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Chemoenzymatic Synthesis of Rengyoside -A, -B, Isorengyoside and Synthesis of their Aglycones

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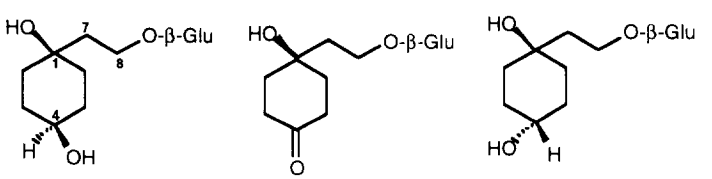
Abstract: The chemoenzymatic synthesis of a group of naturally occurring cyclohexylethanoids, rengyoside-A, -B and isorengyoside, has been performed by enzymatic glucosidation of their chemically synthesized aglycones, rengyol, rengyoxide and isorengyol. © 1997 Published by Elsevier Science Ltd.

Phenylethanoid glycosides (PhGs) are a group of natural products widely distributed in the plant kingdom[1]. Most of the PhGs were isolated from medicinal plants and are contained in crude drugs of plant origin used in Oriental medicine. For example, the crude drug "rengyo", prepared from the fruits of *Forsythia suspensa* Vahl (Oleaceae) and used in Oriental medicine for antiinflammatory, diuretic, drainage and antitodal purposes, contains PhGs as antibacterial principles[2,3]. Another drug which is rich in PhGs is "peep", prepared from the flower of *Millingtonia hortensis* and used in Thailand for the treatment of asthma, sinusitis and as a cholagogue and tonic[4].

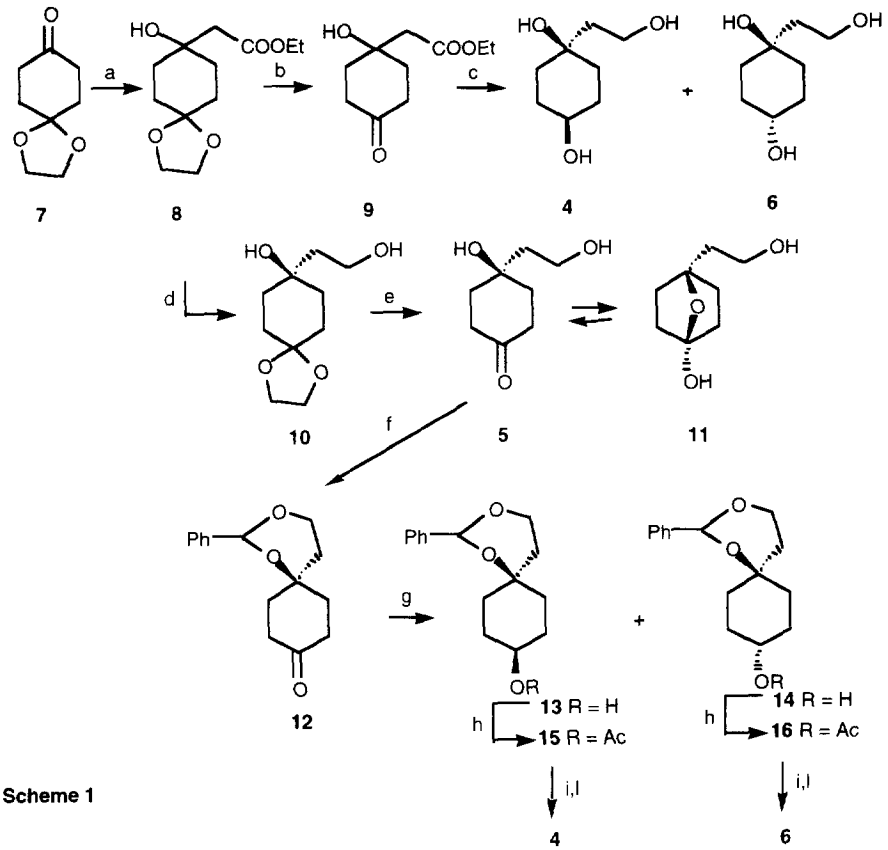
Structurally, PhGs are mostly glycosides of a 2-phenylethanol moiety carrying hydroxy groups on the aromatic ring. A restricted group of biogenetically related compounds is constituted of cyclohexylethanoids such as rengyoside-A (**1**), -B (**2**) and isorengyoside (**3**), also isolated from "rengyo"[5] and "peep"[6], considered to arise from the corresponding aromatic compounds by oxidation at the branched carbon of the aromatic ring followed by extensive reduction[7]. The corresponding aglycones, rengyol (**4**), rengyoxide (**5**) and isorengyol (**6**) have been also isolated from the same sources[3,7].



Rengyoside-A **1** ***Rengyoside-B*** **2** ***Isorengyoside*** **3**

We decided to synthesize **1-3** essentially because rengyol (**4**) and isorengyol (**6**), which bear a primary, a secondary and a tertiary hydroxy group, are ideal substrates for testing the claimed preference of β-glycosidases

towards primary hydroxy groups in enzymatic transglycosidations[8]. This approach was successfully used by us for the synthesis of aleppotrioloside, a naturally occurring glucoside[9].



