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Bioorganic & Medicinal Chemistry Letters 15 (2005) 159–162
Bioorganic & Medicinal Chemistry Letters

Synthesis of digalactosyl diacylglycerols and their structure–inhibitory activity on human lanosterol synthase

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Received 10 August 2004; revised 5 October 2004; accepted 6 October 2004
Available online 22 October 2004

Abstract—Digalactosyl and monogalactocyl diacylglycerols (DGDG and MGDG), which were identified as anti-hyperlipemia active components in *Colocasia esculenta* (Taro), were synthesized. The inhibitory activity of DGDG, MGDG and related compounds on human lanosterol synthase was evaluated as anti-hyperlipemic activity. DGDG with two myristoyl groups at both *sn*-1 and *sn*-2 positions and with an oleoyl group at the *sn*-1 position showed the most potent activity.

Lanosterol synthase is located in the middle stage of the biosynthetic pathway of the cholesterol of mammals, and is a more selective target for suppression of cholesterol biosynthesis. We established an in vitro assay system of lanosterol synthase using recombinant human enzyme and [^{14}C] labeled oxydosqualene, to search for the anti-hyperlipemia active component.[1] In the screening study the ethanol extract of *Colocasia esculenta* (Taro) exhibited the most potent activity among 60 kinds of vegetable extracts. Therefore, we investigated the active principal from Taro and isolated monogalactosyl diacylglycerol (MGDG, **1**) and digalactosyl diacylglycerol (DGDG, **2**) as the active components, after repeated chromatography.[2] MGDG and DGDG, a class of glycolipids, are the major constituents of the chloroplast membrane in the plant kingdom and have attracted much attention in recent years because of their biological activities, such as anti-tumor-promoting activity,[3a] DNA polymerase inhibition,[3b] activity of violaxanthin de-epoxidase in liposomes[3c] and hemolytic activity.[3d] It was reported that MGDG and DGDG with various acyl pairs had been isolated from natural resources. The variations of acyl groups in MGDG and DGDG were supposed to reflect the strength of inhibitory activity against human lanosterol synthase from the viewpoint of structure–activity relationship. However, it is difficult isolate MGDG or DGDG with different acyl groups from Taro, because they have almost the same polarity and size with each other. Thus, we attempted to synthesize selectively MGDG and DGDG with different acyl groups. In this paper, we report the first synthesis of DGDG with the desired acyl groups at the desired positions and elucidation of their inhibitory activity on human lanosterol synthase (Fig. 1).

MGDG was prepared in the same manner as reported before.[8] The synthetic route to DGDG (**2**) is shown in

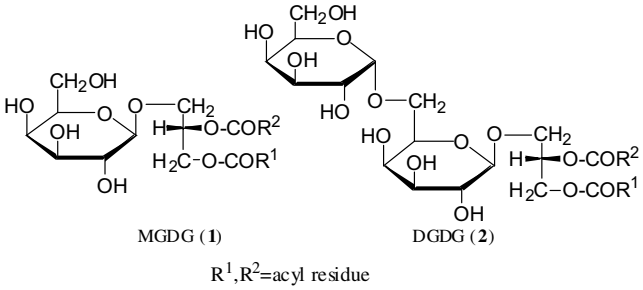

Figure 1. Chemical structures of MGDG and DGDG.

