals, $C_{21}H_{22}O_6$, $M = 370.39$, crystal size $0.55 \times 0.35 \times 0.25$ mm, orthorhombic, space group $P2_12_12_1$ (No. 19): $a = 7.0681(1)$ Å, $b = 10.0781(1)$ Å, $c = 25.7845(3)$ Å, $V = 1836.71(4)$ Å^3, $Z = 4$, $\rho(\text{calcd}) = 1.339$ Mg m^{-3}, $F(000) = 784$, $\mu = 0.098$ mm^{-1}, 29,895 reflections ($2\theta_{max} = 50°$) measured on a Nonius Kappa-CCD diffractometer at 123(2) K using Mo K_α radiation ($\lambda = 0.71073$ Å), 3229 unique [$R_{int} = 0.037$] used for structure solution (Direct Methods, SHELXS-97)[8a] and refinement (full-matrix least-squares on F^2, SHELXL-97)[8b] with 244 parameters, H-atoms with a riding model, *R*1 ($I > 2\sigma(I)$) = 0.025, *wR*2 = 0.064, largest diff. peak and hole 0.113 and −0.201 e Å^{-3}. The absolute configuration could not be determined reliably [$x = -0.2(6)$,[8c] but could be determined from known stereogenic centre (1*S*) and (4*R*) of the camphanic acid unit].

Crystallographic data (excluding structure factors) for the structures reported in this work have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-641978 [(*R*)-**4**]. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge DB2 1EZ, UK (fax: int. code +(1223)336-033; e-mail:deposit@ccdc.cam.ac.uk).

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