Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3323–3326
Bioorganic & Medicinal Chemistry Letters

Synthesis of analogues of a potent antitumor saponin OSW-1

Jacek W. Morzycki,[a,*] Agnieszka Wojtkielewicz[a] and Sławomir Wołczyński[b]

[a]*Institute of Chemistry, University of Bialystok, al. Pilsudskiego 11/4, 15-443 Bialystok, Poland*
[b]*Department of Gynecological Endocrinology, Medical University of Bialystok, Kilinskiego 1, 15-230 Bialystok, Poland*

Received 3 December 2003; revised 8 March 2004; accepted 19 March 2004

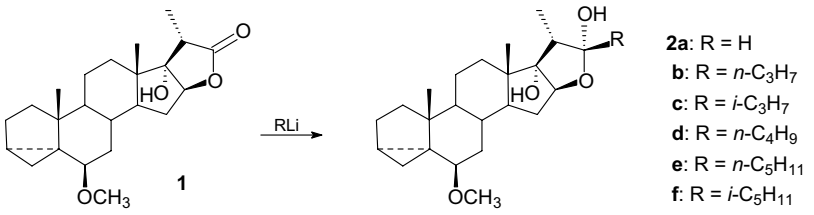
Abstract—A series of side chain analogues (**5a**–**e**), a 22-glycosylated isomer (**10**), and 16β-*O*-L-arabinosyl (**13a**) or 16β-*O*-D-xylosyl (**13b**) analogues of OSW-1 were synthesized. All analogues were found to be less cytotoxic against breast and endometrial cancer cell lines than the natural product.

OSW-1 belongs to a family of saponins isolated 11 years ago by Sashida et al. from the bulbs of *Ornithogalum saundersiae*.[1] The saponins appeared to be strongly cytotoxic against a broad spectrum of malignant tumor cells, such as leukemia HL-60, mouse mastrocarcinoma, human pulmonary adenocarcinoma, large cell carcinoma, and squamous cell carcinoma including adriamycin-resistant and camptothecin-resistant cell lines with IC_{50} between 0.1 and 0.7 nM for the most active OSW-1 (**5f**).[2] This extraordinary cytotoxicity of OSW-1 encouraged several research groups to undertake efforts of its synthesis.[3] The first was Fuchs group which synthesized OSW-1 aglycone, but it proved to be biologically inactive.[4] Fuchs speculated that the 22-oxocarbenium ions might be the active intermediate for the anticancer activity of both OSW-1 and cephalostatins.[5] Then Yu and Hui described complete synthesis of OSW-1.[6] The Chinese chemists employed essentially the same method for the OSW-1 aglycone, but for the first time described synthesis of a sugar part and a glycosylation procedure. A different approach to the synthesis of both parts of OSW-1, which were coupled by the same trichloroacetimidate method, was reported by the American chemists Yu and Jin.[7] A new strategy for synthesizing the aglycone of OSW-1 by using the intact skeleton of diosgenin was recently reported by Tian et al.[8] One more synthesis of OSW-1 was elaborated in our laboratory.[9] Yu and Hui also proved that both parts of OSW-1, the aglycone and the sugar part, are equally important for the biological activity. Several analogues bearing the disaccharide moiety of OSW-1 attached to nonsteroidal aglycones and steroid glycosides with the sugar part at various positions (other than 16β) were prepared and tested for cytotoxicity.[10] All of them were many times less active than OSW-1. Also any alteration in the sugar part, for example, removal of the ester groups on the disaccharide moiety or change of linkage of L-arabinopyranose and D-xylopyranose derivatives (into $1\rightarrow4$), resulted in dramatic decrease of activity. It seems that an α glycoside bond between 16β-hydroxysteroid and acylated sugar moieties is a pharmacophore requirement.

