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Short communication

Glycoglycerolipid analogues active as anti-tumor-promoters: the influence of the anomeric configuration

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Abstract – The in vitro anti-tumor promoting effect of monohexanoates of 2-*O*-α-D-gluco- and galactopyranosyl-*sn*-glycerol on the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced Epstein–Barr virus early antigen (EBV-EA) activation was evaluated and compared to the potencies of the corresponding β-anomers. The results show that the inversion of the anomeric configuration from β to α does not seem to significantly influence the activity, which is present, as for the β-anomers, even at 1×10 mol ratio without any cytotoxicity. © 2000 Éditions scientifiques et médicales Elsevier SAS

glycoglycerolipid analogues / cancer chemoprevention / Epstein–Barr virus activation test / anti-tumor-promoting activity

1. Introduction

Glycoglycerolipid analogues such as 1-*O*-, 3-*O*- and 6′-*O*-acyl derivatives of 2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**1a**) and 2-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2a**) show a significant anti-tumor-promoting activity based on the in vitro inhibition of Epstein–Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [1–3]. At present, the mechanism of the inhibitory effect on tumor promoting activity is not yet known; thus, our attention has been focused on the study of the structure–activity relationship both in vitro and in vivo [4], which might clarify some points of the action mechanism and perhaps the correlation between the in vitro and in vivo effect. In fact, the biological activity of these compounds is modulated by structural features, such as the length of the acyl chain and its position and the nature of the sugar moiety. These last two aspects play a minor role on the activity, which is mainly influenced by variations of the acyl chain length, reaching in any case the best values for the hexanoyl derivatives [2, 3]. An important but not yet taken into account structural characteristic is the anomeric configuration: all the analogues studied upto now are β-glycosylglycerols, according to the β-configuration of the natural bioactive glycoglycerolipids [5].

In order to evaluate the influence of the inversion of the anomeric configuration on the anti-tumor-promoting activity, we decided to synthesize the α-analogues of the most active β-compounds [2, 3], i.e. the monoesters carrying a hexanoyl as acyl group and test their activity.

To prepare these compounds, we employed enzymatic transesterification reactions on 2-*O*-α-D-glucopyranosyl-*sn*-glycerol (**3a**) and 2-*O*-α-D-galactopyranosyl-*sn*-glycerol (**4a**) using the enzymes and the reaction conditions already used in the acylation of the primary hydroxyl groups of the corresponding β-anomers **1a** [6] and **2a** [7] (*figure 1*).

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Table I. Enzymatic transesterification of **3a** and **4a**[a]

Substrate	Enzyme	Time (h)	Monoester yield (%)	Monoester ratio **b**:**c**:**d**
3a	LPS	6.0	61	12:39:49
3a	LCA	0.5	57	28:52:20
4a	LPS	1.5	73	t:t:99
4a	LCA	1.0	62	34:52:14

[a] No acylation took place in the absence of the enzyme; t = traces.

2. Chemistry

2.1. Preparation of monohexanoates of 2-O-α-D-Glucosyl- and 2-O-α-D-galactosylglycerol

2-*O*-α-D-Glucopyranosyl-*sn*-glycerol (**3a**) [8] or 2-*O*-α-D-galactopyranosyl-*sn*-glycerol (**4a**) [9], both prepared through a glycosidation reaction following the trichloroacetimidate method according to Ref. [8], were directly submitted to *Pseudomonas cepacia* (LPS) or *Candida antarctica* (LCA) lipase catalyzed transesterification in the presence of 2,2,2-trifluoroethyl hexanoate as the acyl carrier and pyridine as the solvent.[1]

