Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 607–612

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DERIVATIZATION OF SOYASAPOGENOL A AND THEIR HEPATOPROTECTIVE ACTIVITIES

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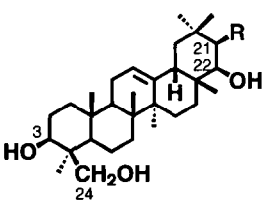
Received 12 December 1997; accepted 2 February 1998

Abstract: Fifteen derivatives of soyasapogenol A (**1**), which is another aglycon moiety of soyasaponins from soybean together with soyasapogenol B (**2**), were prepared and their *in vitro* hepatoprotective effects were evaluated.

Development of new therapeutic medicines for human hepatitis has been awaited because its effective therapy has not yet been established. Recently, we found that soyasapogenol B (**2**),[1] which was obtained from soybean, and its derivatives showed hepatoprotective effect *in vitro* against aflatoxin B_1-induced Hep G2 cells.[2] As our continuing studies on structure-hepatoprotective activity relationship, we have undertaken a screening examination of soyasapogenol A (**1**)[1] derivatives, since only few synthetic study of soyasapogenol A derivatives and their biological activity have hitherto been reported. In this paper, we describe the synthesis of fifteen soyasapogenol A derivatives,[3] in which 3, 21, 22 and 24-hydroxyl groups being regioselectively transformed, as well as the comparison of their *in vitro* hepatoprotective effects.

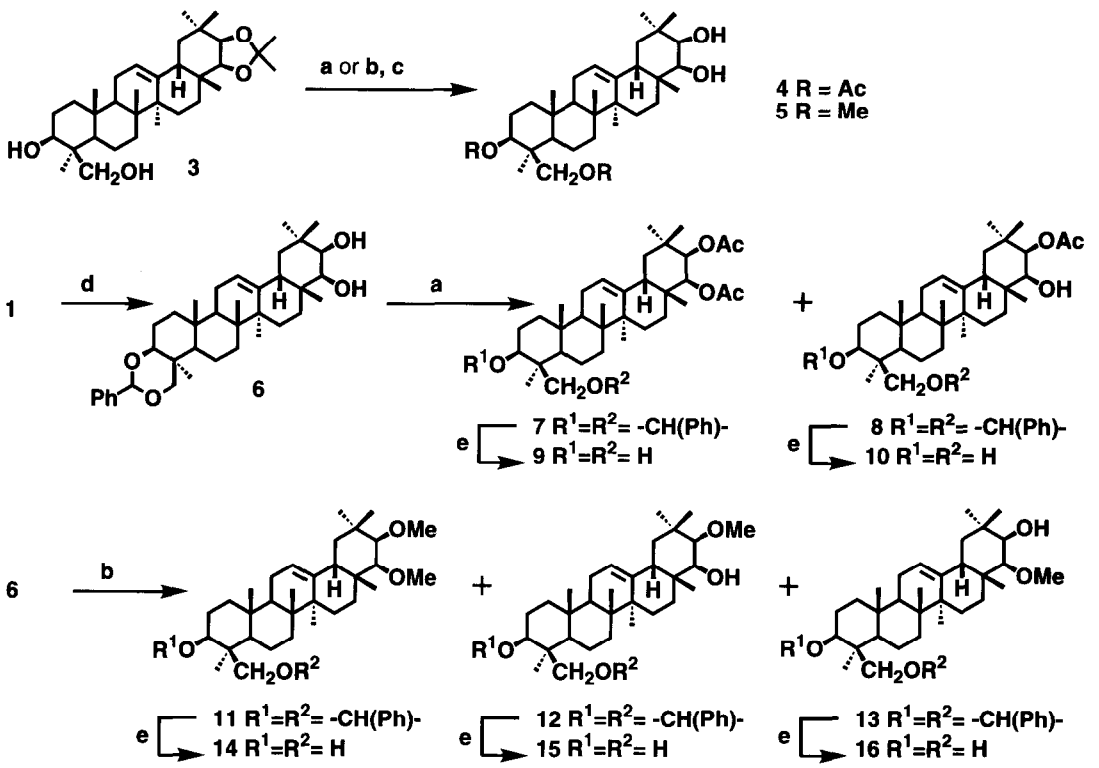


1 R=OH (soyasapogenol A)
2 R=H (soyasapogenol B)

Acetylation and methylation at 3, 21, 22 and/or 24-hydroxyl groups of **1** have been effected by using ketal and acetal intermediates **3**[4] and **6** as shown in Scheme 1. The intermediate **6** was prepared by selective protection of **1** with benzaldehyde dimethyl acetal. Diacetylation at 3, 24-hydroxyl groups of **3** with acetic anhydride followed by deprotection of the acetonide linkage gave the 3, 24-diacetoxy derivative **4**, while treatment of **3** with sodium hydride and methyl iodide provided 3, 24-dimethoxide, which was then deprotected

