Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 10, pp. 1187-1190, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00196-5

1-*O*-, 2-*O*- AND 3-*O*-β-GLYCOSYL-*sn*-GLYCEROLS: STRUCTURE - ANTI-TUMOR-PROMOTING ACTIVITY RELATIONSHIP

Diego Colombo,[a] Antonio Scala,[a] Ida M. Taino,[a] Lucio Toma,[b] and Fiamma Ronchetti[a]*

[a] *Dipartimento di Chimica e Biochimica Medica, Università di Milano, Via Saldini 50, 20133 Milano (Italy), Fax n°: +392-2361407, E-mail: ronchsca@imiucca.csi.unimi.it*
[b] *Dipartimento di Chimica Organica, Università di Pavia, Via Taramelli 10, 27100 Pavia (Italy)*

Harukuni Tokuda,[c] Hoyoku Nishino,[c] Akito Nagatsu,[d] and Jinsaku Sakakibara[d]*

[c] *Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602 (Japan)*
[d] *Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467 (Japan)*

Abstract - The inhibitory activity of synthetic glycosylglycerols on Epstein-Barr virus early antigen (EBV-EA) activation was evaluated. Among the series of 1-*O*-, 2-*O*- and 3-*O*- glycosylglycerols tested, 1-*O*-β-D-galactopyranosyl-*sn*-glycerol showed the highest inhibitory effect toward tumor promotion.

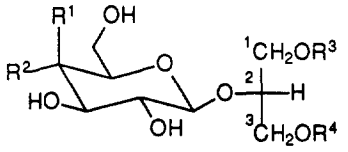
Glyceroglycolipids consist of saccharide residues and fatty acid moieties bound together through glycerol; they are known to exist as cell membrane components[1] in animal tissues, micro-organisms, algae and plants, in which the acyl moiety can merely be an acetyl group.

Owing to the large heterogeneity in their structure, it is probable that glycolipids serve a variety of functions in nature: they are known to have various biologically significant activities,[2,3] and some of them exhibit pharmacological activity.[4] Moreover it has been recently reported that some monoglycosyl- and diglycosyl glycerols, isolated from the cyanobacterium *Phormidium tenue*,[5,6] from *Citrus hystrix*, a traditional herb in Thailand[7] or from the green alga *Chlorella vulgaris*[8] exhibit anti-tumor-promoting activity. Since promotion is the only reversible process during the multistage of carcinogenesis,[9] the search for possible cancer preventive agents, among synthetic and naturally occurring compounds, is an emerging field for cancer control.[10,11]

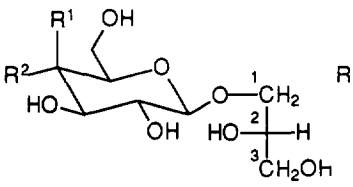
In the course of a project aimed to ascertain the structural features responsible for the cancer chemopreventing activity, we planned to test a series of glycosylglycerols in which no fatty acyl residue was linked to the glycerol subunit. In fact, among the several bioactive glycosylglycerols, 3-*O*-β-D-galactosyl-*sn*-glycerol was one of those exhibiting the strongest activity.[12]

RESULTS AND DISCUSSION

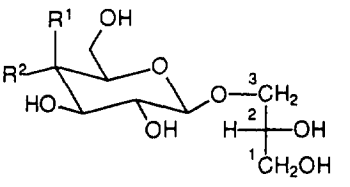
The compounds used in this study,[13,14] 2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**1**), 1-*O*-acetyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**2**), 3-*O*-acetyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**3**), 2-*O*-β-D-galactopyranosyl-*sn*-glycerol (**4**), 1-*O*-β-D-glucopyranosyl-*sn*-glycerol (**5**), 3-*O*-β-D-glucopyranosyl-*sn*-glycerol (**6**),



1: R^1= R^3= R^4= H, R^2= OH
2: R^1= R^4= H, R^2= OH, R^3= Ac
3: R^1= R^3= H, R^2= OH, R^4= Ac
4: R^1= OH, R^2= R^3= R^4= H



5: R^1= H, R^2= OH
7: R^1= OH, R^2= H



6: R^1= H, R^2= OH
8: R^1= OH, R^2= H

1-*O*-β-D-galactopyranosyl-*sn*-glycerol (**7**), 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**8**), methyl β-D-glucopyranoside (**9**) and methyl β-D-galactopyranoside (**10**), differed in the sugar or in the aglycone moiety and in the glycosylation site.

