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NEW ENZYME CATALYZED SYNTHESIS OF MONOACYL GALACTOGLYCERIDES

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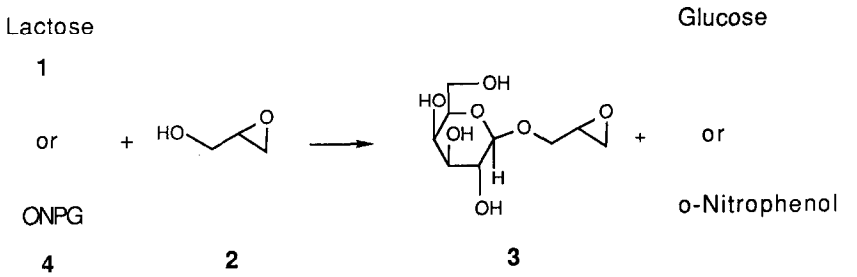
ABSTRACT: 1-Acyl-3-O-β-D-galactopyranosylglycerides have been prepared by β-galactosidase catalysed trans-galactosidation of lactose (1) or o-nitrophenyl galactopyranoside (4) with 2,3-epoxypropanol (2) and subsequent opening of the so formed 1-O-β-D-galactopyranosyl-2,3-epoxypropanol (3) with a fatty acid.

Usually, selective modification and derivatisation of sugars require extensive application of protection and deprotection procedures [1]. Accordingly, large scale preparation of many interesting and potentially useful sugar derivatives as well as detailed studies of such compounds may be quite troublesome. Moreover the production of many sugar derivatives is, to-day, not economically feasible. To overcome such problems several attempts have been made to apply enzymes for regioselective transformation of sugars. The recently described regioselective deacylation of protected sugars as well as enzymatic synthesis of monoacyl sugars [2, 3] are examples of how enzymes can be conveniently applied in the syntheses of sugar derivatives.

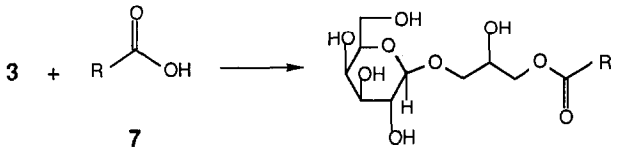
As a part of our interest in the application of enzymes in organic synthesis and in the development of new sugar derivatives for industrial uses we have established a short, convenient enzymatic synthesis of monoacyl galactoglycerides. This naturally occurring type of compound [4] exhibits interesting surfactant properties and may thus find important practical uses.

1-O-β-D-galactopyransyl-2,3-epoxypropanol (3) was found to be a useful intermediate for the syntheses of monoacyl galactoglycerides. It could easily be synthesized by β-galactosidase catalyzed trans-galactosidation using lactose (1) or ortho-nitrophenyl-β-D-galactopyranoside (ONPG) (4) and 2,3-epoxypropanol (2) as substrates [5], cf. Scheme I.



Scheme I

Compound 3 could then be transformed into desired monoacyl galactoglycerides 8a, 8b, and 8c in 61-68% yields simply by heating together the fatty acids 7a, 7b, and 7c respectively and the epoxide 3 in the presence of catalytic amounts of tetraethylammonium bromide (Scheme II)[6].



7 a $R = C_{13}H_{27}$-
b $R = C_{15}H_{31}$-
c $R = C_{17}H_{35}$-

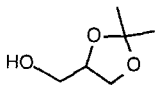
8 a $R = C_{13}H_{27}$-
b $R = C_{15}H_{31}$-
c $R = C_{17}H_{35}$-

Scheme II

Under these conditions only 1-O-acyl-3-O-β-D-galactopyranosyl glycerol and not the corresponding 2-O-acyl derivative was formed. Compared to the previously described synthesis of monoacyl-galactoglycerides [7] this reaction sequence is quite convenient and makes possible synthesis of larger quantities of these sugar derivatives.

The ^1H NMR spectrum of epoxide 3 synthesised indicates that, as expected, only the β-form of the galactoside is generated in the enzymatic synthesis. Furthermore, a slight enantiomeric preference for the formation of 1-O-β-D-galactopyransyl-(2R,3)-epoxypropanol (R/S = 7/3) was detected when ONPG was converted enzymatically into the epoxide 3 in the presence of excess, racemic 2,3-epoxypropanol. No such preference could be detected when lactose was used as substrate in the reaction under similar conditions. Quite similar results were achieved when 2,3-isopro-

pylidene glycerol (5) was used as substrate in the trans-galactosidation reaction.



