New 3-*O*-lauroyl-2-*O*-benzyl-glycerol sulfonate

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The hydroxy groups of D-mannitol were protected by the formation of acetals and benzylethers and then 2-*O*-benzyl-D-glyceraldehyde dimethylacetal was prepared after the deprotection and oxygenolysis of the protected D-mannitol. In the presence of DCC and DMAP, the lauroyl group was introduced at the primary hydroxyl group of the dimethylacetal and 3-O-lauroyl-2-O-benzyl-glycerol was obtained after the deprotection of the dimethylacetal with $FeCl_3{\cdot}6H_2O$ and then reduction with $NaBH_4$. A series of new 3-O-lauroyl-2-O-benzyl-glycerol sulfonates was synthesised by the coupling of different sulfonyl groups with the 3-O-lauroyl-2-O-benzyl- glycerol. The bioactivities of the title compounds were tested and some compounds exhibited fungicidal activity against the tested fungi.

Keywords: D-mannitol, glycerol sulfonate, synthesis, fungicidal activity

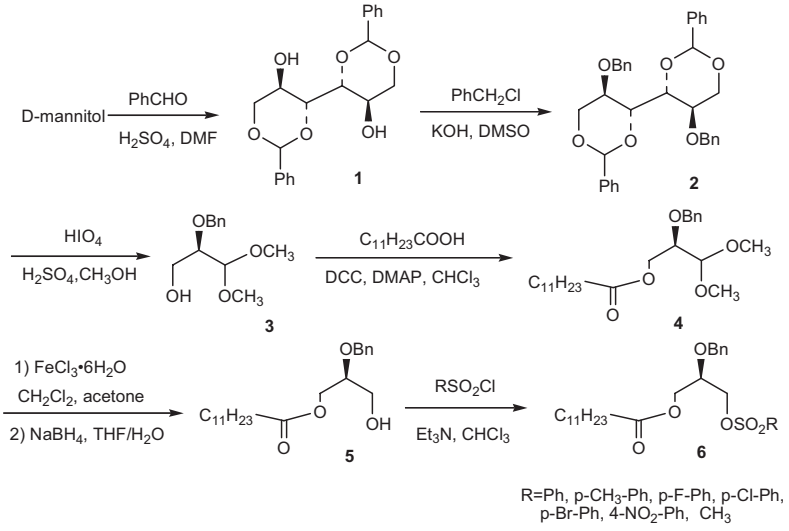
Phospholipids are the primary structural subunits of the bilayers that constitute cell membranes and play critical roles in many biological events through their interactions with other membrane components, including other lipids, proteins, and DNA.[1-5] Lysophospholipids, a kind of phospholipid that occurs in low concentrations in the cell, can be produced through the action of phospholipase on cell membranes and function as key intermediates in diverse biological processes. These compounds display inhibition of cholesterol biosynthesis in Chang liver cells and also exhibit antifungal activity. In addition, lysophosphatidylcholine plays an important role in signal transduction pathway by mediating protein kinase C[6,7] and by inhibition of platelet aggregation.[8] Lysophosphatidylethanolamine (LPE) inhibits plant PLD α and has a profound effect on the physiological symptoms associated with post-harvest senescence of flowers and fruits.[9]

With modification of different parts of the phospholipids, activity varies from one biological process to the other.[10-12] Furthermore, delineation of the structural requirements for the biological activities of phospholipids will not only advance the current level of understanding of the chemistry and biology of these compounds but also provide important insight into the design of new target molecules with the desired activity and potency. To search for new bioactive molecules, we have replaced the phosphodiester headgroup of LPE with sulfonate and introduced the benzyl group into the 2-position of the glycerol backbone to synthesise a series of 3-O-lauroyl-2-O-benzyl- glycerol sulfonates by six steps from D-mannitol. All the target molecules were characterised by ^{1}H NMR and elemental analysis and their bioactivity was tested. The preparation of the title compounds followed the reaction sequence depicted in Scheme 1.