Scheme 1

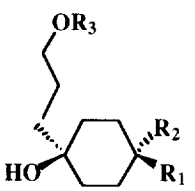
a= Zn, $BrCH_2COOEt$, C_6H_6 (85%); b= AcOH, 90°(67%); c= $LiAlH_4$, Et_2O (76%); d=$LiAlH_4$, Et_2O (80%); e= Dowex, H_2O (100%) f= p-TsOH, C_6H_5CHO (47%); g= LS-Selectride, Et_2O (62%); h= Py,Ac_2O (100%, 15/16= 1:1); i= K_2CO_3, MeOH (70%); l= H_2, Pd/C (100%)

The aglycones **4-6** have been synthesized as reported in Scheme 1. Reformatsky reaction of commercially available 1,4-cycloexanedione mono-ethylene ketal (**7**) with ethyl bromoacetate afforded the β-hydroxyester **8** in 85% yield. Deprotection of **8** with aqueous acetic acid yielded **9** which upon reduction with $LiAlH_4$ in dry diethyl ether gave rengyol (**4**) and isorengyol (**6**) in a 16:1 ratio (by ^{13}C NMR). On the other hand, direct $LiAlH_4$ reduction of **8** afforded **10** (80%) which on subsequent deprotection of the carbonyl function with Dowex[10] gave rengyoxide (**5**; 100%), which exists as a mixture with the monoketal **11**[7]. In order to obtain substantial amounts of isorengyol (**6**), rengyoxide (**5**) was transformed into the benzylideneacetal **12** which upon reduction with LS-Selectride® afforded **13** and **14** in a 1:1 ratio. The stereoselectivity of this reaction was poorer than that expected on the basis of the results on 4-alkyl-cyclohexanones[11] and is probably due to the comparable steric hindrance of the two substituents at C-1. On the other hand, it has been previously reported

that attempts to obtain **6** from **4** by S_N2 type inversion were unsuccessful[12] and therefore the results of the LS-Selectride® reduction were considered satisfactory, also taking into account that rengyol (**4**) and isorengyol (**6**) were previously prepared by a procedure leading to a 3:2 ratio of the two compounds[12]. Thus compounds **13** and **14** were separated by silica gel chromatography as their acetates **15** and **16**, which were converted into **4** and **6**, respectively, by methanolysis followed by hydrogenolysis.

The transglucosidation reaction using rengyol (**4**) as acceptor and 4-nitrophenyl-β-D-glucopyranoside, as glucose donor, and either almond β-glucosidase or a crude homogenate from the thermophilic bacterium *Sulfolobus solfataricus*[13] containing a β-glycosidase activity, gave poor results. In fact, with the almond β-glucosidase a mixture (1.4:1) of rengyoside-A (**1**) and of its isomer **17** glucosylated at the secondary hydroxy group was obtained, in a total yield of 14% with respect to the donor. The yield and the ratio between the two compounds were slightly better using the crude homogenate from *Sulfolobus solfataricus* (25% and 2:1, respectively). Compounds **1** and **17** arising from the above reactions were separated as their acetates **18** and **19** by silica gel column chromatography and their structures assigned by NMR.

Enzymatic glucosylation of isorengyol (**6**) was performed using crude homogenate of *S. solfataricus*; in this case a good preference towards the primary hydroxy group was observed. Infact, after acetylation, isorengyoside acetate (**22**) and its regioisomer glucosylated at the secondary hydroxy group (**23**) were obtained in a 9:1 ratio and in a 44% total yield.



1	R_1 = OH	R_2 = H	R_3 = β-Glu
17	R_1 = O-β-Glu	R_2 = H	R_3 = H
18	R_1 = OAc	R_2 = H	R_3 = β-Glu-Ac_4
19	R_1 = O-β-Glu-Ac_4	R_2 = H	R_3 = H
20	R_1 = OAc	R_2 = H	R_3 = H
21	R_1, R_2 =OCH_2CH_2O		R_3 = β-Glu
22	R_1 = H	R_2 = OAc	R_3 = β-Glu-Ac_4
23	R_1 = H	R_2= O-β-GluAc_4	R_3 = Ac
24	R_1, R_2 = =O		R_3 = β-Glu-Ac_4