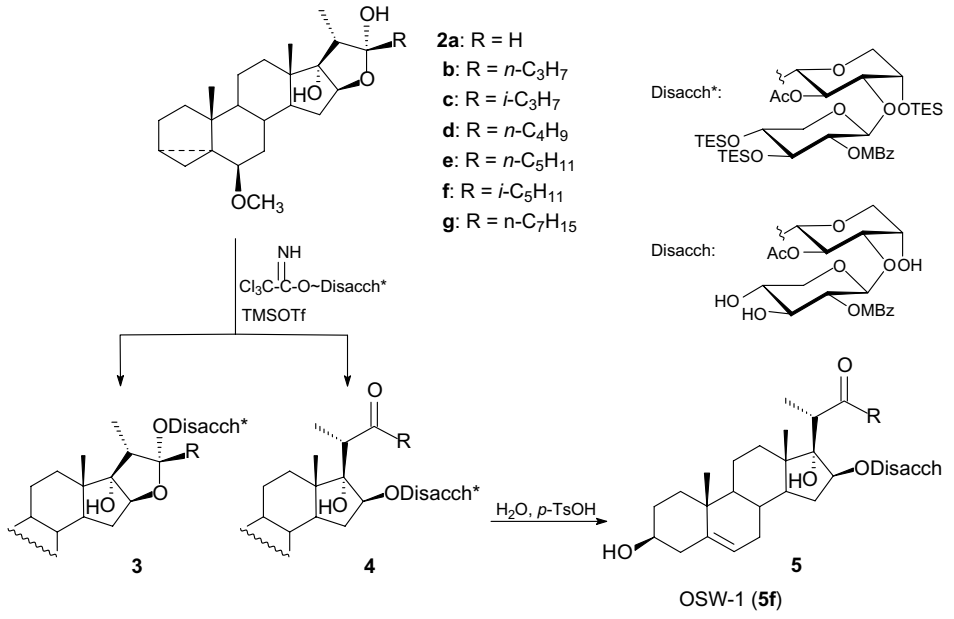
For further study of a structure–activity relationship analogues with structures more close to OSW-1 were designed, among them compounds with different size of a side chain. The method for their synthesis was direct glycosylation of a steroid aglycone in its cyclic form. The required steroid aglycones were prepared by addition of alkyllithium to the easily available[11] hydroxylactone **1** (Scheme 1). Five aglycones (**2a**–**f**) were obtained with 3 (using DIBAL-H), 6 (linear and branched), 7, 8, or 10 carbon atom side chain. Each of the aglycones was coupled with the OSW-1 disaccharide. An alternative synthesis of the side chain analogues of OSW-1 consisting of addition of alkyllithium to the 16β-glycoside 22-aldehyde (compound **4a** in Scheme 2) failed due to the undesired retro-aldol reaction leading to a formation of 17-ketone, a product devoid of side chain. All aglycones existed in a cyclic hemiketal form. The products were stereochemically pure, but configuration of the new stereogenic center at C-22 could not be elucidated from the spectra. Presumably, the thermodynamically favored 22*R* isomers

* Corresponding author. Tel.: +48-85-745-76-04; fax: +48-85-745-75-81; e-mail: morzycki@uwb.edu.pl

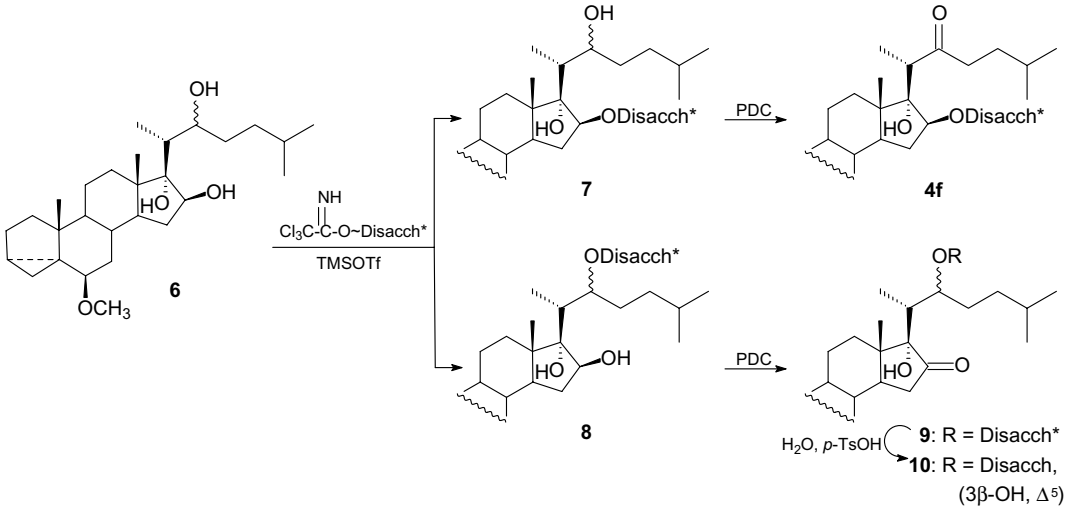
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.03.102



Scheme 1.

