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CHEMOENZYMATIC SYNTHESIS AND STEREOCHEMISTRY OF ALEPPOTRIOLOSIDE, A NATURALLY OCCURRING GLUCOSIDE

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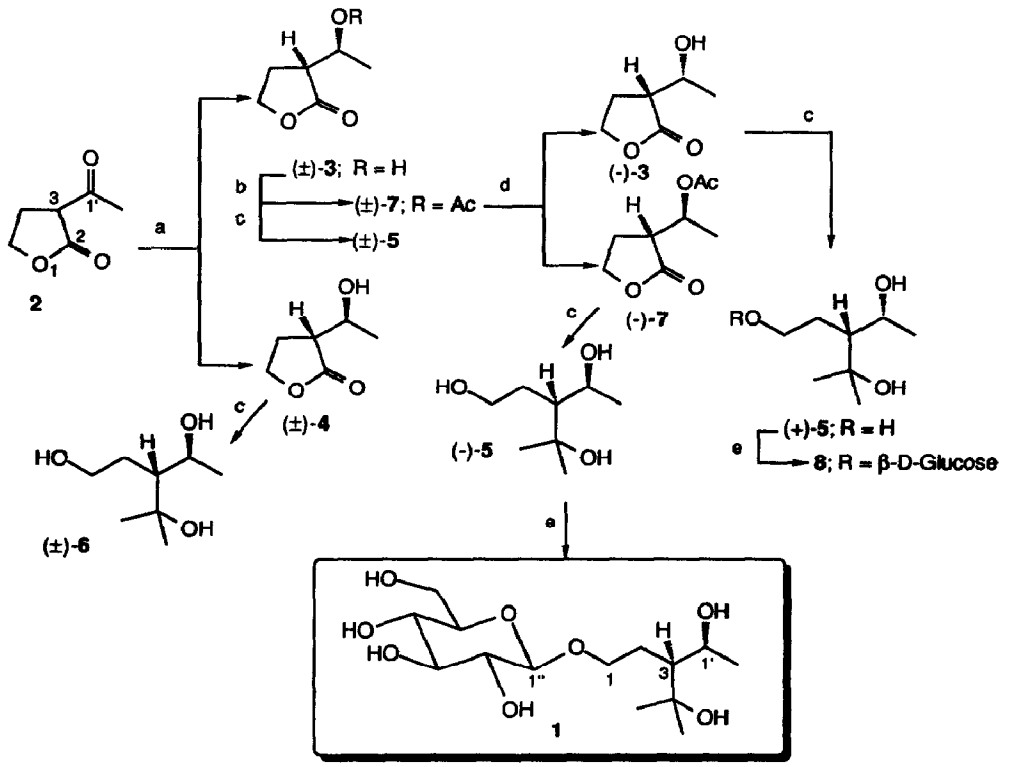
Abstract The stereochemistry of aleppotrioloside, a naturally occurring glucoside, has been determined as (3S)-3-[(1S)-1-hydroxyethyl]-4-methyl-1,4-pentanediol-1-*O*-β-D-glucopyranoside by its synthesis based on two key enzymatic reactions: *Pseudomonas fluorescens* lipase (PFL) catalyzed enantioselective resolution of an aglycone precursor and transglucosylation of the aglycone by thermophilic β-glycosidase from *Sulfolobus solfataricus*.

Enzymatic transglycosylation of polyols by β-glycosidases proceeds with preference towards primary hydroxyl groups, the relative percentage of functionalization at secondary hydroxyl groups depending on the complexity of the molecule (i.e. its interactions with the enzymatic site of glycosylation)[1]. Recently the gross structure of aleppotrioloside, a naturally occurring β-glucoside, has been reported[2] (**1**; no stereochemistry of the aglycone implied); the aglycone of **1**, bearing at the same time a primary, a secondary and a tertiary hydroxyl group, offers a unique opportunity for testing the regiospecificity of transglycosylation. We report here the synthesis of aleppotrioloside based on two enzymatic steps which allowed also the determination of its stereochemistry.

$NaBH_4$ reduction of the commercially available α-acetyl-γ-butyrolactone (**2**) afforded the two diastereomic pairs (±)-**3** (43%) and (±)-**4** (38%) which were separated by silica gel column chromatography. The relative configuration of the diastereomers was determined as previously described[3] by ^1H NMR analysis of the 1,3-dioxane derivatives of the corresponding triols obtained by $LiAlH_4$ reduction of (±)-**3** and (±)-**4**. In order to preliminarly infer the relative stereochemistry of the aglycone moiety in **1**, both (±)-**3** and (±)-**4** were reacted with CH_3MgBr affording (±)-**5** and (±)-**6** which, in turn,were subjected to transglucosylation using phenyl-β-D-glucoside as carbohydrate donor and crude homogenate of *Sulfolobus solfataricus*, containing a β-glycosidase activity[4]. The comparison of the ^{13}C NMR spectra of the derived glucosides with that of **1** showed that the relative stereochemistry of aleppotrioloside should be 3*R**,1'*R**, since the resonances reported for **1**[2], in particular those of the aglycone moiety, were found only in the spectrum of the diastereomeric glucosides arising from (±)-**5**. Glucosylation at secondary hydroxyl group was detected only in a trace amount, while the tertiary hydroxyl group was not glucosylated.