Results and discussion

Synthesis

D-mannitol was used to construct the glycerol skeleton. Firstly, D-mannitol was converted into 1,3;4,6-di-*O*-benzyliden-D-mannitol **1** by protection of the 1,3- and 4,6-hydroxyl groups as benzylidenacetals using benzaldehyde with sulfuric acid catalysis. The desired product **1** was isolated as white crystals in a yield of 40% after extracting the byproducts with boiling chloroform.[13] The 2- and 5-hydroxyl groups in **1** were alkylated with benzyl chloride in DMSO using KOH as base to give 1,3;4,6-di-*O*-benzyliden-2,5-di-*O*-benzyl-D-mannitol **2** in yield 68%.[14] The benzyl ether was reacted with 1.2 mole equivalents of periodic acid in refluxing methanol containing catalytic amounts of sulfuric acid and cleaved to give 2-O-benzyl-D-glyceraldehyde dimethylacetal **3**.[13] Thus the C-3 building block was prepared in three steps



Scheme 1

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from D-mannitol. Acylation of the primary 1-hydroxyl group in **3** with lauric acid in the presence of DCC and DMAP gives 3-*O*-lauroyl-2-*O*-benzyl glyceraldehyde dimethylacetal **4** in 82% yield. To deprotect the dimethylacetal, **4** was first dispersed in 1 M hydrochloric acid and the reactant solution was stirred vigorously at 70°C, which gave a complex mixture of inseparable products. Considering mild reaction conditions and catalyst availability, $FeCl_3{\cdot}6H_2O$, $SnCl_2{\cdot}2H_2O$ or acetone containing molecular iodine were applied to cleave the dimethylacetal of **4**, among which $FeCl_3{\cdot}6H_2O$ gave the best result. Therefore, dimethylacetal **4** was easily converted into 3-*O*-lauroyl-2-*O*-benzyl glycerol **5** in 50% yield by deacetalisation using $FeCl_3{\cdot}6H_2O$ in dichloromethane/acetone, followed by reduction with sodium borohydride. In the presence of Et_3N, **5** was tosylated by different sulfonyl chlorides to obtain 3-*O*-lauroyl-2-*O*-benzyl-glycerol sulfonates **6** in yields 40–90%.

Bioactivity

The antifungal activities of the title compounds in vitro against *Gibberella zeae*, *Alternaria solani*, *Phoma asparagi*, *Physalospora piricola*, and *Cercospora arachi dicola* were tested. The inhibition percentages in these assays over control samples without fungicides are listed in Table 1. The results showed that compounds **6a**, **6b**, **6f** and **6g** exhibited some fungicidal activity against the tested fungi.

Experimental

General

Melting points were determined on a Yanaco melting point apparatus and are uncorrected. Specific rotation was determined on a PE MODEL 341 polarimeter. ^{1}H NMR spectra were recorded on a Bruker Avance 300 M NMR spectrometer in $CDCl_3$ with TMS as internal standard and coupling constants (J) are expressed in Hz. Elemental analyses were done on a Yanaco CHN Corder MT-3 by Jian-Xin Ma in Nankai University. The reaction progress is followed with TLC plates run in PE-EtOAc solvent systems. Spots were visualised by exposure to UV light (254 nm) followed by I_2 vapour. Column chromatographies were carried out with silica gel 200–300 mesh with PE-EtOAc mixtures under positive pressure.

Biological assay

The fungicidal activities of the title compounds were evaluated using the mycelium growth rate test. 1 ml solution of the tested compound in acetone was added to a culture plate and then 9 ml PDA culture medium was added to obtain the 'flat' containing 50 ppm of tested compound. A bacterium tray of 4 mm diameter, cut along the external edge of the hypase, was added to the 'flat' containing the tested compound and put in an equilateral triangular style. Later, the culture plate was cultured at 24±1°C and the expanded diameter of the bacterium tray was measured after 48 h and compared with that treated with sterilised water to estimate the activity. Two replicates were included in the evaluation.

3-O-Lauroyl-2-O-benzyl-D-glyceraldehyde dimethylacetal (**4**)