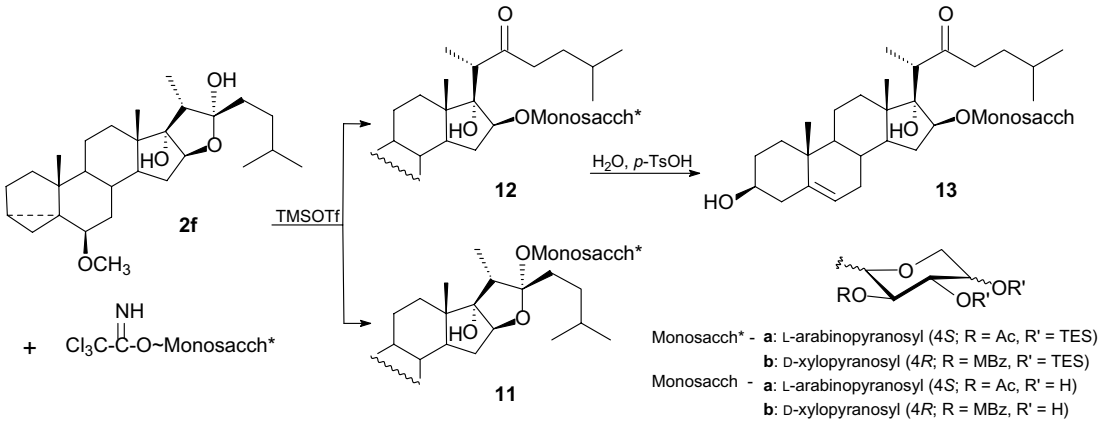


Scheme 2.



Scheme 3.

were formed. Glycosylation reactions were carried out with the disaccharide trichloroacetimidate under the standard conditions (TMSOTf was used as a promoter).[12] The conversion was about 20% and usually two types of products were formed (Scheme 2), 22-*O*-glycosides (**3**) and 16β-*O*-glycosides (**4**). The configuration of glycosides at the anomeric position was found to be α. The ratio of products depended strongly on size of a side chain. In the case of compound **2b** with a short side chain the only product was 16β-*O*-glycoside, whereas the reaction of aglycone with the longest side chain (compound **2g**) afforded exclusively 22-*O*-glycoside.



Scheme 4.

Table 1. Growth inhibition ratio (%) and IC_{50} for the cancer cell necrosis

Compound conc. (mol/L)	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
OSW-1 (**5f**)					
MCF-7	87%	100	100	100	100
MDA–MB-231	100	100	100	100	100
Ishikawa cells 24 h	95%	100	100	100	100
*Compound **5b***					
MCF-7	0	0	0	5	12
MDA–MB-231	0	0	0	7	15
Ishikawa cells 24 h	0	0	0	10	18
*Compound **5d***					
MCF-7	0	0	0	15	27
MDA–MB-231	0	0	0	12	23
Ishikawa cells 24 h	0	0	0	17	28
*Compound **5e***					
MCF-7	0	0	0	10	18
MDA–MB-231	0	0	0	14	26
Ishikawa cells 24 h	0	0	0	12	17

OSW-1 (**5f**)	MCF-7	MDA–MB-231	Ishikawa cells 24 h
IC_{50} for necrosis	7×10^{-6}	5×10^{-7}	3×10^{-6}

Other glycosylation reactions afforded both products, which were relatively easy to separate by flash chromatography and to distinguish by spectroscopic methods. The most characteristic was signal of a 20-H proton (q) deshielded by a neighboring carbonyl group to $\delta \sim 3.1$ ppm in the ^{1}H NMR spectra of compounds **4** (in **3** it appeared at ~2.4 ppm). The signal of arabinopyranose anomeric proton (~4.8 ppm in **4**) was shifted to $\delta \sim 5.4$ ppm in compounds **3**. Also characteristic was shape of a 16α-H proton at ~4.2 ppm (t, $J = 7.5$ Hz) in compounds **3**. Acetate protons appeared at $\delta \sim 2.0$ ppm in 16β-*O*-glycosides (**4**), whereas at ~1.7 ppm in the 22-*O*-glycosylated isomers (**3**). An obvious difference in the ^{13}C NMR spectra was observed in the chemical shift of C-22 ($\delta \sim 218$ ppm for **4** and ~115 ppm for **3**).