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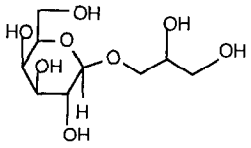
This latter result is in contrast to that previously reported on enzymatic synthesis of isopropylidene-galactosyl-glycerol which was found to provide diastereomerically pure 1-O-β-D-galactopyranosyl-(2R)-glycerol (6) after deprotection [8].



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We believe, however, that this earlier observed preferred generation of diastereomerically pure galactoside 6 is due to diastereomeric enrichment during crystallisation procedures employed in the synthesis rather than to an actual enantioselectivity of the applied enzyme. As described in the experimental section we avoided a possible diastereomeric enrichment during work up by purifying, in high yield, simply by use of a short pad chromatography on silicium oxide followed by acetylation of the isolated material. The products were then analysed by ^{1}H NMR. For this, reference samples were prepared in a similar way using enantiomerically pure 2,3-isopropylidene glycerol of the R and S configuration as well as by using the two enantiomerically pure forms of 2,3-epoxypropanol[9]. In all cases diastereomerically pure products were obtained from these enantiomerically pure building blocks.

The second step in the reaction sequence was found to take place with retention of configuration at C-2 of the aglycon. This was deduced from the ^{1}H NMR spectrum of the hexaacetate of the galactosyl-glycerol obtained by base catalysed removal of the fatty acid residue followed by acetylation.

Further work on the stereoselectivity of the enzymatic reaction is in progress. Also the surfactant properties of the products are under investigation.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM 400 spectrometer with TMS as internal standard. Analytical high pressure liquid chromatography (HPLC) was performed on a Shimadzu LC-4A instrument using a SiO_2-NH_2 column and EtOH (96%) or CH_3CN:H_2O as eluent. Mass spectra were recorded on a Varian 311 A instrument. Thin layer chromatography (TLC) was performed on silica gel plates with 2-propanol:acetone:0.1 M acetic acid (6:3:1) or toluene:ethyl acetate:methanol (8:6:3) as eluents. Liquid chromatography was performed using Merck silica gel (0.040-0.063 mm) and

gradient elution with petroleum ether, containing increasing amounts of ethyl acetate and methanol. β-Galactosidase, derived from E. coli, was purchased from Boehringer Mannheim Co. either as suspension or as freeze dried material.

1-O-β-D-galactopyranosyl-2,3-epoxypropanol (3): a) Using o-nitrophenyl-galactopyranoside as substrate β-galactosidase 830 µl, (about 50 U, suspended in $(NH_4)_2SO_4$) was added to a mixture of 5 g (16.6 mmol) of o-nitrophenyl-galactopyranoside and 17.5 ml (0.26 mol) of 2,3-epoxy-1-propanol in 400 ml of buffer (0.07 M phosphate buffer, 10 mM $MgCl_2$, pH 7). The reaction was followed by TLC and HPLC. After 4 hours the mixture was extracted with ether (4 x 100 ml) to remove nitrophenol and the water phase was concentrated at reduced pressure. The product thus obtained was taken up in ethanol and filtered to remove inorganic salts. Subsequent evaporation yielded 6.4 g crude product. This product was subject to chromatography on SiO_2 and further crystallized from absolute ethanol yielding 1.1 g (28%) of product. mp: 126-127°C. ^{1}H NMR (DMSO-d^6) δ; 2.6 (m, 1H), 2.74 (m, 1H), 3.14 (m, 1H), 3.2 - 3.4 (m, 3H), 3.45 - 3.55 (m, 2H), 3.56 - 3.65 (m, 2H), 3.73 (dd, 1H), 4.13 (d, 1H), 4.38 (d, 1H), 4.56 (m, 1H), 4.72 (d, 1H), 4.94 (d, 1H). ^{13}C NMR (DMSO-d^6) δ; 43.8, 50.1, 60.5, 68.2, 69.3, 70.5, 73.4, 75.2, 103.4.
b) Using lactose as substrate 12 mg freeze dried β-galactosidase (about 2400 U), was added to a mixture of 5 g (13.9 mmol) of lactose and 20 ml (0.3 mol) of 2,3-epoxy-1-propanol in 300 ml of buffer (0.07 M phosphate buffer, 10 mM $MgCl_2$, pH = 7). The reaction mixture was stirred for 15 hours at room temperature and subsequently heated to 80°C for 10 minutes. The water phase was evaporated at reduced pressure (pump, 30°C). The product was taken up in ethanol and filtered to remove inorganic salts. Evaporation yielded the crude product as a yellow syrup. Further purification was made as in example a) and the data were as given above.