Keywords: *Colocasia esculenta*; Glycolipid; Digalactosyl diacylglycerols; Lanosterol synthase; Cholesterol biosynthesis.
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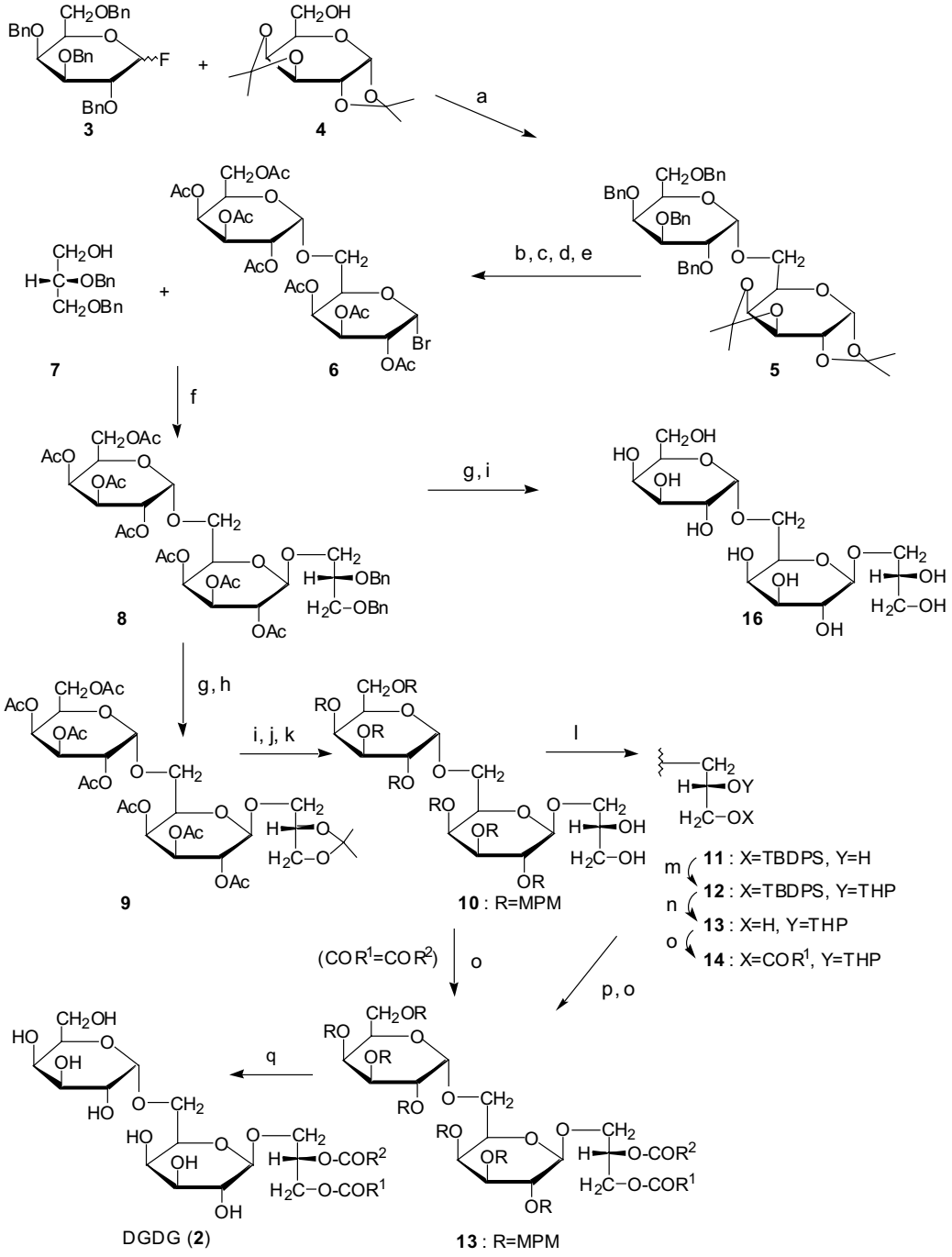
doi:10.1016/j.bmcl.2004.10.013



Scheme 1. Reagents and conditions: (a) $SnCl_2$, $AgClO_4$, MS4A, Et_2O, −30 °C, 48 h; (b) H_2, 10% Pd–C, EtOH, rt, 22 h; (c) Dowex 50WX8–100, H_2O, 60 °C, 12 h; (d) Ac_2O, Py, rt, 5 h; (e) 30% HBr–AcOH, CH_2Cl_2, −10 °C, 0.5 h; (f) HgO, $HgBr_2$, $ClCH_2CH_2Cl$, rt, 4 h; (g) H_2, 10% Pd–C, EtOH, rt, 3 h; (h) $Me_2C(OMe)_2$, *p*-TsOH, DMF, rt, 2 h; (i) NaOMe, MeOH, rt, 10 min; (j) MPM-Cl, NaH, DMF, rt, 17 h; (k) *p*-TsOH, MeOH, rt, 2 h; (m) TBDPS-Cl, pyridine, rt, 3 h; (m) DHP, PPTS, CH_2Cl_2, rt, 6.5 h; (n) TBAF, THF, rt, 4 h; (o) fatty acid, DMAP, EDCI–HCl, CH_2Cl_2, rt, 4 h; (p) PPTS, MeOH, rt, 12 h; (q) CAN, CH_3CN–H_2O, rt, 45 min.

Scheme 1. We selected linolate, linolenate, oleic, and palmitate as introduction acyl groups, because they are components of DGDG isolated from Taro,[2] and myristrate, that is, a major acyl component of DGDG isolated from microalgae.[4] Compound **3**[5] as a mixture of α- and β-anomers was reacted with **4** in the presence of $SnCl_2$ and $AgClO_4$[6] to give **5** as a 10:9 mixture of α- and β-anomers. The mixture was separated by silica gel chromatography to obtain the α-anomer (44%). The benzyl groups and isopropylidene acetals were cleaved followed by protection of the resulting hydroxyl groups as acetate to afford **6**. Dibenzyl glycerol (**7**)[7] derived from D-mannitol was reacted with **6** in the presence of HgO and $HgBr_2$ to give **8** (64%) as the β-anomer.