A final step of saponin synthesis (compounds **5**) was simultaneous deprotection of functional groups of both steroid (cycloreversion) and sugar (desilylation) moieties in 16β-*O*-glycosides. It was easily achieved by treatment with *p*-TsOH in dioxane-aqueous solution at 80 °C. All novel compounds **5a**–**g** (except for earlier prepared OSW-1 **5f**) were fully characterized (selected spectral and analytical data for **5e** are given in Ref. 13) and subjected to biological evaluation.

A synthesis of OSW-1 isomer with a sugar moiety attached to oxygen atom at C-22 was also attempted (Scheme 3). The OSW-1 aglycone (**2f**) was reduced with lithium aluminum hydride to afford 16β,17α,22ξ-triol **6**, which was glycosylated with 1 equiv of the disaccharide trichloroacetimidate. A regioselective glycosylation at O-22 was expected on the basis of the previous study on benzylation of this compound.[9] However, the reaction was not regioselective and glycosylation occurred equally at both secondary positions (O-16 and O-22). The products **7** and **8** were separated and oxidized with pyridinium dichromate to the corresponding ketones. One of them appeared to be identical in all

respects with the previously obtained protected saponin OSW-1 (**4f**). Interestingly, the 22-*O*-glycoside **9** proved to be stereochemically pure compound, though the starting triol **6** was a mixture of epimers at C-22. Probably, an opposite epimer reacted faster at O-16. However, the configuration at C-22 in the isolated product could not be concluded from its spectra. The protecting groups were removed in the same way as described above and the novel OSW-1 analogue **10** was obtained.[13]

The aglycone of OSW-1 in its hemiketal form (**2f**) was also treated with the monosaccharide (L-arabinopyranose and D-xylopyranose derivatives) trichloroacetimidates (Scheme 4). The conversions were slightly higher (about 30%) than in the previous glycosylation reactions. Again a mixture of 22-*O*- (**11**) and 16β-*O*-glycosylated (**12**) products was formed in almost equal amounts. The former products were subjected to deprotection in a usual manner to afford new analogues **13a** and **13b**.[13]

All described analogues were tested for cytotoxicity against two breast cancer cell lines (MCF-7 and MDA–MB-231) and endometrial cancer Ishikawa cell line. OSW-1 (**5f**) influenced significantly [^{3}H]thymidine incorporation, cell viability, and growth. The saponin induced necrosis of the cells without apoptosis. The analogues with linear side chain **5b**, **5d**, and **5e** showed much lower cytotoxicity that the saponin OSW-1. Other analogues were not biologically active. Table 1 shows the results of the growth inhibition tests and IC_{50} values determined for the necrosis induced by OSW-1 in three lines of cancer. IC_{50}'s for the analogues (**5b**, **5d**, **5e**) were more than 1000 times higher than these determined for OSW-1.

Acknowledgements

This work was supported by the State Committee for Scientific Research (Grant No. 3 P05F 014 23). One of the authors (A.W.) thanks Foundation for Polish Science for a domestic grant for young scholars.