The synthesis of **1** thus required resolution of (±)-**3**, which was achieved by enantioselective hydrolysis of the acetyl derivative (±)-**7** using *Pseudomonas fluorescens* lipase[5] that yielded (3*R*,1'*R*)-**3** (40%; 90 % ee[6]; $[\alpha]_D$ -33.8°; *c* 3, $CHCl_3$) and (3*S*,1'*S*)-**7** (41%; >98 % ee[6]; $[\alpha]_D$ -8.6°; *c* 3, $CHCl_3$). It should be noted that (3*S*,1'*S*)-**3** has already been prepared by baker's yeast reduction of **2**, along with its 3*R*,1'*S* isomer[3]. Both (3*R*,1'*R*)-**3** and (3*S*,1'*S*)-**7** were treated with an excess of CH_3MgBr yielding (+)-**5** (36%; $[\alpha]_D$ +2.3°; *c* 1.2, MeOH) and (-)-**5** (50%; $[\alpha]_D$ -3.7°; *c* 1.5, MeOH), respectively. Transglucosylation of (-)-**5** as above yielded a glucoside (22%; $[\alpha]_D$ -29.5; *c* 1.6, MeOH; lit.[2] $[\alpha]_D$ -30; *c* 0.69, MeOH) whose ^1H and ^{13}C NMR spectra were superimposable to those of naturally occurring **1**, while transglucosylation of (+)-**5** afforded **8** (23%; $[\alpha]_D$ -12.4; *c* 0.8, MeOH). In the NMR spectra[7] of **1** and **8** the presence of the minor diastereomer was not detected.



Reaction conditions: a, $NaBH_4$ in MeOH, 0°C; *b*, Ac_2O, pyridine; *c*, CH_3MgBr in anhydrous Et_2O; *d*, *Pseudomonas fluorescens* lipase (1300 U/mmole of (±)-7) in 200 mM phosphate buffer, pH 7, 6 days, r.t.; *e*, crude homogenate of *Sulfolobus solfataricus*[1b], 75°C, phenyl-β-D-glucoside in a 1:28 molar ratio with respect to (+)-5 or (-)-5; at total donor consumption another aliquot was added (two times); yields are calculated with respect to the total amount of donor added.

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6.The absolute configuration and the enantiomeric excess of (-)-3 were established by ^1H NMR analysis of Mosher esters[8]. 7 was hydrolyzed (0.1N HCl in MeOH; 24h; r.t.) to (+)-3 ($[\alpha]_D$ +36.8; *c* 0.7, $CHCl_3$; lit.[3] $[\alpha]_D$ +36.9; 84% ee; *c* 1.3, $CHCl_3$) and analyzed as above.

7. 1 ^{13}C NMR, δ (CD_3OD): 104.41, 78.15, 77.93, 75.32, 75.14, 71.63, 71.10 (2C), 62.64, 52.46, 29.32, 29.12, 25.62, 21.88. ^1H NMR, δ (CD_3OD): 4.26 (d, J 7.8Hz; H-1"), 3.93 (m; H-1a+H-1'), 3.85 (dd, J 12.0 and 2.1Hz; H-6"a), 3.66 (dd, J 11.9 and 5.3 Hz; H-6"b), 3.59 (m; H-1b), 3.34 (t, J 8.8Hz; H-3"), 3.26 (m; H-4"+ H-5"), 3.15 (dd, J 9.0 and 7.8Hz H-2"), 1.62 (m; H-2a), 1.44 (m; H-2b), 1.41 (m; H-3), 1. 22 (s $2CH_3$), 1.21 (d, J 6.1Hz; H-2'). 8 ^{13}C NMR, δ (CD_3OD): 104.33, 78.17, 77.95, 75.31, 75.14, 71.64, 71.10(2C), 62.74, 52.54, 29.27, 28.18, 25.61, 21.84. ^1H NMR, δ (CD_3OD): 4.26 (d, J 7.9Hz; H-1"), 3.92 (m; H-1a+H-1'), 3.85 (dd, J 12.0 and 1.7Hz; H-6"a), 3.66 (dd, J 11.9 and 5.2 Hz; H-6"b), 3.63 (m; H-1b), 3.34 (t, J 8.9Hz; H-3"), 3.26 (m; H-4"+ H-5"), 3.16 (t, J 8.4Hz H-2"), 1.62 (m; H-2a), 1.47 (m; H-2b), 1.42 (m; H-3), 1.21 (s $2CH_3$), 1.21 (d,J 6.0Hz; H-2').

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