To a solution of **3** (5 g, 0.022 mol), lauric acid(5.31 g, 0.027 mol) and DMAP(0.27 g, 0.002 mol) in 100 ml of $CHCl_3$ was added dropwise the solution of DCC(5.47 g, 0.027 mol) in 50 ml of $CHCl_3$. The reactant solution was stirred for 2 h at room temperature. The precipitate was filtered off and the filtrate was concentrated to dryness. The residue was purified by column chromatography with PE/EtOAc (15:1, v/v) to obtain colourless liquid in yield 82%. $[\alpha]^{20}_D$ + 18.63° (c0.8, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.37–7.31 (m, 5H, Ar<u>H</u>), 4.72 (s, 2H, PhC<u>H</u>$_2$), 4.43–4.37 (m, 2H, 1-<u>H</u>, 3-<u>H</u>), 4.16–4.10 (m, 1H, 3-<u>H</u>'), 3.70–3.65 (m, 1H, 2-<u>H</u>), 3.45 (s, 3H, OC<u>H</u>$_3$), 3.43 (s, 3H, OC<u>H</u>$_3$), 2.32 (t, 2H, J = 7.5 Hz, C<u>H</u>$_2$CO), 1.63 (t, 2H, J = 6.8 Hz, COCH$_2$C<u>H</u>$_2$), 1.27 (s, 16H, (C<u>H</u>$_2$)$_8$), 0.90 (t, 3H, J = 6.9 Hz, C<u>H</u>$_3$). Anal calcd for $C_{24}H_{40}O_5$: C 70.55, H 9.87; found: C 70.2, H 9.5

3-O-Lauroyl-2-O-benzyl-D-glycerol (**5**)

To a solution of **4** (5.04 g, 0.012 mol) in 80 ml of $CHCl_3$ was added $FeCl_3{\cdot}6H_2O$ (11.67 g, 0.043 mol) and 20 ml of acetone. The reactant solution was stirred for 6 h at room temperature. Saturated $NaHCO_3$ solution was added and the water phase was extracted with CH_2Cl_2 three times. After combination the organic phases were washed with water until colourless and the solvent was removed. The solution of the residue in 40 ml of THF was added at 0°C to the solution of $NaBH_4$ (0.93 g, 0.025 mol) in 10 ml of water. The reactant solution was stirred for 4 h at room temperature and water was added. The solution was neutralised with 10% HCOOH and extracted three times with ether. After combination, drying with magnesium sulfate, evaporation of the solvent, the product was obtained through column chromatography with PE/EtOAc (4:1,v/v) as a colourless liquid in 50% yield. $[\alpha]^{20}_D$ –2.2° (c1.4, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.29–7.19 (m, 5H, Ar<u>H</u>), 4.65 (d, 1H, J = 12 Hz, PhC<u>H</u>$_a$H$_b$), 4.53 (d, 1H, J = 12 Hz, PhCH$_a$<u>H</u>$_b$), 4.17 (d, J = 4.5 Hz, 2H, 3-<u>H</u>, 3-<u>H</u>'), 3.66–3.52 (m, 3H, 2-<u>H</u>,1-<u>H</u>, 1-<u>H</u>'), 2.25 (t, 2H, J = 7.5 Hz, C<u>H</u>$_2$CO), 1.97 (O<u>H</u>), 1.55 (t, 2H, J = 6.8 Hz, COCH$_2$C<u>H</u>$_2$), 1.29–1.14 (m, 16H, (C<u>H</u>$_2$)$_8$), 0.81 (t, 3H, J = 6.9 Hz, C<u>H</u>$_3$). Anal calcd for $C_{22}H_{36}O_4$: C 72.49, H 9.95; found: C 72.1, H 9.9.

3-O-Lauroyl-2-O-benzyl-D-glycerol sulfonate (**6**)

To a solution of **5** (1 mmol) and the sulfonyl chloride (1.2 mmol) in 5 ml of $CHCl_3$ was added dropwise at 0°C a solution of Et_3N (1.2 mmol) in 5 ml of $CHCl_3$. The reactant solution was stirred for 12 h at room temperature. The solution was washed three times with water and dried with $MgSO_4$. After evaporation of solvent the product was obtained through column chromatography with PE/EtOAc (10:1,v/v).

