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Synthesis of Aglycon Analogues of Sarmentosin and Their Bioactivity of Lymphocyte Proliferation

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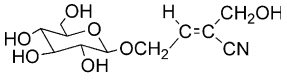
Abstract—A number of aglycon analogues of sarmentosin were prepared and their bioactivities on the proliferation of T-lymphocytes and B-lymphocytes were assayed.

Sarmentosin (**1**), a cyano-ethylene glucoside isolated from the folk medicine *Sedum sarmetosum* Bunge, is an active principle for treatment of hepatitis B in China.[1,2] Clinical trials of sarmentosin showed a good effect in lowering serum glutamate-pyruvate transaminase.[3] The content of sarmentosin in plant varies greatly with the growing region and the collecting season. It is known that cyanogenic glycosides are readily hydrolyzed in aqueous media, so sarmentosin is chemically rather unstable. Our group accomplished the first total synthesis of sarmentosin,[4] and is interested in searching new potential analogues for treatment of hepatitis B. In this paper, we would like to report the preparation and bioassay of eight analogues of sarmentosin aglycon.



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We supposed that the aglycon, an unsaturated cyano-containing moiety of sarmentosin was the pharmacophoric group, and the simplified analogues were thus designed, in which the acetal group in sarmentosin was changed into more stable ether and ester groups. Therefore, β-D-glucopyranosyl in sarmentosin was replaced by aryl (**5**, **6**), alkyl (**14**) and acyl (**7**, **8**, **15**, **16**, **17**), respectively. The synthetic approach to these analogues is depicted in Scheme 1. The starting material butane-1,2,4-triol-1,2-acetonide (**2**) was converted into iodide **3**, which was treated with phenols in the presence of potassium carbonate to afford aryl ethers **4a**. Esters **4b** were obtained by the reaction of **2** with aromatic acids in the presence of DCC and DMAP. Subsequently, analogues **5**, **6**, **7** and **8** were obtained from ethers **4a** or esters **4b** via a reaction sequence similar to the construction of aglycon in the synthesis of sarmentosin itself[4,7] (Scheme 1).

Meantime, a more convenient and convergent route to the aglycon analogues of sarmentosin has been developed too (Scheme 2). The protected aglycon **12** was first synthesized as the common intermediate, then it was esterified respectively with various acids to yield ester analogues **15**, **16** and **17**. For preparation of intermediate **12**, the starting material **2** was first protected as *p*-methoxy benzyl(PMB) ether **9**. Hydrolysis of acetonide **9** and the selective protection of the primary alcohol with *t*-butyldiphenylsilyl chloride (TPSCl) afforded **10**, which was oxidized by Dess–Martin method to give ketone **11**. Ketone **11** was further converted into the corresponding cyanohydrin by treatment with acetone cyanohydrin in the presence of Et_3N followed by dehydration with thionyl chloride in pyridine, giving the desired E-olefin, the protected aglycon **12** as the only product. Selective cleavage of PMB group in **12** by DDQ afforded alcohol **13**. Esterification of alcohol **13** with the corresponding acids and removal of TPS group of the esters with TBAF furnished analogues **15**, **16** and **17**, respectively.[7] This approach proved to be more facile for peparation of various analogues of

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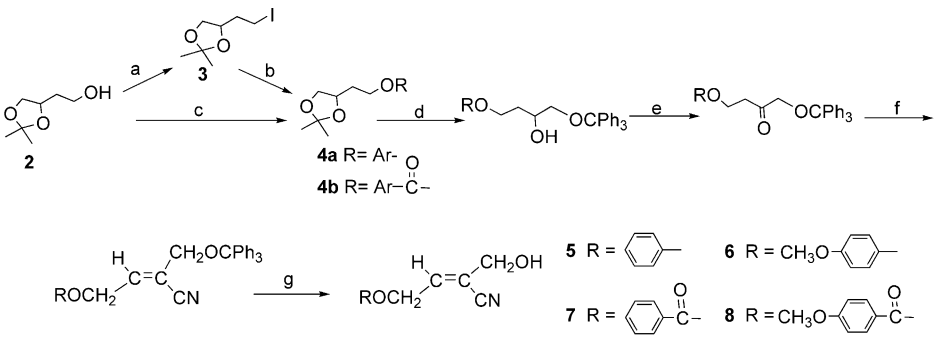
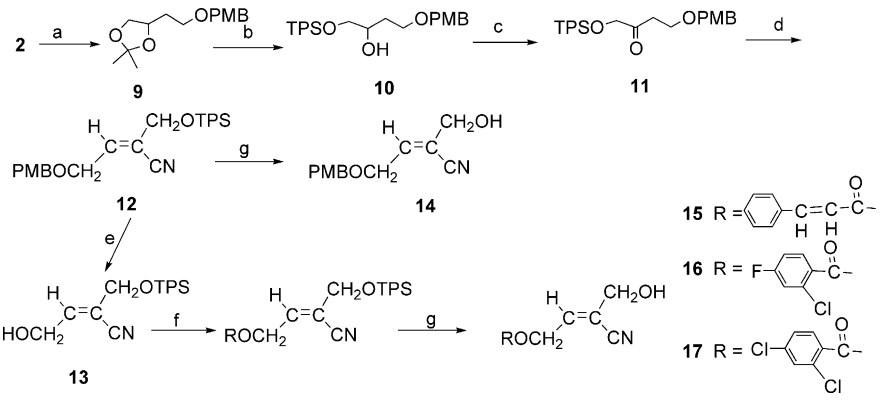