The route to DGDG from **8** was carried out in essentially similar way to the synthesis of MGDG previously

reported.[8] After the benzyl groups on the glycerol moiety of **8** were converted to isopropylidene acetal groups, the acetyl groups at the sugar moiety were cleaved followed by protection of the resulting hydroxyl groups as MPM ethers. Then, the isopropylidene acetal groups were removed to afford **10**. The hydroxyl groups at *sn*-1 and -2 were protected individually as *t*-butyldiphenylsilyl (TBDPS) ether and tetrahydropyranyl (THP) ether, respectively, to give **12** (98%). The TBDPS ether was cleaved to **13** (97%), then acyl groups were incorporated at the *sn*-1 position to give the corresponding **14** (79–98%). After removal at the THP ether of **14**, the corresponding acyl groups were introduced at the *sn*-2 position to afford **15** (81–98%). Finally, the MPM ethers of **15** were removed by treatment with ceric ammonium nitrate (CAN), and DGDG with different acyl groups (**2c**–**j**, **2l**, **2n**–**o**) were obtained (11% from **3** for 18 steps).[9]

DGDG with the same acyl groups at the *sn*-1 and -2 positions (**2a**,**b**,**k**,**m**) were obtained by acylation of **10** followed by cleavage of the MPM ethers. Digalactosyl glycerol (**16**) was obtained by removal of the protective groups of **8**. In total, we prepared 25 kinds of DGDG (**2**) and digalactosyl glycerol (**16**).

Inhibitory activity on human lanosterol synthase of MGDG and DGDG was determined by using the same method as described before.[2] The result is shown in Table 1. MGDG showed 23% inhibition (ca. 0.4 μmol/assay) or less, while most DGDG showed higher activity than MGDG. DGDG with two myristoyl groups (**2a**) showed the most potent inhibitory activity, 72% at the concentration of 0.5 μ mol/assay. The dioleoyl derivative (**2b**) also potent and showed 57% inhibition at the same concentration. DGDG with an oleoyl group at *sn*-1 (**2c**,**f**,**i**) tend to inhibit enzyme more effectively than the others except for that with two myristoyl groups at both *sn*-1 and *sn*-2 positions. Both dioleoyl and dimyristoyl DGDG showed potent inhibitory effect, probably because the distance between the terminal methyl and carbonyl groups of the oleoyl group, which is bent double at *cis*-olefine, is close to that of the linear myristoyl group. Digalactosyl glycerol (**16**) showed no inhibition against human lanosterol synthase. From these data, it was revealed acyl groups in the digalactosyl glycerol were essential for the inhibitory activity, and the whole molecular conformation of DGDG seemed to affect the inhibitory activity on human lanosterol synthase.

DGDG with myristoyl group(s) were not reported as the component of higher plants, but were isolated from microalgae such as *Chlorella* and *Spirulina* species, which are commercially available health food. DGDG with any acyl groups as well as MGDG are known as the component of not only Taro but also of most daily ingestive vegetables and cereals such as spinach, wheat, runner bean, cucumber, etc.[11] Our result suggests that the taking these vegetables should be effective and practical for avoiding hypercholesterolemia, because even DGDG with acyl groups other than a myristoyl group indicated moderate activity. As there were few reports about the acyl moiety of DGDG in each vegetable,[11] it may be important to know the acyl composition of DGDG in the vegetables for evaluating the efficacy of them against hypercholesterolemia.

In summary, we have established the first sufficient synthetic route to DGDG (**2**) with the desired acyl groups at the desired positions, and obtained 25 kinds of DGDG. As the result of the evaluation of their inhibitory activity on human lanosterol synthase, we clarified that the myristoyl group contributed the most to the

Table 1. Inhibitory activity of synthetic MGDG (**1p**–**r**)[a], DGDG (**2a**–**o**)[b] and digalactosyl glycerol (**16**) on Human Lanosterol Synthase