3-O-Lauroyl-2-O-benzyl-D-glycerol benzene sulfonate(**6a**): Yellow liquid, $[\alpha]^{20}_D$ + 3.63° (c 1.6, $CHCl_3$), yield 69%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.90 (d, 2H, J = 8.3 Hz, Ar<u>H</u>), 7.64 (d, 1H, J = 6.0 Hz, Ar<u>H</u>), 7.53 (t, 2H, J = 7.5 Hz, Ar<u>H</u>), 7.33–7.24 (m, 5H, Ar<u>H</u>), 4.59 (d, 1H, J = 11 Hz, PhC<u>H</u>$_2$), 4.54 (d, 1H, J = 11 Hz, PhC<u>H</u>$_2$), 4.22–4.06 (m, 4H, 1-<u>H</u>, 1-<u>H</u>', 3-<u>H</u>, 3-<u>H</u>'), 3.85–3.80 (m, 1H, 2-<u>H</u>), 2.24 (t, 2H, J = 7.5 Hz, C<u>H</u>$_2$CO), 1.56 (t, 2H, J = 6.8 Hz, COCH$_2$C<u>H</u>$_2$), 1.25 (s, 16H, C<u>H</u>$_2$), 0.88 (t, 3H, J = 6.9 Hz, C<u>H</u>$_3$). Anal calcd for $C_{28}H_{40}O_6S$: C 66.44, H 7.99; found: C 66.4, H 8.0

3-O-Lauroyl-2-O-benzyl-D-glycerol p-methylbenzene sulfonate (**6b**): Yellow liquid, $[\alpha]^{20}_D$ + 4° (c 0.8, $CHCl_3$), yield 20%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.80 (d, 2H, J = 8.3 Hz, Ar<u>H</u>), 7.37–7.26 (m, 7H, Ar<u>H</u>), 4.61 (d, 1H, J = 12 Hz, PhC<u>H</u>$_2$), 4.57 (d, 1H, J = 12 Hz, PhC<u>H</u>$_2$), 4.23–4.08 (m, 4H, 1-<u>H</u>, 1-<u>H</u>', 3-<u>H</u>, 3-<u>H</u>'), 3.87–3.80 (m, 1H, 2-<u>H</u>), 2.46 (s, 3H, ArC<u>H</u>$_3$), 2.27 (t, 2H, J = 7.5 Hz, C<u>H</u>$_2$CO), 1.59 (t, 2H, J = 7.5 Hz, COCH$_2$C<u>H</u>$_2$), 1.28 (s, 16H, C<u>H</u>$_2$), 0.90 (t, 3H, J = 6.9 Hz, C<u>H</u>$_3$). Anal calcd for $C_{29}H_{42}O_6S$: C 67.15, H 8.16; found: C 66.9, H 8.2

3-O-Lauroyl-2-O-benzyl-D-glycerol p-fluorobenzene sulfonate (**6c**): Yellow liquid, $[\alpha]^{20}_D$ + 4° (c 0.4, $CHCl_3$), yield 67%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.92–7.88 (m, 2H, Ar<u>H</u>), 7.36–7.15 (m, 7H, Ar<u>H</u>), 4.62 (d, 1H, J = 12 Hz, PhC<u>H</u>$_2$), 4.56 (d, 1H, J = 12 Hz, PhC<u>H</u>$_2$), 4.23–4.07 (m, 4H, 1-<u>H</u>, 1-<u>H</u>', 3-<u>H</u>, 3-<u>H</u>'), 3.86–3.79

Table 1 Antifungal activity of title compounds *in vitro* (%, 50 mg l^{-1})

Compd	R	Ratio(%)				
		Gibberella zeae	*Alternaria solani*	*Phoma asparagi*	*Physalospora piricola*	*Cercospora arachi dicola*
6a	C_6H_5	6.0	34.5	10.7	33.3	13.0
6b	*p*-$CH_3C_6H_4$	12.1	24.1	10.7	28.2	13.0
6c	*p*-FC_6H_4	0	0	0	7.7	0
6d	*p*-ClC_6H_4	0	0	0	20.5	13.0
6e	*p*-BrC_6H_4	9.0	10.3	0	15.4	0
6f	*p*-$NO_2C_6H_4$	12.1	27.6	0	38.5	13.0
6g	CH_3	12.1	31.0	10.7	35.9	0

(m, 1H, 2-H), 2.26 (t, 2H, J = 7.5 Hz, CH_2CO), 1.58 (t, 2H, J = 6.8 Hz, $COCH_2CH_2$), 1.26 (s, 16H, CH_2), 0.88 (t, 3H, J = 6.9 Hz, CH_3). Anal calcd for $C_{28}H_{39}FO_6S$: C 64.34, H 7.52; found: C 64.3, H 7.5