1-O-Tetradecanoyl-3-O-β-D-galactopyranosyl-glycerol (8a): In a typical procedure 1-O-β-D-galactopyransyl-2,3-epoxypropanol (1 g, 4.2 mmol) was added to myristic acid (1.06 g, 1.1 equivalent) at 80°C. Then, tetraethylammonium bromide (40 mg, 0.05 ml equivalent) was added. The semi solid mixture was stirred for 3 hours. The product was taken up in acetone/methanol (50:50) and evaporated on SiO_2 followed by a chromatography yielding 1.2 g (61%) of product which was crystallised from acetone. ^{1}H NMR(DMSO-d^6) δ; 0.88 (t, 3H), 1.25 (bs, 20H), 1.54 (m, 2H), 2.32 (t, 2H), 3.25-3.6 (mm, 6H), 3.65 (bs, 1H), 3.72 (dd, 1H), 3.83 (m, 1H), 4.0 (m, 1H), 4.08 (m, 2H), 4.4 (d, 1H), 4.6 (t, 1H), 4.75 (d, 1H), 4.9 (d, 1H), 5.0 (d, 1H). ^{13}C NMR(DMSO-d^6) δ;13.8, 22.0, 24.4, 28.5, 28.6, 28.7, 28.8, 28.9-29.0 (4C), 31.2, 33.5, 60.4, 65.4, 67.5, 68.1, 70.4, 70.6, 73.4, 75.3, 104.0, 172.8. MS. Fast atom bombardment FAB (substance dissolved in glycerol) MS: m/z 929 (M_2H^+), 465 (MH^+), 303, 211 ($C_{13}H_{27}$-CO^+); calculated for MH^+ ($C_{23}H_{45}O_9$) 465.3064, measured 465.3066.

1-O-Hexadecanoyl-3-O-β-D-galactopyranosyl-glycerol (8b): Yield 64%. ^{1}H NMR(DMSO-d^6) δ; 0.86 (t, 3H), 1.25 (bs, 24H), 1.5 (m, 2H), 2.3 (t, 2H), 3.25-3.55 (mm, 6H), 3.62 (bs, 1H), 3.7 (m, 1H), 3.8 (m, 1H), 3.96 (m, 1H), 4.05 (m, 2H), 4.4 (d, 1H), 4.55 (t, 1H), 4.7 (d, 1H), 4.85 bs, 1H), 4.98 (d, 1H). ^{13}C NMR(DMSO-d^6) δ; 13.8, 22.0, 24.4, 28.5, 28.7 (2C), 28.9 (2C), 29.0 (5C), 31.2, 33.5, 60.4, 65.4, 67.5, 68.1, 70.4, 70.6, 73.4, 75.3, 104.0, 172.8. MS: m/z 985 (M_2H^+), 493 (MH^+), 331, 239 ($C_{15}H_{31}$-CO^+); calculated for MH^+ ($C_{25}H_{49}O_9$) 493.3377, measured 493.3371.

1-O-Octadecanoyl-3-O-β-D-galactopyranosyl-glycerol (8c): Yield 68%. ^{1}H NMR(DMSO-d^6) δ; 0.85 (t, 3H), 1.24 (bs, 28H), 1.5 (m, 2H), 2.29 (t, 2H), 3.25-3.55 (mm, 6H), 3.62 (bs, 1H), 3.68 (m, 1H), 3.8 (m, 1H), 3.95 (m, 1H), 4.05 (m, 2H), 4.35 (d, 1H), 4.56 (t, 1H), 4.71 (d, 1H), 4.86 (d, 1H), 4.96 (d, 1H). ^{13}C NMR(DMSO-d^6) δ; 13.8, 22.0, 24.4, 28.5, 28.6, 28.7, 28.9, 29.0, (8c), 31.3, 33.5, 60.4, 65.4, 67.5, 68.2, 70.5, 70.7, 73.4, 75.3, 104.0, 172.8. MS: m/z 1041 (M_2H^+), 521 (MH^+), 359, 267 ($C_{17}H_{35}$-CO^+); calculated for MH^+ ($C_{27}H_{53}O_9$) 521.3690, measured 521.3681.