Compounds	Acyl group		Inhibition (%)	
	sn-1 (COR^1)	*sn*-2 (COR^2)	ca. 0.4 μmol/assay	
1p	Myristoyl	Linoleoyl	23	
1q	Linoleoyl	Oleoyl	19	
1r	Palmitoyl	Linoleoyl	16	
			0.25 μmol/assay	0.5 μmol/assay
2a	Myristoyl	Myristoyl (14:0)	51	72
2b	Oleoyl	Oleoyl (18:1)	42	57
2c	Oleoyl	Palmitoyl (16:0)	24	46
2d	Linoleoyl	Linolenoyl (18:3)	18	45
2e	Myristoyl	Palmitoyl	35	45
2f	Oleoyl	Linoleoyl (18:2)	9	40
2g	Linoleoyl	Myristoyl	22	38
2h	Linoleoyl	Oleoyl	8	31
2i	Oleoyl	Linolenoyl	7	29
2j	Linoleoyl	Palmitoyl	3	27
2k	Linoleoyl	Linoleoyl	n.d.	26
2l	Linolenoyl	Palmitoyl	5	26
2m	Palmitoyl	Palmitoyl	4	24
2n	Palmitoyl	Oleoyl	4	21
2o	Palmitoyl	Linoleoyl	n.d.	20
16	H	H	n.d.	n.d.

[a] MGDG showimg more than 15% inhibition (ca. 0.4 μmol/assay) are shown. n.d.: not detected.
[b] DGDG showing more than 20% inhibition (0.5 μmol/assay) are shown.[10]

activity of DGDG among various acyl groups we tested, and a dimyristoyl derivative (**2a**) and DGDG with an oleoyl group at *sn*-1 (**2b**,**c**,**f**,**i**) indicated the strong activity.

Acknowledgements

This work was supported by a grant from the Japan Health Sciences Foundation.

References and notes

1. (a) Sakano, Y.; Shibuya, M.; Matsumoto, A.; Takahashi, Y.; Tomoda, H.; Ômura, S.; Ebizuka, Y. *J. Antibiot.* **2003**, *56*, 817; (b) Sakano, Y.; Shibuya, M.; Yamaguchi, R.; Tomoda, H.; Ômura, S.; Ebizuka, Y. *Antibiot.* **2004**, *57*, 564.
2. Sakano, Y.; Mutsuga, M.; Tanaka, R.; Suganuma, H.; Inakuma, T.; Toyoda, M.; Goda, Y.; Shibuya, M.; Ebizuka, Y. *Biol. Pharm. Bull.*, submitted.
3. (a) Shirahashi, H.; Murakami, N.; Watanabe, M.; Nagatsu, A.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A. *Chem. Pharm. Bull.* **1993**, *41*, 1664; (b) Murakami, C.; Kumagai, T.; Hada, T.; Kanekazu, U.; Nakazawa, S.; Kamisuki, S.; Maeda, N.; Xu, X.; Yoshida, H.; Sugawara, F.; Sakaguchi, K.; Mizushina, Y. *Biochem. Pharmacol.* **2003**, *65*, 259; (c) Latowski, D.; Kostecka, A.; Strzalka, K. *Biochem. Soc. Trans.* **2000**, *28*, 810; (d) Parrish, C. C.; Bodennec, G.; Gentien, P. *Phytochemistry* **1998**, *47*, 783.
4. Murakami, N.; Morimoto, T.; Imamura, H.; Ueda, T.; Nagai, S.; Sakakibara, J.; Yamada, N. *Chem. Pharm. Bull.* **1991**, *39*, 2277.
5. (a) Ferrier, R. J.; Furneaux, R. H. *Methods Carbohydr. Chem.* **1980**, *8*, 251; (b) Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 4189.
6. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 43.
7. (a) Mannock, D. A.; Lewis, R. N. A. H.; McElhaney, R. N. *Chem. Phys. Lipids* **1987**, *43*, 113; (b) Takano, S.; Ogasawara, K. *Yuki Gosei Kagaku Kyokaishi* **1987**, *45*, 1987; (c) van Boeckel, C. A. A.; Visser, G. M.; van Boom, J. H. *Tetrahedron* **1985**, *41*, 4557.
8. Nagatsu, A.; Watanabe, M.; Ikemoto, K.; Hashimoto, M.; Murakami, N.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A.; Yazawa, K. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1619.
9. All compounds were characterized by NMR and MS spectra.
10. The following DGDGs were also synthesized and assayed, but inactive (<20% inibition at 0.5 μmol/assay): (*sn*-1 (COR^1), *sn*-2 (COR^2)) = (myristoyl, oleoyl), (myristoyl, linoleoyl), (myristoyl, linoleoyl), (oleoyl, myristoyl), (linolenoyl, myristoyl), (linolenoyl, oleoyl), (linolenoyl, linolenoyl), (palmitoyl, myristoyl), and (palmitoyl, linolenoyl).
11. (a) Allen, C. F.; Good, P.; Davis, H. F.; Fowler, S. G. *Biochem. Biophys. Res. Commun.* **1964**, *15*, 424; (b) Sugawara, T.; Miyazawa, T. *Lipids* **1999**, *34*, 1231.