3-O-Lauroyl-2-O-benzyl-D-glycerol p-chlorobenzene sulfonate (**6d**): Yellow liquid, $[\alpha]^{20}_D$ + 5º (c 0.6, $CHCl_3$), yield 84%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.81 (d, 2H, J = 8.3 Hz, ArH), 7.47 (d, 2H, J = 9.0 Hz, ArH), 7.33–7.23 (m, 5H, ArH), 4.62 (d, 1H, J = 12 Hz, $PhCH_2$), 4.55 (d, 1H, J = 12 Hz, $PhCH_2$), 4.22–4.07 (m, 4H, 1-H, 1-H', 3-H, 3-H'), 3.85–3.78 (m, 1H, 2-H), 2.25 (t, 2H, J = 7.5 Hz, CH_2CO), 1.57 (t, 2H, J = 6.8 Hz, $COCH_2CH_2$), 1.27 (s, 16H, CH_2), 0.88 (t, 3H, J = 6.9 Hz, CH_3). Anal calcd for $C_{28}H_{39}ClO_6S$: C 62.38, H 7.29; found: C 62.2, H 7.45

3-O-Lauroyl-2-O-benzyl-D-glycerol p-bromobenzene sulfonate (**6e**)**:** Yellow liquid, $[\alpha]^{20}_D$ + 1.92º (c 1.2, $CHCl_3$), yield 66%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.72 (d, 2H, J = 9.0 Hz, ArH), 7.65–7.62 (d, 2H, J = 9.0 Hz, ArH), 7.37–7.23 (m, 5H, ArH), 4.60 (d, 1H, J = 12 Hz, $PhCH_2$), 4.53 (d, 1H, J = 12 Hz, $PhCH_2$), 4.22–4.07 (m, 4H, 1-H, 1-H', 3-H, 3-H'), 3.85–3.78 (m, 1H, 2-H), 2.25 (t, 2H, J = 7.5 Hz, CH_2CO), 1.57 (t, 2H, J = 6.8 Hz, $COCH_2CH_2$), 1.26 (s, 16H, CH_2), 0.93 (t, 3H, J = 6.9 Hz, CH_3). Anal calcd for $C_{28}H_{39}BrO_6S$: C 57.63, H 6.74; found: C 57.7, H 7.0

3-O-Lauroyl-2-O-benzyl-D-glycerol p-nitrobenzene sulfonate (**6f**): Yellow solid, m.p. 63-64ºC, $[\alpha]^{20}_D$ + 4º (c 0.4, $CHCl_3$), yield 52%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.26 (d, 2H, J = 8.3 Hz, ArH), 8.05–8.02 (d, 2H, J = 9.0 Hz, ArH), 7.34–7.20 (m, 5H, ArH), 4.59(d, 1H, J = 12 Hz, $PhCH_2$), 4.48 (d, 1H, J = 12 Hz, $PhCH_2$), 4.32–4.10 (m, 4H, 1-H, 1-H', 3-H, 3-H'), 3.87–3.80 (m, 1H, 2-H), 2.28 (t, 2H, J = 7.5 Hz, CH_2CO), 1.58 (t, 2H, J = 6.8 Hz, $COCH_2CH_2$), 1.26 (s, 16H, CH_2), 0.88 (t, 3H, J = 6.9 Hz, CH_3). Anal calcd for $C_{28}H_{39}NO_8S$: C 61.18, H 7.15, N 2.55; found: C 61.1, H 7.1, N 2.4

3-O-Lauroyl-2-O-benzyl-D-glycerol methyl sulfonate (**6g**): Colourless liquid, $[\alpha]^{20}_D$ 0º (c 0.8, $CHCl_3$), yield 95%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.35–7.30 (m, 5H, ArH), 4.69 (d, 1H, J = 12 Hz, $PhCH_2$), 4.64 (d, 1H, J = 12 Hz, $PhCH_2$), 4.40–4.10 (m, 4H, 1-H, 1-H', 3-H, 3-H'), 3.92–3.85 (m, 1H, 2-H), 3.0 (s, 3H, CH_3SO_2), 2.32 (t, 2H, J = 7.5 Hz, CH_2CO), 1.62 (t, 2H, J = 6.8 Hz, $COCH_2CH_2$), 1.26 (s, 16H, CH_2), 0.88 (t, 3H, J = 6.9 Hz, CH_3); Anal calcd for $C_{23}H_{38}O_6S$: C 62.41, H 8.65; found: C 62.9, H 8.15.

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