Since the regioselectivity of the transglucosidation reaction was poorer than expected, we tried to glucosylate a substrate having a protected or masked secondary hydroxy group. Rengyol acetylated at the secondary hydroxy group (**20**), obtained from hydrogenolysis of **15**, was not substrate of both almond and *S. solfataricus* enzymes. However, both rengyoxide (**5**) and its ethylidene acetal **10** were successfully glucosylated using the *S. solfataricus* crude homogenate, yielding rengyoside-B (**2**) and its ethylidene acetal (**21**), respectively. In both case transglucosylation at tertiary hydroxy group was not observed. In the synthesis of

rengyoside-B (**2**) a 1:1 molar ratio donor/acceptor resulted in a 23% yield while a four fold molar excess of acceptor lead to a 35% yield. In the synthesis of **21** a stoichiometric amount of donor was added in different aliquots (molar excess 9) obtaining 18% yield. Glucoside **21** was converted into rengyoside-B (**2**) by Dowex removal of the protecting group, while rengyoside-A (**1**) can be obtained by $NaBH_4$ reduction of **2** as previously described[7]. On the other hand, acetylation of rengyoside-B (**2**) and reduction of the acetate with LS-Selectride®, followed by acetylation of the reaction products, afforded rengyoside-A acetate (**18**) and isorengyoside acetate (**22**) in a 1:1.5 ratio. The two compounds were separated by silica gel column chromatography and converted by methanolysis into rengyoside-A (**1**) and isorengyoside (**3**), identified by comparison of their spectral and optical rotation data with those reported for the natural products[5,6].

Alcohols and acyclic polyols of different structures can be glycosylated by thermophilic glycosidases[14]; as shown here, also different cyclohexylethanoids aglycones can act as substrates. Using rengyol (**4**) and isorengyol (**6**) primary and secondary hydroxy groups functionalization was observed during enzymatic glucosylation, with satisfactory (9:1) preference for primary hydroxy group only in **6** confirming the role of the stereochemistry of the acceptor on the regioselectivity of enzymatic transfer[15]. The yields of these reactions are strongly dependent upon structure, molar excess of acceptors and their concentration in the reaction medium. The use of thermophilic glycosidase increases to 25% the yield with respect to that obtained with mesophilic almond enzyme (14%) in glucosylation of rengyol (**4**); however, the reaction conditions were not optimized and the conversion of the aglycones was in general unsatisfactory.

In the widely accepted mechanism for the transglucosidation reactions (see ref. 8 for a review), the enzyme-bound glycosyl cation intermediate is captured by the acceptor to form the glycoside; alternatively, it can be captured by water leading to hydrolysis or by the donor itself forming disaccharides. Accordingly, as usually done, we have initially used a large excess of acceptors, as in the synthesis of **1**. The conversion of the aglycones has been later improved by using a stoichiometric amount of donor, as in the synthesis of rengioside-B (**2**) and of the derivative **21**. High concentration (0.4-1.38 mmoles/ml) of acceptors (**5**) and (**6**) have been used to increase the yield from 23% to 44% in the reactions leading to **2** and **22**, respectively, without affecting the regioselectivity of the reaction.

EXPERIMENTAL

Optical rotations were obtained with a JASCO DIP 1000 polarimeter. NMR spectra were recorded on a Bruker AM 250 (250.13 MHz for ^1H and 62.89 MHz for ^{13}C) and a Bruker AMX 500 (500.13 MHz for ^1H and 125.75 MHz for ^{13}C) spectrometers. Chemical shifts are given in ppm (δ) scale; for the spectra in $CDCl_3$ the $CHCl_3$ signal was used as internal standard (δ 7.26 ^1H; δ 77.0 ^{13}C), while for the spectra in C_5D_5N the downfield signal of pyridine was used as internal standard (δ 8.80 ^1H; δ 150.0 ^{13}C); for the spectra in CD_3OD the MeOH signal was used as internal standard (δ 3.34 ^1H; δ 49.0 ^{13}C). J values are given in Hz. Mass spectra were taken on a VG TRIO 2000 instrument and VG TRIO ZAB2SE instrument. Column chromatographic separations were carried out using Silica gel 60 (70-230 mesh and 230-400 mesh Merck).

Biocatalysts and Glucoside Synthesis

The thermophilic archaeon *Sulfolobus solfataricus* strain MT4 (DSM 5833), isolated[13] from an acid hot spring in Agnano, Naples, was grown at 88 °C and at pH 3.5 in 90 L fermenter. Standard culture medium and work-up for obtaining a crude homogenate were previously reported[14]. Almond β-glucosidase was obtained from Fluka (5.7 U/mg).

Enzymatic synthesis of glucosides **1**, **2**, **3**, **21** and **22** was performed using *Sulfolobus solfataricus* or almond β-glucosidase at 75 or 30 °C, respectively, under stirring. The reactions were monitored by TLC ($CHCl_3$/MeOH 8:2 or $CHCl_3$/MeOH/H_2O, 65:25:4) and after donor consumption the mixture of glucosides were purified on silica gel column ($CHCl_3$/MeOH 8:2). Acetylation (Ac_2O/Pyr) and further purification on silica gel column ($CHCl_3$/MeOH 98:2) were used to obtain the peracetates.