Stereochemistry in the transgalactosidation reaction

In a typical procedure freeze dried β-galactosidase (0.5 mg) was added to ONPG (150 mg) and 2,3-epoxy-propanol (450 µl) in 4.5 ml buffer (0.07 M phosphate buffer, 10 mM $MgCl_2$, pH = 7). The reaction was followed by TLC. After approx. 1 h, the mixture was extracted with Et_2O and the the water phase concentrated in vacuo. The resulting residue was taken up in MeOH, concentrated on SiO_2, and chromatographed on a short SiO_2 column. All fractions containing the product were collected, concentrated and, subsequently, acetylated in acetic-anhydride/pyridine (5°C, 12 hours). After work up the crude acetate was subjected to NMR-analysis. The enantioselectivity could best be determined by comparison of the diastereotopic protons in the acetylated compounds.
The peaks corresponding to the Z-protons at carbon 3 in the epoxy-propanol aglycon appears at δ=2.56 in (S)-3-acetate and at δ=2.66 in (R)-3-acetate, respectively.
When lactose and racemic 2 were used as substrates the ^{1}H NMR analysis of the resulting 2',3',4',6'-tetra-O-acetyl-1'-O-β-D-galactopyranosyl-2,3-epoxypropanol, indicated the formation of a 1:1 diastereomeric mixture of 3.

When ONPG and racemic 2 were used as substrates the ^{1}H NMR analysis of the 2',3',4-',6'-tetra-O-acetyl-1'-O-β-D-galactopyranosyl-2,3-epoxypropanol, indicated formation of a 50% d.e. of (R)-3.

When lactose was used as a substrate β-galactosidase (1.5 mg) was added to lactose (250 mg) and 750 µl 2,3-epoxy-propanol in 7.5 ml buffer. The reaction was run overnight at room temperature. Work up was performed as described above. 1-O-β-D-galactopyranosyl-2,3-epoxypropanol did not separate totally from glucose in the chromatography but the glucose penta acetate thus formed in the acetylation step did not interfere with the NMR-analysis. In the NMR of a diastereomeric mixture of (S)-9-acetate and (R)-9-acetate the two dubletts at approx. 2=4.55 (d,1H,J=8Hz), corresponding to the protons at C-1 in the sugar units, separate with 12 Hz and the ratio of (R) and (S) could thereby be determined.
When lactose and racemic 5 was used as substrates the ^{1}H NMR analysis of the 2',3',4',6'-tetra-O-acetyl-1'-O-β-D-galactopyranosyl-2,3-isopropylidene-glycerol indicated a formation of a 1:1 diastereomeric mixture of 9.

When ONPG and racemic 5 were used as substrates the ^{1}H NMR analysis of the 2',3',4-',6'-tetra-O-acetyl-1'-O-β-D-galactopyranosyl-2,3-isopropylidene-glycerol indicated the formation of a 20% d.e. of (S)-9.

2',3',4',6'-tetra-O-Acetyl-1'-O-β-D-galactopyranosyl-(2R,3)-epoxypropanol: ^{1}H NMR ($CDCl_3$) δ; 5.40 (d, 1H), 5.21 (dd, 1H), 5.02 (dd, 1H), 4.51 (d, 1H), 4.26-4.10 (mm), 3.92 (dd, 1H), 3.88 (dd, 1H), 3.18 (m, 1H), 2.78 (dd, 1H), 2.66 (dd, 1H, J_{vic} = 2.8 Hz, J_{gem} = 5.2 Hz), 2.16 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H).

2',3',4',6'-tetra-O-Acetyl-1'-O-β-D-galactopyranosyl-(2S,3)-epoxypropanol: ^{1}H NMR ($CDCl_3$) δ; 5.40 (d, 1H), 5.24 (dd, 1H), 5.04 (dd, 1H), 4.61 (d, 1H), 4.30-4.08 (mm, 3H), 3.92 (dd, 1H), 3.50 (dd, 1H), 3.18 (m, 1H), 2.8 (dd, 1H), 2.56 (dd, 1H, J_{vic} = 2.6 Hz, J_{gem} = 4.8 HZ), 2.16 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H).

2',3',4',6'-tetra-O-Acetyl-1'-O-β-D-galactopyranosyl-(2R,3)-isopropylidene-glycerol: ^{1}H NMR ($CDCl_3$) δ; 5.39 (d, 1H), 5.20 (dd, 1H), 5.02 (dd, 1H), 4.55 (d, 1H, J = 8 Hz), 4.23 (m, 1H), 4.15 (m, 2H), 4.04 (dd, 1H), 3.92 (dd, 1H), 3.82 (dd, 1H), 3.73 (dd, 1H), 3.65 (dd, 1H), 2.20 (s, 3 H), 2.11 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 1.44 (s, 3H), 1.38 (s, 3H).