References and notes

1. (a) Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969; (b) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633; (c) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y.; Beutler, J. A. *J. Nat. Prod.* **2001**, *64*, 88; (d) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y. *Chem. Pharm. Bull.* **2001**, *49*, 1042.
2. (a) Rouhi, A. M. *Chem. Eng. News* **1995** (September 11), 28; (b) *Advances in Experimental Medicine and Biology; Saponins Used in Traditional and Modern Medicine*; Waller, G. R., Yamasaki, K., Eds.; Plenum: New York, 1996; Vol. 404.
3. Gryszkiewicz-Wojtkielewicz, A.; Jastrzebska, I.; Morzycki, J. W.; Romanowska, D. B. *Curr. Org. Chem.* **2003**, *7*, 1257.
4. Guo, C.; Fuchs, P. L. *Tetrahedron Lett.* **1998**, *39*, 1099.
5. Guo, C.; LaCour, T. G.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 419.
6. Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202.
7. Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576.
8. Xu, Q.; Peng, X.; Tian, W. *Tetrahedron Lett.* **2003**, *44*, 9375.
9. Morzycki, J. W.; Wojtkielewicz, A. *Carbohydr. Res.* **2002**, *337*, 1269.
10. (a) Ma, X.; Yu, B.; Hui, Y.; Xiao, D.; Ding, J. *Carbohydr. Res.* **2000**, *329*, 495; (b) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Carbohydr. Res.* **2001**, *334*, 159; (c) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2153.
11. (a) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzebska, I. *Tetrahedron* **2001**, *57*, 2185; (b) Morzycki, J. W.; Gryszkiewicz, A. *Polish J. Chem.* **2001**, *75*, 983.
12. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353.
13. Selected analytical data for compounds **5e**, **10**, and **13a**. **5e**: amorphous solid; IR($CHCl_3$): 3453, 1728, 1692, 1606, 1512, 1259, 1170, 1033; ^{1}H NMR (200 MHz): 8.09 (d, $J = 8.9$, 2H), 6.98 (d, $J = 8.9$, 2H), 5.35 (m, 1H), 4.94 (dd, $J = 8.0$, 7.2, 1H), 4.69 (m, 2H), 4.22 (brs, 1H), 4.17 (m, 1H), 4.15 (m, 1H), 3.88 (s, 3H), 3.75 (m, 1H), 3.66–3.73 (m, 2H), 3.40–3.55 (m, 3H), 2.65 (q, $J = 7.4$, 1H), 1.95 (s, 3H), 1.03 (s, 3H), 1.01 (d, $J = 7.4$, 3H), 0.86 (m, 3H), 0.80 (s, 3H); ^{13}C NMR (50 MHz): 218.8 (C), 169.4 (C), 166.0 (C), 164.2 (C), 140.6 (C), 132.2 (2×CH), 121.4 (CH), 121.3 (C), 114.0 (2×CH), 102.2 (CH), 99.1 (CH), 88.5 (CH), 85.6 (C), 80.0 (CH); ESI-MS: 895.5 (MNa^+); HR-MS calcd for $C_{47}H_{68}O_{15}Na$ (MNa^+): 895.4456; found: 895.4472. **10**: IR($CHCl_3$): 3461, 1736, 1606, 1512, 1170, 1259, 1081; ^{1}H NMR (200 MHz): 7.95 (d, $J = 8.7$, 2H), 6.90 (d, $J = 8.7$, 2H), 5.34 (m, 1H), 4.97 (m, 3H), 4.64 (d, $J = 6.4$, 1H), 4.52 (d, $J = 6.8$, 1H), 3.96–4.04 (m, 2H), 3.94 (brs, 1H), 3.86 (s, 3H), 3.70–3.82 (m, 4H), 3.32–3.58 (m, 3H), 1.89 (s, 3H), 1.01 (s, 3H), 0.93 (d, $J = 7.0$, 3H), 0.86 (d, $J = 6.2$, 6H), 0.75 (s, 3H); ^{13}C NMR (50 MHz): 218.8 (C), 169.7 (C), 166.3 (C), 163.9 (C), 141.0 (C), 132.2 (2×CH), 121.4 (C), 121.0 (CH), 113.8 (2×CH), 101.9 (CH), 100.6 (CH), 82.9 (C), 82.8 (CH), 80.7 (CH); ESI-MS: 895.4 (MNa^+); HR-MS calcd for $C_{47}H_{68}O_{15}Na$ (MNa^+): 895.4456; found: 895.4469. **13a**: mp 199–202 °C; IR ($CHCl_3$): 3489, 1741, 1692, 1234, 1053. ^{1}H NMR (200 MHz): 5.33 (d, $J = 4.6$, 1H), 4.73 (dd, $J = 6.2$, 4.3, 1H), 4.34 (m, 2H), 3.74–3.96 (m, 4H), 3.58 (dd, $J = 11.6$, 3.0, 1H), 3.54 (m, 1H), 3.24 (d, $J = 7.6$, 1H), 3.01 (q, $J = 7.3$, 1H), 2.14 (s, 3H), 1.23 (d, $J = 7.3$, 3H), 1.00 (s, 3H), 0.90 (d, $J = 6.2$, 6H), 0.82 (s, 3H); ^{13}C NMR (50 MHz): 218.7 (C), 170.7 (C), 140.7 (C), 121.3 (CH), 99.5 (CH), 89.9 (CH), 85.4 (C); ESI-MS: 629.4 (MNa^+); Anal. calcd for $C_{34}H_{54}O_9$: C 67.30, H 8.97; found: C 67.17, H 9.02.