Synthesis of Rengyol (4)

Reformatsky reaction on 7

To a suspension of activated Zn powder (2.0 g; 30.6 mmol) in 6 ml of dry benzene, 1.0 g (6.40 mmol) of 1,4-cycloexanedione mono-ethylene ketal (**7**) was added; 0.85 ml (7.64 mmol) of $BrCH_2COOEt$ in 10 ml of dry benzene were slowly added and the reaction mixture was heated at reflux for 30 min. The reaction was then stirred for 1 h at r.t., AcOH (3 ml) was added and the suspension was diluted with water and extracted with AcOEt. The extract was washed with brine and dried over Na_2SO_4. Removal of solvent gave a residue which was chromatographed on a silica gel column. Elution with Et_2O/Petroleum ether 2:1 gave ester **8** (1.324 g; 5.42 mmol; 85%). ^{1}H-NMR ($CDCl_3$): δ 1.25 (3H, t, J=7.0 Hz), 1.5-2.0 (8H, m), 2.46 (2H, s), 3.46 (1H, s), 3.94 (4H, m), 4.16 (2H, q, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 14.1 (Me), 30.1 (C-3, C-5), 34.7 (C-2, C-6), 45.2 (C-7), 60.6 (O*CH*$_2$Me), 64.1, 64.2 (O*CH*$_2$*CH*$_2$O), 68.7 (C-1), 108.5 (C-4), 172.9 (C-8). EIMS, m/z 244 (M^+), 227, 226, 199, 181.

Deprotection of 8

A 33% aqueous AcOH solution (6 ml) was added to the ester **8** (1.324 g). The mixture was stirred at 90 °C for 12 h and then quenched with a saturated $NaHCO_3$ solution and extracted with Et_2O. The extract was washed with brine and dried over Na_2SO_4. Removal of solvent gave a residue which was flash chromatographed on a silica gel column. Elution with Et_2O/Petroleum Ether 3:1 gave the ketone **9** (726 mg; 67%). ^{1}H-NMR ($CDCl_3$): δ 1.28 (3H, t, J=7.0 Hz), 1.75 (2H, dt, J=13.4 and 4.7 Hz), 2.11 (2H, m), 2.4 (2H, m), 2.54 (2H, s), 2.77 (2H, dt, J=14.1 and 6.3 Hz), 3.91 (1H, s, OH), 4.20 (2H, q, J=7.1 Hz). ^{13}C-NMR ($CDCl_3$): δ 14.1 (Me), 36.7, 36.8 (C-2, C-3, C-5, C-6), 44.7 (C-7), 61.0 (O*CH*$_2$Me), 68.4 (C-1), 172.8 (C-8), 211.4 (C-4). EIMS, m/z 200 (M^+), 183, 182, 155, 154, 137, 136.

$LiAlH_4$ reduction of 9

A solution of **9** (726 mg;) in ether (30 ml) was added dropwise to a cooled solution of $LiAlH_4$ in Et_2O (1M; 16 ml) during 20 min and the mixture stirred at r.t. for 30 min. The suspension was then heated at reflux for 2 h. The excess $LiAlH_4$ was decomposed by adding in sequence AcOEt and 25% aqueous NH_4OH (1 ml) under ice-cooling. The precipitate was filtered off and washed with $CHCl_3$/ MeOH 8:2. Removal of solvents gave rengyol (**4**) contaminated by isorengyol (**6**) (16:1; by ^{13}C-NMR) as a white solid (439 mg; 76%) which was not further purified.

*Rengyol (**4**).* ^{1}H-NMR (MeOD): δ 1.3-1.7 (8H, m), 1.67 (2H, t, J=7.3 Hz), 3.53 (1H, m,), 3.72 (2H, t, J=7.5 Hz).^{13}C-NMR (C_5D_5N): δ 31.9 (C-2, C-6), 36.3 (C-3, C-5), 45.3 (C-7), 59.0 (C-8), 69.9 (C-4), 70.1 (C-1). Signals due to isorengyol (**6**) were found at δ 31.3, 34.6, 43.3, 67.5, 71.3. EIMS, m/z 161 $(M+H)^+$, 143, 142, 115, 97.

Synthesis of Rengyoxide (5)

*$LiAlH_4$ reduction of **8***

A solution of **8** (1.3 g; 5.42 mmol) in ether (40 ml) was added dropwise to a cooled solution of $LiAlH_4$ in Et_2O (1M; 16 ml) during 20 min and the mixture stirred at r.t. for 30 min. The suspension was then heated at reflux for 2 h. The excess $LiAlH_4$ was decomposed by adding in sequence AcOEt and 25% aqueous NH_4OH (1 ml) under ice-cooling. The precipitate was filtered off and washed with $CHCl_3$/MeOH 8:2. Removal of solvent gave the alcohol **10** (870 mg; 80%). ^{1}H-NMR (MeOD): δ 1.77-1.91 (10H, m), 3.93 (2H, t, J=7.5 Hz), 4.11 (4H, bs). ^{13}C-NMR (MeOD): δ 31.4 (C-3, C-5), 35.7 (C-2, C-6), 44.9 (C-7), 59.1 (C-8), 65.2 (OCH_2CH_2O), 71.0 (C-1), 109.9 (C-4). EIMS, m/z 202 (M^+), 185, 167, 139.