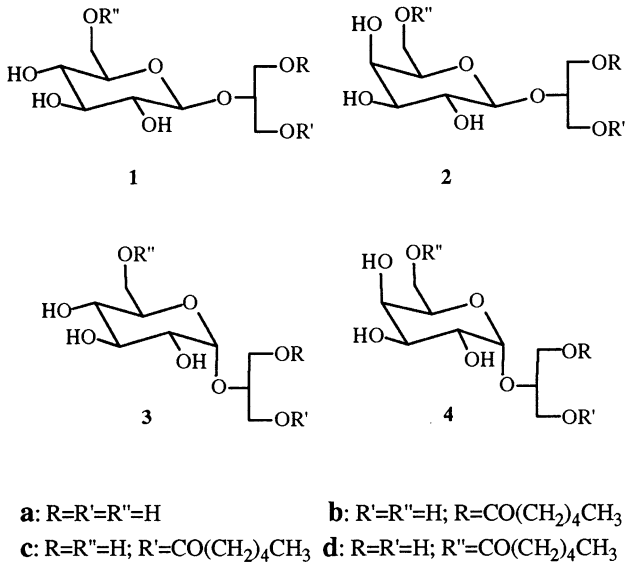

Figure 1. Structures of α- and β-glucosyl- and galactosylglycerols.

[1] Owing to the low selectivity displayed by LPS and LCA (*table I*), other enzymes and reaction conditions were screened. However, no significant improvement was obtained.

The reactions were performed in order to obtain the maximum yield of the monoester fraction (see *table I*), which was recovered by flash chromatography; the ratio among the 1-*O*-, 3-*O*- and 6′-*O*-isomers was determined by ^{1}H-NMR analysis coupled with chiral GLC analysis (Section 5.2) for the assignment of the configuration at C-2.

Additional chromatographic purification of the monoester mixtures could afford pure 2-*O*-(6-*O*-hexanoyl-α-D-glucopyranosyl)-*sn*-glycerol (**3d**) and 2-*O*-(6-*O*-hexanoyl-α-D-galactopyranosyl)-*sn*-glycerol (**4d**) (see Section 5).

2.2. Assignment of configuration at C-2

The configuration at C-2 of the 1-*O*- and 3-*O*-hexanoates **3b,c** and **4b,c** was determined by correlation to authentic samples of 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol, exploiting chiral GLC analysis, after enzymatic hydrolysis with α-gluco- or galactosidase as described for the corresponding β-anomers [1, 7].

3. Pharmacology

As a primary screening for anti-tumor-promoters, the 6′-*O*-hexanoates **3d** and **4d,** together with the non-acylated compounds **3a** and **4a,** were tested for their anti-tumor-promoting activity using a short-term in vitro assay for Epstein–Barr virus activation in Raji cells induced by TPA [10, 11]. In order to make an easy comparison between the potency of the α- and β-anomers, *table II* shows the in vitro tumor inhibitory effect of α-glycosylglycerols **3a**, **4a,** **3d** and **4d** and of the isomeric mixtures **3b–d** and **4b–d**, besides the previously obtained data for the corresponding 6′-*O*-hexanoates **1d** and **2d** of the β-series.

Table II. Inhibitory effects of **1**, **2**, **3** and **4** on TPA-induced EBV-EA activation

	Concentration (mol ratio/TPA)			
	1000	500	100	10
	% to positive control ± S.E. ($n = 3$) (% viability)[a]			
3a	0 ± 0.8 (70)	46.8 ± 1.1 (>80)	79.5 ± 2.0 (>80)	100 ± 0.2 (>80)
3d	0 ± 0.5 (70)	13.2 ± 1.1 (>80)	43.6 ± 2.0 (>80)	76.6 ± 0.3 (>80)
3b–d[b]	0 ± 0.6 (70)	12.4 ± 1.6 (>80)	40.6 ± 2.1 (>80)	72.3 ± 0.1 (>80)
4a	0 ± 0.6 (70)	41.2 ± 1.3 (>80)	76.7 ± 1.9 (>80)	94.6 ± 0.4 (>80)
4d	0 ± 0.3 (70)	10.7 ± 1.2 (>80)	38.6 ± 2.5 (>80)	69.4 ± 0.1 (>80)
4b–d[c]	0 ± 0.2 (70)	16.3 ± 1.5 (>80)	49.8 ± 2.3 (>80)	80.4 ± 0.5 (>80)
1d[d]	0 ± 0.0 (70)	19.3 ± 0.3 (>80)	37.6 ± 1.5 (>80)	78.1 ± 1.8 (>80)
2d[e]	0 ± 0.0 (70)	10.7 ± 0.1 (>80)	30.1 ± 0.9 (>80)	67.8 ± 1.2 (>80)

[a] TPA 32 pmol, 100%.
[b] **3b**:**3c**:**3d** = 12:39:49.
[c] **4b**:**4c**:**4d** = 34:52:14.
[d] From Ref. [1].
[e] From Ref. [3].