Scheme 1. Reagents and conditions: (a) (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (ii) NaI, K_2CO_3; (b) ArOH, K_2CO_3, acetone; (c) RCOOH, DCC, DMAP, CH_2Cl_2; (d) (i) *p*-TsOH, MeOH; (ii) Ph_3CCl, Et_3N, CH_2Cl_2; (e) PCC, NaOAc, CH_2Cl_2; (f) (i) acetone cyanohydrin, $NaHCO_3$, MeOH; (ii) $SOCl_2$, pyridine; (g) *p*-TsOH·H_2O, CH_3OH.



Scheme 2. Reagents and conditions: (a) PMBOC(=NH)CCl_3, CSA(cat.), CH_2Cl_2, 91%; (b) (i) TsOH·H_2O, MeOH; (ii) TPSCl, imidazole, CH_2Cl_2, 87%; (c) Dess–Martin periodinane, pyridine, CH_2Cl_2, 89%; (d) (i) acetone cyanohydrin, Et_3N, MeOH; (ii) $SOCl_2$, pyridine, 32%; (e) DDQ, CH_3Cl, H_2O, 90%; (f) RCOOH, DCC, DMAP, toluene, 88%; (g) TBAF, HOAc, THF, 82%.

Table 1. Effects of sarmentosin aglycon analogues on the proliferation of T and B lymphocytes[a]

Compd	T cell proliferation (%) μmol/L			B cell proliferation (%) μmol/L		
	20	2	0.2	20	2	0.2
5	131	113	100	45↓	48↓↓↓	51↓↓↓
6	80	114	101	79	77	81
7	23↓↓↓	68	97	67	73	137
8	25↓↓	63	100	31↓	86	109
14	37↓↓	54↓	93	38↓↓↓	71↓↓	169↑
15	38↓	62↓↓	80	20↓	69	96
16	29↓↓	98	123	113	151↑	151
17	131	94	119↑	73	69	68

[a]Compared to DMSO as control (%): ↓deceased $P<0.05$; ↓↓$P<0.01$; ↓↓↓$P<0.001$. Values are means of three experiments.

aglycon and sarmentosin itself as well. Analogue **14** was directly yielded by selective cleavage of TPS group in **12**.[7]

It was reported that sarmentosin showed good and rapid effects in lowering serum transaminase levels and improved liver function by an immunomodulating mechanism.[3] The analogues mentioned above were tested for their activities on the proliferation of T lymphocytes to show the cell-mediated immunity, and B lymphocytes to show the antibody formation ability by ^{3}H-thymidine incorporation test of mouse splenocytes in vitro. A brief description of the proliferation test is provided below.[5,6] The results of the proliferation test are listed in Table 1.

The results indicate that within the concentration range tested, **14** and **15** showed the distinct suppressive effect on T cell proliferation while **5** showed the most significant effect on B-lymphocytes. Further pharmacological studies are under way.

Acknowledgements

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References and Notes

1. Fang, S. D.; Yan, X. Q.; Li, J. F.; Fan, Z. Y.; Xu, X. Y.; Xu, R. S. *Chinese Science Bulletin* **1979**, *24*, 431.
2. Fang, S. D.; Yan, X. Q.; Li, J. F.; Fan, Z. Y.; Xu, X. Y.; Xu, R. S. *Acta Chim. Sin.* **1982**, *40*, 273.
3. Zhai, S. K.; Shen, M. L.; Xiong, Y. L.; Li, J. F.; Ding, Y. E.; Gao, Y. S. *Chin. J. Microbiol. Immun.* **1982**, *2*, 145.
4. Chu, G.; Zhou, Q.; Bai, D. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1343.

5. Coligan, J. E., Kruisbeek, A. M., Margulies, D. M., Shevach, E. M., Strober, W., Eds. *Current Protocols in Immunology*. Greene Publishing Associates & Wiley-Interscience: New York, 1991.

6. T cell and B cell function assay: Fresh spleen cells were obtained from ICR mice (male, 7–9 weeks old). 5×10^5 of spleen cells were cultured in 96-well flat plates with 200 μL of RPMI 1640 media containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified, 37 °C, 5% CO_2 incubator for 48 h. The cultures were either unstimulated or stimulated with 5 μg/mL of concanavalin A (ConA) or 10 μg/mL of lipopolysaccharide (LPS) to induce T cells or B cells proliferative responses respectively. The compounds were added in cultures with indicated concentrations to test their bioactivities. Proliferation was assessed in terms of uptake of [^{3}H]-thymidine during 6–12 h pulsing with 20 kBq [^{3}H]-thymidine/well, then the cells were harvested by a Basic 96 harvester, and counted in a 1540 MicroBeta Trilux (PerkinElmer Life Sciences).