*Deprotection of **10***

To a suspension of **10** (870 mg; 4.34 mmol) in 40 ml of water, 3.2 g of Dowex 50X8-100 (H^+ form) were added. The mixture was stirred at room temperature for 24 h. The resin was filtered off and washed with AcOEt. The organic layer was separated and dried over Na_2SO_4 and the solvent was evaporated under vacuo to afford rengioxide **5** as an yellow oil (100%). ^{1}H-NMR (MeOD): δ 1.84 (2H, t, J=4.3 Hz), 1.85 (2H, dt, J=8.3 and 3.3 Hz), 2.03 (2H, m), 2.22 (2H, m), 2.71 (2H, dt, J=8.5 and 4.0 Hz), 3.81 (2H, t, J=4.3 Hz). ^{13}C-NMR (MeOD): δ 32.0 (C-2, C-6), 35.0 (C-3, C-5), 44.5 (C-7), 59.1 (C-8), 70.6 (C-1), 214.9 (C-4). Signals due to monoketal **11** were found at δ 31.7, 34.7, 34.9, 37.7, 37.8, 44.9. EIMS, m/z 158 (M^+), 140, 113, 95.

Synthesis of Isorengyol (6)

*Protection of **5** with Benzaldehyde*

To 100 mg of **5**, 64 µl of C_6H_5CHO and 1 mg of *p*-TsOH were added. The mixture was stirred at room temperature for 2 h and then chromatographed on a silica gel column. Elution with AcOEt/Petroleum Ether 1:2 gave **12** (71 mg; 47%). ^{1}H-NMR ($CDCl_3$): δ 1.36-2.86 (overlapping protons), 4.15 (2H, m), 5.77 (1H, s), 7.35-7.54 (5H, m, Ph). ^{13}C-NMR ($CDCl_3$): δ 28.3, 33.9, 35.6, 36.1, 39.2, 62.9 (C-8), 71.1 (C-1), 94.5 (*O*C*H*O), 125.7, 127.9, 128.1, 128.5, 130.0, 211.5 (C-4). EIMS, m/z 246 (M^+), 77.

*Reduction of **12** with LS-Selectride®*

In an oven-dried flask, at room temperature and under a dry stream of nitrogen, a lithium trisiamylborohydride (LS-Selectride®) solution in THF (1M; 0.6 ml) was introduced and cooled to -78°C (dry ice-acetone). 103 mg (0.419 mmol) of **12**, dissolved in 3 ml of THF (maintained at 0°), were added. The resulting mixture was stirred vigorously at -78° for 2 h and then allowed to equilibrate to room temperature (1h). The reaction mixture was hydrolyzed with 40 µl of water and 0.15 ml of EtOH added; the remaining organoborane was oxidized with 0.1 ml of 6N NaOH and 0.15 ml of 30% hydrogen peroxide. The reaction

mixture was diluted with water saturated with K_2CO_3 and extracted with Et_2O. The organic extract was dried over Na_2SO_4. Removal of solvent gave 100 mg (0.40 mmol) of an yellow oil containing the mixture of **13** and **14**.

A crude mixture of **13** and **14**. (204 mg; 0.823 mmol; coming from different runs), acetic anhydride (1 ml) and pyridine (1.5 ml) were added and the reaction was stirred at room temperature for 2h. Removal of solvents under a dry stream of nitrogen, gave an oil that after purification on silica gel colum (elution with AcOEt/Petroleum Ether 1: 9) gave 84 mg (35%) of **15** and 82 mg (34%) of **16**.

15. ^{1}H NMR ($CDCl_3$): δ 1.3-2.0 (8H, m), 2.04 (3H, s), 2.62 (2H, m) 4.10 (2H, m), 4.77 (1H, m), 5.71 (1H, s), 7.32-7.53 (5H, m). ^{13}C NMR ($CDCl_3$): δ 21.4 (Me), 26.0, 26.1, 26.8, 34.9, 37.9, 63.1 (C-8), 71.3 (C-1), 72.5 (C-4), 77.0, 94.1 (*OCHO*), 126.0, 128.2, 128.6, 139.1, 171.0.

16. ^{1}H NMR ($CDCl_3$): δ 1.47-1.90 (8H, m), 2.02 (3H, s), 2.30 (2H, m) 4.12 (2H, m), 5.01 (1H, m), 5.71 (1H, s), 7.31-7.51(5H, m). ^{13}C NMR ($CDCl_3$): δ 21.4 (Me), 24.2, 25.2, 35.1, 35.3, 62.9 (C-8), 69.9 (C-4), 72.2 (C-1), 77.0, 94.3(*OCHO*), 125.9, 128.1, 128.6, 139.0, 170.6. EIMS, m/z 290 (M^+), 230, 77.

*Methanolysis of **15** and subsequent hydrogenolysis*

In a flask containing 235 mg of **15** (coming from different runs), K_2CO_3 (24 mg) and MeOH (1 ml) were added. The reaction was stirred at room temperature for 3h, and then filtered to remove K_2CO_3 and taken to dryness. The residue was placed under hydrogen (lattice) and 2ml of EtOH and 25 mg of Pd/C were added. The mixture was stirred at room temperature for 3h. The catalyst was filtered off and the removal of solvent under reduced pressure afforded 89.6 mg (70%) of rengyol **4**.