4. Results and discussion

All the tested compounds were significantly active and, as already observed in the case of the corresponding β-glycosylglycerols [2, 3, 12], the hexanoates were more potent than the non-acylated compounds **3a** and **4a**. In particular, the activities of the 6′-*O*-hexanoyl-α-glucoside **3d** and of the 6′-*O*-hexanoyl-α-galactoside **4d** were comparable and similar to those of the corresponding hexanoates of the β-series, **1d** and **2d**. The α-glucosylglycerols and α-galactosylglycerols mixtures **3b–d** and **4b–d** were very potent too, showing an inhibitory effect similar to that of the 6′-monohexanoates **3d** and **4d**. The similarity of the values suggests that the 1- and 3-monoesters **3b,c** and **4b,c** are also active and that the different monoester isomers, when in mixture, do not significantly influence the activities of one another.

In conclusion, as evidenced by compound **3d** and **4d** activities, the anomeric configuration seems to play a negligible role on the potency of 2-*O*-glycoglycerolipids, while once again the presence of a hexanoyl chain is effective in enhancing it.

5. Experimental protocols

^{1}H-NMR spectra were recorded with a Bruker AM-500 spectrometer on pyridine-d_5 solutions at 303 K; chemical shifts are reported as δ(ppm) relative to tetramethylsilane as internal standard. Mass experiments were performed through chemical ionization mass spectrometry (CI-MS) as described in Ref. [13]. Melting points were recorded on a Büchi 510 capillary melting point apparatus and were uncorrected. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in methanol solutions in a 1 dm cell at 20°C. Analytical thin layer chromatography (TLC) was carried out on Merck 60 F_{254} silica gel plates (0.25 mm thickness) and the spots were detected by spraying with 50% aqueous H_2SO_4 and heating at 110°C. Flash chromatography was performed with Merck 60 silica gel (230–400 mesh). *Pseudomonas cepacia* (lipase PS) was a generous gift from Amano Pharmaceutical Co; *Candida antarctica* SP 435 L, (Novozym® 435) was a generous gift from Novo Nordisk A/S; α-glucosidase from brewers yeast and α-galactosidase from green coffee beans were purchased from Sigma. 2,2,2-Trifluoroethyl hexanoate was synthesized according to Ref. [6]. Evaporation under reduced pressure was always effected with the bath temperature kept below 40°C. Pyridine was distilled over calcium hydride prior to use. Analyses of the new compounds, indicated by the symbols of the elements, were within ± 0.4% of the theoretical values.

5.1. General procedure for the enzymatic synthesis of monoesters *3b–d* and *4b–d*

3a or **4a** (0.2 g, 0.79 mmol) were dissolved in 4 mL of pyridine; 2,2,2-trifluoroethyl hexanoate (2.36 mmol) and the proper lipase (1 g of LPS supported on celite [14] or 0.3 g of LCA) were added in the order and the suspension was stirred at 45°C. The reaction was monitored by TLC (dichloromethane–methanol 8:2, v/v) and was stopped by filtering off the enzyme which was washed with pyridine. The solvent was removed under reduced pressure, the residue submitted to flash chromatography (dichloromethane–methanol from 9:1 to 8:2, v/v) and the monoester fraction characterized through ^{1}H-NMR analysis and chemical ionization MS [13] (m/z 370 [M + NH_4]$^+$).

^{1}H-NMR anomeric signals for **3b–d** and **4b–d**: δ 5.61 (d, 1H, $J_{1',2'} = 4.0$ Hz) for **3b**, 5.60 (d, 1H, $J_{1',2'} = 4.0$ Hz)

for **3c**, 5.65 (d, 1H, $J_{1',2'} = 4.0$ Hz) for **3d**; δ5.64 (d, 1H, $J_{1',2'} = 4.0$ Hz) for **4b**, 5.62 (d, 1H, $J_{1',2'} = 4.0$ Hz) for **4c**, 5.68 (d, 1H, $J_{1',2'} = 4.0$ Hz) for **4d**.