The anti-tumor-promoting activities of compounds **1-10** were determined using a short-term *in vitro* assay for Epstein-Barr virus activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[6,15] The inhibitory effect on the activation and the viabilities of Raji cells are summarized in Table 1.

Table 1. Inhibitory effects of the compounds **1-10** on TPA-induced EBV-EA* activation.

Compound	Concentration (mol ratio/TPA)			
	1000	500	100	10
	% to positive control† ± s.e.‡ (% viability)			
1	0 ± 0.7 (70)	45.8 ± 2.8	81.0 ± 2.0	100 ± 1.6
2[16]	0 ± 0.6 (70)	32.9 ± 2.3	71.5 ± 1.9	94.7 ± 1.1
3	0 ± 0.6 (70)	36.6 ± 2.2	77.3 ± 2.3	100 ± 1.6
4	0 ± 0.1 (80)	17.5 ± 0.7	88.1 ± 1.4	100 ± 0.3
5[17]	0 ± 0.4 (70)	25.8 ± 1.4	64.6 ± 1.8	87.5 ± 1.3
6	0 ± 0.5 (70)	27.4 ± 1.2	68.9 ± 1.9	90.4 ± 1.1
7	0 ± 0 (70)	16.5 ± 0.6	58.2 ± 1.5	79.1 ± 1.0
8	0 ± 0.2 (70)	20.7 ± 0.9	62.4 ± 1.2	82.5 ± 1.1
9	22.8 ± 1.5 (70)	54.1 ± 1.3	70.7 ± 1.0	100 ± 0.5
10	20.3 ± 1.4 (80)	43.9 ± 1.2	76.5 ± 1.1	93.7 ± 0.3

* Epstein-Barr virus early antigen.
† TPA 32 pmol = 100%.
‡ Standard error (n = 3).

No sample exhibited cytotoxicity at the concentration of 1000 mol ratio/TPA. The inhibitory effect of the galactosyl type compounds (**4**, **7** and **8**) was more potent with respect to the corresponding glucosyl ones (**1**, **5** and **6**) (see Table 1) at the dose of 500 mol ratio toward TPA, so the orientation of the 4-hydroxyl in the sugar moiety plays a role in eliciting the activity.

Also the site of attachment of the sugar to the glycerol moiety affects the anti-tumor-promoting activity: in both the galacto- and gluco-series the 1-*O*-β derivatives (**5** and **7**) showed a more potent effect than the corresponding 3-*O*-β ones (**6** and **8**) which were in turn more active than their 2-*O*-isomers (**1** and **4**). The introduction of an acetyl group on one of the diastereotopic hydroxymethyl groups of **1** (compounds **2** and **3**, Table1) only slightly enhances its low activity.

At last, the role of the glycerol moiety was investigated in order to ascertain if this subunit was really essential for the inhibitory activity or this effect was merely due to the sugar. To this purpose methyl β-D-glucopyranoside (**9**) and methyl β-D-galactopyranoside (**10**) were tested; Table 1 shows that **10** was more potent than **9**, confirming that the galacto-subunit is once more more potent than the gluco- one in the compounds we tested. The glycosylglycerols **1-8** also completely inhibited Epstein-Barr virus associated early antigen (EBV-EA) activation at the concentration of 1000 mol ratio per TPA; conversely the corresponding methylglycosides **9** and **10** showed a reduced inhibitory effect at the same concentration. So there is only a weak tendency to inhibition in the *O*-methyl derivatives.

In conclusion this study shows that the glycerol moiety is important for the inhibitory activity, since the substitution of the glyceryl with a methyl group greatly reduces it. Moreover the galacto-subunit gives a more pronounced activity to the compound. The evaluation of anti-tumor-promoting activity of the glycosylglycerols we tested, combined with good viability (70-80%) and low toxicity, has shown that 1-*O*-β-D-galactopyranosyl-*sn*-glycerol (**7**) exhibits the strongest activity against the tumor promoting stage of EBV-EA, even higher than that of its diastereoisomer **8**, which displays the basic skeleton of naturally occurring monogalactosyldiacylglycerols.[18]

Detailed investigations of the pharmacological activities *in vivo* are now in progress.

Acknowledgement This study was supported in part by Ministero dell'Università e della Ricerca Scientifica e Tecnologica and Consiglio Nazionale delle Ricerche (Rome, Italy) and in part by Grants-in-Aid from the Ministry of Education, Science and Culture, and Ministry of Health and Welfare for the Second Term Comprehensive 10-Year Strategy for Cancer Control, Japan.