*Methanolysis of **16** and subsequent hydrogenolysis*

Treatment of **16** (200 mg; coming from different runs) as above afforded 88.3 mg (80%) of isorengyol (**6**). ^{1}H-NMR (MeOD): δ 1.5 (4H, m), 1.71-192 (6H, m), 3.78 (2H, t, J=7.5 Hz), 3.82 (1H, m). ^{13}C NMR, identical to that previously reported[6]. EIMS, m/z 161 (MH^+); 143, 142, 115, 97.

Synthesis of Rengyoside A (1).

a) By glucosidation of rengyol using Sulfolobus solfataricus crude homogenate

440 mg of rengyol (**4**; 2.75 mmol) and 41.2 mg (0.137 mmol) of *p*-nitrophenyl-β-D-glucoside were dissolved in 5 ml of crude homogenate of *Sulfolobus solfataricus*. The reaction was stirred at 75° and monitored by TLC. After total donor consumption, the reaction mixture was purified on silica gel column. The elution with $CHCl_3$/MeOH/H_2O 65:25:4 gave 11 mg (0.034 mmol; 25% in respect to the donor) of the glucosides **1** and **17** (2:1; by ^{13}C-NMR). To the mixture of **1** and **17**, 200 μl of Ac_2O and 250 μl of pyridine were added and the reaction was stirred at room temperature for 2h. Removal of solvents under a dry stream of nitrogen, gave an oil that after purification on silica gel colum (elution with $CHCl_3$/MeOH 98:2) gave 4.2 mg of **18** and 3.3 mg of **19**.

b) By glucosidation of rengyol using almond β-glucosidase

To 300 mg of **4** (1.88 mmol) and 30 mg (0.1 mmol) of *p*-nitrophenyl-β-D-glucoside in 5 ml of phosphate buffer (pH=7), 1 mg of almond β-glucosidase was added. The reaction was stirred at room temperature and monitored by TLC. After total donor consumption, two further aliquots of 30 mg of donor were added. Workup as above and acetylation afforded 12 mg of a mixture of **18** and **19** (1.4:1) in a total yield of 13%.

18. $[\alpha]_D$ = -13.4 (c=0.42, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) : δ 1.80 (m, H-7), 2.00, 2.02, 2.03, 2.05, 2.09 (acetyl methyls), 3.69 (m, H-8), 4.11 (m, H-5'), 4.16 (dd, J=12.5 and 2.2 Hz, H-6'a), 4.23 (dd, J=12.5 and 4.5 Hz, H-6'b), 4.50 (d, J=7.8 Hz, H-1'), 4.67 (m, H-4), 4.97 (t, J=9.4 Hz, H-2'), 5.08 (t, J=9.6 Hz, H-4'), 5.21 (t, J=9.5 Hz, H-3'). ^{13}C NMR ($CDCl_3$): δ 20.6, 20.7, 21.4 (acetyl methyls), 26.7 (C-3 and C-5), 35.0 and 35.1 (C-2 and C-6), 40.5 (C-7), 61.8 (C-6'), 66.5 (C-8), 68.4 (C-4'), 69.3 (C-1), 71.2 (C-2'), 71.9 (C-5'), 72.4 (C-4), 72.6 (C-3'), 100.6 (C-1'), 169.4, 170.2, 170.6 (acetyl carbonyls). FABMS, m/z 533 (MH^+), 515, 473, 455.

19. $[\alpha]_D$= -12.5 (c=0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$) : δ 1.80 (t, J=6.6 Hz, H-7), 2.00, 2.02, 2.03, 2.04, 2.08 (acetyl methyls), 3.61 (m, H-4), 3.69 (m, H-5'), 4.13 (dd, J=12.2 and 2.3 Hz, H-6'a), 4.25 (m, H-8 and H-6'b), 4.60 (d, J=8 Hz, H-1'), 4.96 (t, J=9.5 Hz, H-2'), 5.08 (t, J=9.7 Hz, H-4'), 5.20 (t, J=9.5 Hz, H-3'). ^{13}C NMR ($CDCl_3$): δ 20.6, 21.06 (acetyl methyls), 27.0 (C-5), 28.4 (C-3), 34.8 and 35.1 (C-2 and C-6), 40.1 (C-7), 60.8 (C-8), 62.1 (C-6'), 68.6 (C-4'), 69.6 (C-1), 71.5 (C-2'), 71.7 (C-5'), 72.9 (C-3'), 77.0 (C-4), 99.2 (C-1'), 169.2, 169.4, 170.3 (acetyl carbonyls). FABMS, m/z 533 (MH^+), 515, 473.