2',3',4',6'-tetra-O-Acetyl-1'-O-β-D-galactopyranosyl-(2S,3)-isopropylidene-glycerol: ^{1}H NMR ($CDCl_3$) δ; 5.39 (d, 1H), 5.21 (dd, 1H), 5.01 (dd, 1H), 4.59 (d, 1H, J = 8 Hz), 4.26 (m, 1H), 4.15 (m, 2H), 4.02 (dd, 1H), 3.92 (dd, 1H), 3.89 (dd, 1H), 3.80 (dd, 1H), 3.64 (dd, 1H), 2.18 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.44 (s, 3H), 1.36 (s, 3H).

Stereochemistry in the epoxy ring opening

Hexaacetyl galactosyl glycerol: In a typical procedure 1-O-hexadecanoyl-3-O-β-D-galactopyranosyl glycerol (8b) (100 mg) (prepared from crystalline 1-O-β-D-galactopyranosyl-2,3-epoxypropanol (3) of 2R/2S-ratio = 4/1) in 5 ml MeOH (dry) and 0.2 ml MeONa (1 M) was stirred at room temperature for 7 h. Carbon dioxide was added and the solvent evaporated. The semi-solid was dissolved in 5 ml H_2O and extracted with hexane (2 x 5 ml). The water-phase was then evaporated to dryness and the residue acetylated with acetic anhydride in pyridine.

^{1}H NMR analysis of this product indicated a similar R/S ratio (2R/2S = 4/1) as in the starting epoxide 3.

Reference samples of pure (R) and (S) hexa acetyl galactosyl glycerol was prepared from the corresponding pure diastereomers of 1-O-β-D-galactopyranosyl-2,3-isopropylidene-glycerol via acetic acid hydrolysis followed by acetylation.

Hexa-O-acetyl-β-D-galactosyl-(S)-glycerol: ^{1}H NMR ($CDCl_3$) δ ; 5.39 (d, 1H), 5.25-5.15 (m, 2H), 5.00 (dd, 1H), 4.50 (d, 1H, J=7.5 Hz), 4.29 (dd, 1H), 4.2-4.08 (mm, 3H), 3.98 (dd, 1H), 3.92 (t, 1H), 3.69 (dd, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H).

Hexa-O-acetyl-β-D-galactosyl-(R)-glycerol: ^{1}H NMR ($CDCl_3$) δ; 5.39 (d, 1H), 5.22-5.17 (m, 2H), 5.02 (dd, 1H), 4.49 (d, 1H, J=7.9 Hz), 4.31 (dd, 1H), 4.2-4.09 (mm, 3H), 3.97 (dd, 1H), 3.92 (t, 1H), 3.70 (dd, 1H), 2.16 (s, 3H), 2.08 (s, 3H), 2.07 (s, 6H), 2.06 (s, 3H), 1.99 (s, 3H).

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REFERENCES

1 a) Sugihara, J.M. Adv. Carbohydr. Res. (1953) 8, 1-44.
b) Kovac, P; Sokoloski, E.A.; Glaudemans, C.P.J. Carbohydr. Res. (1984) 128, 101-109.
2 Sweers, H.M.; Wong C-H. J. Am. Chem. Soc. (1986), 108, 6421.
3 Therisod, M.; Klibanov, A.M. J. Am. Chem. Soc. (1986), 108, 5638.
4 Herz, W. et al. Phytochemistry (1983), 22, 1741.
5 Wallenfels, K.; Malhotra, P. Adv. Carbohydr. Chem. (1961), 16, 239.
6 Lok, C.M.; Ward, J.P.; van Dorp D.A. Chem. Phys. Lipids (1976), 16, 115.
7 a) Heisig, O.M.R.A.; Heinz, E. Phytochemistry (1972), 11, 815.
b) Beier, R.C.; Mundy, B.P.; Strobel, G.A. Carbohydrate Res. (1981), 93, 141.
8 Boos, W. Methods in Enzymology (1982) 89, 59.
9 Baldwin, J.J.; Raab, A.W.; Mensler, K.; Arison, B.H.; McClure, D.E. J. Org. Chem. (1978), 43, 4876.