7. Analytical data: Compound **5**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (t, $J=7.4$ Hz, 2H), 7.00 (t, $J=7.4$ Hz, 1H), 6.92 (d, $J=8.5$ Hz, 2H), 6.72 (t, $J=6.0$ Hz, 1H), 4.85 (d, $J=6.0$ Hz, 2H), 4.30 (d, $J=1.4$ Hz, 2H); 3425, 2223, 1587, 1494, 1238, 1033, 756, 692; HRMS (*m*/*z*): calcd for $C_{11}H_{11}NO_2$: 189.0789, found 189.0776; **6**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.83 (s, 4H), 6.66 (t, $J=6.0$ Hz, 1H), 4.77 (d, $J=6.0$ Hz, 2H), 4.26 (d, $J=2.9$ Hz, 2H), 3.75 (s, 3H); IR (film, cm^{-1}) 3442, 2925, 2223, 1508, 1228, 1035, 825; HRMS (*m*/*z*): calcd for $C_{12}H_{13}NO_3$: 219.0896, found 219.0907; **7**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06 (dd, $J=7.3$, 1.4 Hz, 2H), 7.59 (m, 1H), 7.45 (dd, $J=7.6$, 7.3 Hz, 2H), 6.68 (m, 1H), 5.13 (dt, $J=6.4$, 1.4 Hz, 2H), 4.32 (d, $J=2.8$ Hz, 2H); IR (film, cm^{-1}) 3456, 2225, 1722, 1600, 1452, 1272, 1116, 713; HRMS (*m*/*z*): calcd for $C_{12}H_{11}NO_3$: 217.0739, found 217.0747; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, $J=6.8$ Hz, 2H), 6.91 (d, $J=7.0$ Hz, 2H), 6.65 (tt, $J=6.3$, 1.6 Hz, 1H), 5.08 (dt, $J=6.3$, 1.2 Hz, 2H), 4.30 (d, $J=1.2$ Hz, 2H), 3.85 (s, 3H); IR (film, cm^{-1}) 3470, 2223, 1716, 1606, 1511, 1259, 1170, 1105, 1027, 769; HRMS (*m*/*z*): calcd for $C_{13}H_{13}NO_4$: 274.0844, found 274.0844; **14**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (m, 2H), 6.90 (dd, $J=6.6$, 2.1 Hz, 2H), 6.58 (tt, $J=6.4$, 1.3 Hz, 1H), 4.50 (s, 2H), 4.32 (d, $J=6.4$ Hz, 2H), 4.24 (d, $J=2.8$ Hz, 2H), 3.82 (s, 3H); IR (film, cm^{-1}) 3430, 2221, 1612, 1513, 1249, 1176, 1033, 821; HRMS (*m*/*z*): calcd for $C_{13}H_{15}NO_3$: 233.1052, found 233.1050; **15**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.73 (d, $J=16.0$ Hz, 1H), 7.51 (m, 2H), 7.39 (m, 3H), 6.60 (tt, $J=6.3$, 1.6 Hz, 1H), 6.43 (d, $J=16.0$ Hz, 1H), 4.98 (d, $J=6.3$ Hz, 2H), 4.29 (d, $J=2.9$ Hz, 2H); IR (film, cm^{-1}) 3446, 2223, 1714, 1635, 1450, 1311, 1166, 981, 769, 684: calcd for $C_{14}H_{13}NO_3$: 243.0895, found 243.0891; **16**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95 (m, 1H), 7.20 (m, 1H), 7.03 (m, 1H), 6.65 (tt, $J=6.4$, 1.7 Hz, 1H), 5.10 (dt, $J=6.4$, 1.3 Hz, 2H), 4.30 (d, $J=1.6$ Hz, 2H); IR (film, cm^{-1}) 3461, 2225, 1731, 1602, 1490, 1251, 1114, 1043, 910, 769; HRMS (*m*/*z*): calcd for $C_{12}H_9NFClO_3$: 269.0255, found 269.0264; **17**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.84 (d, $J=8.4$ Hz, 1H), 7.47 (d, $J=1.9$ Hz, 1H), 7.30 (dd, $J=8.4$, 1.9 Hz, 1H), 6.65 (t, $J=6.4$ Hz, 1H), 5.10 (d, $J=6.4$ Hz, 2H), 4.28 (d, $J=1.2$ Hz, 2H); IR (film, cm^{-1}) 3430, 2231, 1708, 1587, 1376, 1286, 1137, 1026, 765; MS (*m*/*z*): 145 (17), 173 (100), 175 (63), 285 (M^+, 26), 287 ($M^+ + 2$, 17).