REFERENCES AND NOTES

1. Curatolo, W. *Biochim. Biophys. Acta* **1987**, *906*, 111-136.
2. Koynova, R. D.; Tenchov, B. G.; Kuttenreich, H.; Hinz, H. J. *Biochemistry* **1993**, *32*, 12437-12445.
3. Curatolo, W. *Biochim. Biophys. Acta* **1987**, *906*, 137-160.
4. Kikuchi, H.; Tsukitani, Y.; Manda, T.; Fujii, T.; Nakanishi, H.; Kobayashi, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1982**, *30*, 3544-3547.
5. Murakami, N.; Imanura, H.; Sakakibara, J.; Yamada, N. *Chem. Pharm. Bull.* **1990**, *38*, 3497-3499.
6. Shirahashi, H.; Murakami, N.; Watanabe, M.; Nagatsu, A.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A. *Chem. Pharm. Bull.* **1993**, *41*, 1664-1666.
7. Murakami, A.; Nakamura, Y.; Koshimizu, K.; Ohigashi, H. *J. Agric. Food Chem.* **1995**, *43*, 2779-2783.

8. Morimoto, T.; Nagatsu, A.; Murakami, N.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A. *Phytochemistry* **1995**, *40*, 1433-1437.
9. Hennings, H.; Glick, A. B.; Greenhalgh, D. A.; Morgan, D. L.; Strickland, J. E.; Tennenbaum, T.; Yuspa, S. H. *Proc. Soc. Exp. Biol. Med.* **1993**, *202*, 1-18.
10. Greenwald, P.; Nixon, D. W.; Malone, W. F.; Kelloff, G. J.; Stern, H. R.; Witkin, K. M. *Cancer* **1990**, *65*, 1483-1490.
11. Wattenberg, L. W. *Cancer Res.* **1993**, *53*, 5890-5896.
12. 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*, 1619-1622.
13. The synthesis of **1**,[19] **2**,[20] and **3**[20] has been performed according to known procedures; the synthesis of **4**[21] has been carried out as described for **1**;[15] **5**, **6**, **7** and **8** were prepared by conventional deprotection of the corresponding tetra- or hexaacetates;[21] **9** and **10** were purchased from Fluka.
14. The physicochemical properties of **1**,[22] **2**,[22] **3**,[22] **5**, [23] **6**,[24]and **8**[5] were consistent with the literature; **4**:[25] mp 118-120 °C (from ethanol), $[\alpha]_D$ -5.0° (c 1.0, H_2O); **7**: oil, $[\alpha]_D$ +1.5° (c 1.0, H_2O).
15. Tokuda, H.; Konoshima, T.; Kozuka, M.; Kimura, T. *Cancer Lett.* **1988**, *40*, 309-317.
16. In mixture with 15% of its diastereoisomer **3**.
17. In mixture with 4% of its diastereoisomer **6**.
18. It is worthy of note that compounds **1**, **4-8** showed only moderate antibacterial and antifungal *in vitro* activity (Marinone Albini, F.; Albini, E. personal communication).
19 Marinone Albini, F.; Murelli, C.; Patritti, G.; Rovati, M. *Synth. Commun.* **1994**, *24*, 1651-1661.
20. Colombo, D.; Ronchetti, F.; Scala, A.; Taino, I. M.; Marinone Albini, F.; Toma, L. *Tetrahedron: Asymmetry* **1994**, *5*, 1377-1384.
21. Colombo, D.; Ronchetti, F.; Scala, A.; Taino, I. M.; Marinone Albini, F.; Toma, L. *Tetrahedron Lett.* **1995**, *36*, 4865-4868;
22. Kaneda, M. *Phytochemistry* **1990**, *29*, 3559-3564.
23. Kaneda, M.; Kobayashi, K.; Nishida, K.; Katsuta, S. *Phytochemistry* **1984**, *23*, 795-798.
24. Kaneda, M.; Mizutani, K.; Tanaka, K. *Phytochemistry* **1982**, *21*, 891-893.
25. Nakano, H.; Takenishi, S.; Watanabe, Y. *Agric. Biol. Chem.* **1988**, *8*, 1913-1921.

(*Received in Belgium* 16 *February* 1996; *accepted* 22 *April* 1996)