To 24 mg of **18**, coming from different runs, dissolved in MeOH (2ml), K_2CO_3 (20 mg) was added and the mixture was stirred at room temperature for 24h. The mixture was filtered off on a short silica gel column obtaining 15 mg (100%) of rengyoside A (**1**). $[\alpha]_D$ = -19.4 (c=1.0, MeOH); lit.[5] $[\alpha]_D$ = -11.0 (c=0.18, MeOH); lit. [6] $[\alpha]_D$ = -21 (c=0.18, MeOH).^{1}H NMR (C_5D_5N; 400 MHz): δ 1.55 (m, H-7), 4.99 (d, J= 7.7, H-1'). ^{13}C NMR (C_5D_5N): δ 32.0 (C-3 and C-5), 36.3 and 36.5 (C-2 and C-6), 43.1 (C-7), 62.9 (C-6'), 66.8 (C-8), 69.2 (C-4), 70.0 (C-1), 71.8 (C-4'), 75.3 (C-2'), 78.7 and 78.8 (C-3' and C-5'), 105.0 (C-1'). FABMS, m/z 323 (MH^+).

Synthesis of Isorengyoside (3).

a) By glucosidation of isorengyol using Sulfolobus solfataricus crude homogenate

80 mg of isorengyol (**6**; 0.5 mmoles) and 26.5 mg of phenyl-β-D-glucoside (0.1 mmoles) were dissolved in 0.5 ml of crude homogenate of *Sulfolobus solfataricus*. At total donor consumption (4.5 h) purification of the mixture of glucosides and acetylation afforded isorengyoside acetate (**22**) and its regioisomer **23** functionalized at the secondary hydroxy group (9:1; 23.3 mg; 44%). The mixture of **22** and **23** was separated by silica gel column (*n*-hexane:EtOAc, 7:3 to 1:1) affording 16.7 mg of pure **22** and 6.6 mg of a 2:1 mixture of **22** and **23**.

b) By reduction of rengyoside B

In an oven-dried flask, under a dry stream of nitrogen, lithium trisiamylborohydride solution in THF (1.0M; 0.63 ml) was introduced and cooled to -78°C (dry ice-acetone). 110 mg (0.225 mmol) of **24** dissolved in 2 ml of THF (maintained at 0°) were then added. The resulting mixture was stirred vigorously for 2 h at -78° and then allowed to equilibrate to room temperature (1h). The reaction mixture was hydrolyzed with 40 µl of water and 0.15 ml of EtOH added; the organoborane was oxidized with 0.1 ml of 6N NaOH and 0.15 ml of 30% hydrogen peroxide. Removal of solvents under reduced pressure gave a residue which was directly treated with acetic anhidryde (0.5 ml) and pyridine (1 ml). The reaction was stirred at room temperature for 12h. The removal of volatile solvents under a dry stream of nitrogen, gave an oil that, after purification on silica gel colum ($CHCl_3$/MeOH 98:2) gave 17 mg (14%) of **18** and 34 mg (28%) of **22** .

22. $[\alpha]_D$ = -15.3 (c=1.7, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) : δ 1.80 (m, H-7), 2.00, 2.02, 2.03, 2.04, 2.08, (acetyl methyls), 3.70 (m, H-8), 4.12 (m, H-5'), 4.15 (dd, J=12.3 and 2.4 Hz, H-6'a), 4.23 (dd, J=12.4 and 4.6 Hz, H-6'b), 4.50 (d, J=7.8 Hz, H-1'), 4.94 (m, H-4), 4.97 (t, J=9.7 Hz, H-2'), 5.07 (t, J=9.6 Hz, H-4'), 5.20 (t, J=9.5 Hz, H-3'). ^{13}C NMR ($CDCl_3$): δ 20.6, 20.7, 21.4 (acetyl methyls), 26.0 (C-3 and C-5), 32.6 (C-2 and C-6), 40.0 (C-7), 61.8 (C-6'), 66.2 (C-8), 68.4 (C-4'), 70.0 (C-1), 70.1 (C-4), 71.2 (C-2'), 71.9 (C-5'), 72.6 (C-3'), 100.6 (C-1'), 169.3, 169.4, 170.2, 170.6 (acetyl carbonyls).

To 29 mg of **22** dissolved in MeOH (2ml), K_2CO_3 (20 mg) was added and the mixture was stirred at room temperature for 24h. The mixture was filtered off on a short silica gel column ($CHCl_3$/MeOH 8:2) obtaining 18 mg (100%) of isorengyoside (**3**). $[\alpha]_D$ = -19.7 (c=1.2, MeOH); lit.[6] $[\alpha]_D$ = -21.0 (c=0.4, MeOH). ^{1}H NMR (C_5D_5N): δ 1.8-2.3 (10H, m), 4.0-4.7 (7H, m), 5.05 (d, J= 8.2 Hz; H-1'). ^{13}C NMR (C_5D_5N): δ 31.2 (C-3 and C-5), 34.5 and 34.6 (C-2 and C-6), 41.3 (C-7), 62.9 (C-6'), 66.8 (C-8), 67.3 (C-4), 70.4 (C-1), 71.8 (C-4'), 75.3 (C-2'), 78.6 and 78.8 (C-5' and C-3'), 105.0 (C-1').