*5.1.1. 2-O-(6′-O-hexanoyl-α-D-glucoyranosyl)-sn-glycerol **3d***

Flash chromatography (two runs, dichloromethane–methanol from 8.5:1.5 to 8:2, v/v) of the monoester fraction (0.169 g) from LPS catalyzed transesterification on **3a** yielded **3d** (0.063 g, 90% pure by ^{1}H-NMR). Crystallization from chloroform allowed pure **3d** to be obtained: mp. 106–107°C; $[\alpha]_D$ +84° (c 1); ^{1}H-NMR: δ 0.75 (t, 3H, $J = 7.0$ Hz, CH_3), 1.15 (m, 4H, $2CH_2$), 1.58 (m, 2H, CH_2), 2.25–2.35 (m, 2H, CH_2), 4.05 (dd, 1H, $J_{3',4'} = 9.5$ Hz, $J_{4',5'} = 9.5$ Hz, H-4′), 4.11–4.26 (m, 5H, H-2′, 2H-1 and 2H-3), 4.34 (m, 1H, H-2), 4.58 (dd, 1H, $J_{2',3'} = 9.5$ Hz, H-3′), 4.72–4.80 (m, 2H, H-5′ and H-6′a), 5.02 (m, 1H, H-6′b), 5.65 (d, 1H, $J_{1',2'} = 4.0$ Hz, H-1′); MS (CI/NH_3): m/z 370 $[M + NH_4]^+$; $C_{15}H_{28}O_9$ (C, H, O).

*5.1.2. 2-O-(6′-O-hexanoyl-α-D-galactopyranosyl)-sn-glycerol **4d***

Flash chromatography (dichloromethane–methanol from 9:1 to 8:2, v/v) of the monoester fraction (0.203 g) from LPS catalyzed transesterification on **4a** yielded **4d** (0.142 g): mp. 77–78°C (isopropyl ether–methanol); $[\alpha]_D$ + 98.0° (c 1); ^{1}H-NMR: δ 0.75 (t, 3H, $J = 7.0$ Hz, CH_3), 1.20 (m, 4H, $2CH_2$), 1.60 (m, 2H, CH_2), 2.35 (m, 2H, CH_2), 4.13–4.28 (m, 4H, 2H-1 and 2H-3), 4.33 (m, 1H, H-2), 4.40 (d, 1H, $J_{3',4'} = 3.0$ Hz, H-4′), 4.49 (dd, 1H, $J_{2',3'} = 10.0$ Hz, H-3′), 4.65 (dd, 1H, $J_{1',2'} = 4.0$ Hz, H-2′), 4.76–4.82 (m, 2H, H-5′ and H-6′a), 4.87 (dd, 1H, $J_{5',6'b} = 9.0$ Hz, $J_{6'a,6'b} = 12.0$ Hz, H-6′b), 5.68 (d, 1H, H-1′); MS (CI/NH_3): m/z 370 $[M + NH_4]^+$; $C_{15}H_{28}O_9$ (C, H, O).

5.2. Assignment of configuration at C-2

The configuration at C-2 of compounds **3b,c** and **4b,c** was determined by correlation to authentic samples of 1-*O*-hexanoyl-2,3-*O*- and 3-*O*-hexanoyl-1,2-*O*-isopropylidene-*sn*-glycerol, using chiral GLC analysis (dimethylpentyl-β-cyclodextrin capillary column), after enzymatic hydrolysis with α-glucosidase from brewers yeast or α-galactosidase from green coffee beans in the conditions described for the corresponding β-anomers [1, 7]. In the chromatogram, the lower retention time peak corresponded to the 1-*O*-hexanoyl-2,3-*O*-isopropylidene-*sn*-glycerol and the higher retention time peak to the 3-*O*- hexanoyl-isomer.

6. Short term in vitro bioassay for anti-tumor promoters

Epstein–Barr virus (EBV) is known to be activated by tumor promoters to produce viral early antigens (EA) and evaluation of its inhibition is often used as a primary screening for anti-tumor-promoting activities in vitro [15]. The anti-tumor-promoting activity was tested using a short-term in vitro assay for Epstein–Barr virus activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [10, 11]. The assays were performed in triplicate for each compound. No sample exhibited significant toxicity against Raji cells. The viability of the cells was assayed against treated cells using the Trypan blue staining method. The results are reported in *table II*.

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