Synthesis of Rengyoside B (2)

a) By glucosidation of rengyoxide using Sulfolobus solfataricus crude homogenate

109 mg of **5** (0.69 mmol) and 47 mg (0.18 mmol) of phenyl-β-D-glucoside were dissolved in 5 ml of crude homogenate of *Sulfolobus solfataricus*. The reaction was stirred at 75 °C and monitored by TLC. After total donor consumption, the glucosi de was purified on silica gel column. The elution with $CHCl_3$/MeOH 8:2 gave 20 mg (62.5 μmol; 35%) of **2.**

Alternatively, 127.9 mg of **5** (0.80 mmoles) and 205 mg of phenyl-β-D-glucoside (0.80 mmoles) were dissolved in 1 ml of crude homogenate of *Sulfolobus solfataricus*. After 16h 1ml of crude homogenate was added to secure the complete conversion of the donor and, after additional 24 h of stirring, the usual work-up afforded 59.6 mg (186 μmoles; 23%) of rengyoside (**2**) were obtained. $[\alpha]_D$ = -20.0 (c=0.2; MeOH); lit.[5] $[\alpha]_D$=-10.4 (c=0.28, EtOH). lit.[6] $[\alpha]_D$ =-17.6 (c=0.80, EtOH). ^{1}H NMR (C_5D_5N): δ 1.87 (2H, m, H-2b, H-6b), 2.17 (4H, m, H-7, H-2a, H-6a), 2.34 (2H, d, J=14 Hz, H-3b, H-5b), 2.98 (2H, m, H-3a, H-5a), 4.03 (1H, m, H-5'), 4.09 (1H, t, J=8 Hz, H-2'), 4.15 (1H, m, H-8a), 4.30 (2H, m, H-3', H-4'), 4.45 (1H, dd, J=12.0 and 5.0 Hz, H-6'b), 4.56 (1H, m, H-8b), 4.61 (1H, dd, J=12.0 and 2.5 Hz, H-6'a), 4.96 (1H, d, J=8 Hz, H-1'). ^{13}C NMR (C_5D_5N): δ 37.7, 37.8, 37.9 (C-2, C-3, C-5, C-6), 42.1 (C-7), 62.9 (C-6'), 66.6 (C-8), 69.1 (C-1), 71.9 (C-4'), 75.3 (C-2'), 78.7, 78.8 (C-3' and C-5'), 105.0 (C-1'), 211.5 (C-4). FABMS, m/z 325 (MNa^+-H_2O).

*b) Through enzymatic synthesis of **21***

346 mg of **10** (1.71 mmol) and 450 mg (1.75 mmol) of phenyl-β-D-glucoside were dissolved in 5 ml of crude homogenate of *Sulfolobus solfataricus*. The reaction was stirred at 75°C and monitored by TLC. After total donor consumption, the glucoside was purified on silica gel column. The elution with $CHCl_3$/MeOH 9:1 gave 114 mg (0.3 mmol; 18%) of **21.** $[\alpha]_D$ = -15.0 (c=5.4, MeOH). ^{1}H NMR (C_5D_5N): δ 1.78-2.34 (overlapping protons), 3.97 (5H, m, H-5' and OCH_2CH_2O), 4.08 (t, J= 8 Hz, H-2'), 4.17 (1H, m, H-8a), 4.30 (2H, m, H-3', H-4'), 4.43 (1H, dd, J=12.0 and 5.2 Hz, H-6'b), 4.57 (2H, m, H-8b and H-6'a), 4.94

(1H, d, J=7.7 Hz, H-1'). ^{13}C NMR (C_5D_5N): δ 30.2,31.4 (C-3 and C-5), 35.9 and 36.0 (C-2 and C-6), 42.6 (C-7), 62.9 (C-6'), 64.5 and 64.6 (OCH_2CH_2O), 66.8 (C-8), 69.4 (C-1), 71.9 (C-4'), 75.4 (C-2'), 78.6, 78.8 (C-3' and C-5'), 105.0 (C-1'), 109.5 (C-4). FABMS, m/z 387 (MNa^+), 365 (MH^+), 347.

113 mg of **21** were acetylated with 450 µl of Ac_2O and 500 µl of pyridine and the reaction was stirred at r.t. for 3h. Removal of solvents under a dry stream of nitrogen, gave an oil (163 mg) wich was treated with Dowex (237 mg) as above to afford 110 mg of pure rengyoside B acetate (**24**). ^1H NMR ($CDCl_3$): δ 1.86 (t, J=7.0 Hz, H-7), 2.00, 2.01, 2.04, 2.08 (acetyl methyls), 4.51 (d, J=8 Hz, H-1'), 4.99 (t, J=9.4 Hz, H-2'), 5.07 (t, J=9.7 Hz, H-4'), 5.21 (t, J=9.4 Hz, H-3'). ^{13}C NMR ($CDCl_3$): δ 20.6, 36.8, 37.2, 39.9, 61.7, 66.4, 68.3, 69.1, 71.1, 71.9, 72.4, 100.5, 169.4, 169.5, 170.2, 170.6, 212.1. FABMS, m/z 489 (MH^+), 471.

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