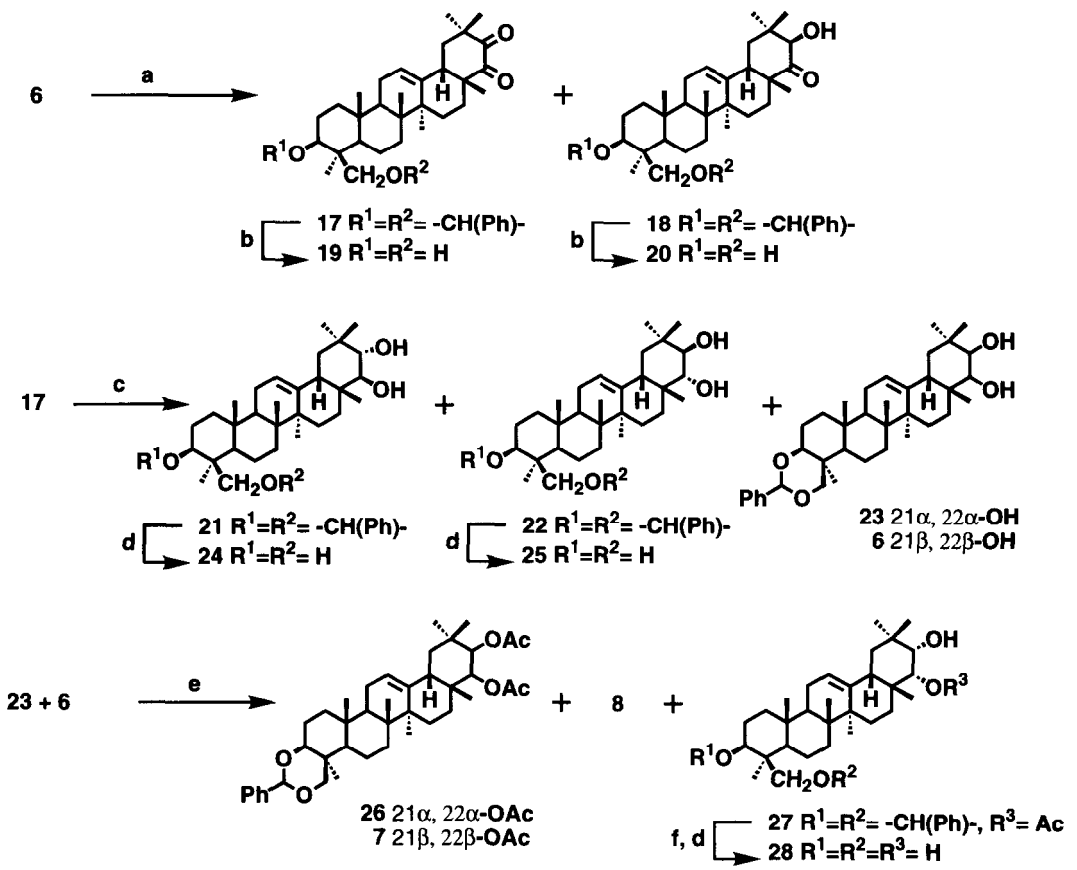
0960-894X/98/$19.00
PII: S0960-894X(98)00077-8

to afford **5**. On the other hand, treatment of **6** with acetic anhydride in pyridine at room temperature for 3 h yielded 21-acetoxy and 21, 22-diacetoxy derivatives (**8** and **7**) in 65 % and 20% yields, respectively. When the reaction was continued for 15 h at room temperature, the diacetate **7** was obtained in 68% yield as a single product. Removal of the benzyliden moieties from **7** and **8** afforded **9** and **10**, respectively. Methylation of **6** gave three products: the 21, 22-dimethoxy derivative **11** (in 28%), the 21-methoxy derivative **12** (in 26%), and the 22-methoxy derivative **13** (in 14% yield). Deprotection of the benzylidene moieties of **11**, **12** and **13** afforded **14**, **15** and **16**, respectively.



Scheme 1: (a) Ac_2O, Py., rt; (b) NaH, CH_3I, THF, rt; (c) 1NHCl, MeOH:CH_2Cl_2 (2:1), rt; (d) $PhCH(OMe)_2$, CSA, DMF, rt; (e) H_2, 10%Pd/C, MeOH:CH_2Cl_2 (1:1), rt.

Next, the 21, 22-diketo derivative and the diastereoisomers of 21, 22-dihydroxy groups have been prepared as shown in Scheme 2. Oxidation of **6** under Swern conditions gave two products: the 21, 22-diketo derivative **17** in 37% yield and the 22-keto derivative **18** in 15% yield. The structure of **18** was confirmed by the X-ray crystallographic analysis[5] as shown in Figure 1. Removal of the protecting groups in **17** and **18** with 1N HCl afforded **19**[6] and **20**,[7] respectively. Reduction of the 21, 22-diketo derivative **17** with lithium aluminum hydride (LAH) gave four diastereoisomers. Chromatographic separation of the isomers gave 21α, 22β-diol **21**, 21β, 22α-diol **22** and a mixture of 21α, 22α-diol **23** and 21β, 22β-diol **6**. Catalytic hydrogenation of **21** and **22** with Pd-C afforded **24** and **25**, respectively. On the other hand, acetylation of the mixture of diols **23** and **6** gave a mixture of four acetates **7**, **8**, **26** and **27**. Compound **27** could be isolated from this mixture by column chromatography. Removal of the protecting groups from **27** gave 21α, 22α-diol **28**.



Scheme 2: (a) Swern oxidation; (b) 1NHCl, MeOH:CH_2Cl_2 (2:1), rt; (c) $LiAlH_4$, THF, 0°C~rt; (d) H_2, 10%Pd/C, MeOH:CH_2Cl_2 (1:1), rt; (e) Ac_2O, Py., rt; (f) DIBAL-H, THF, rt.

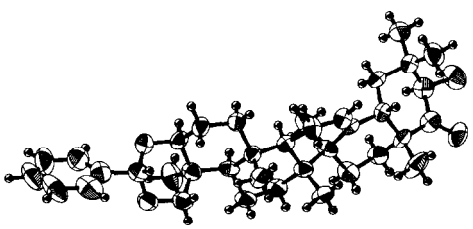
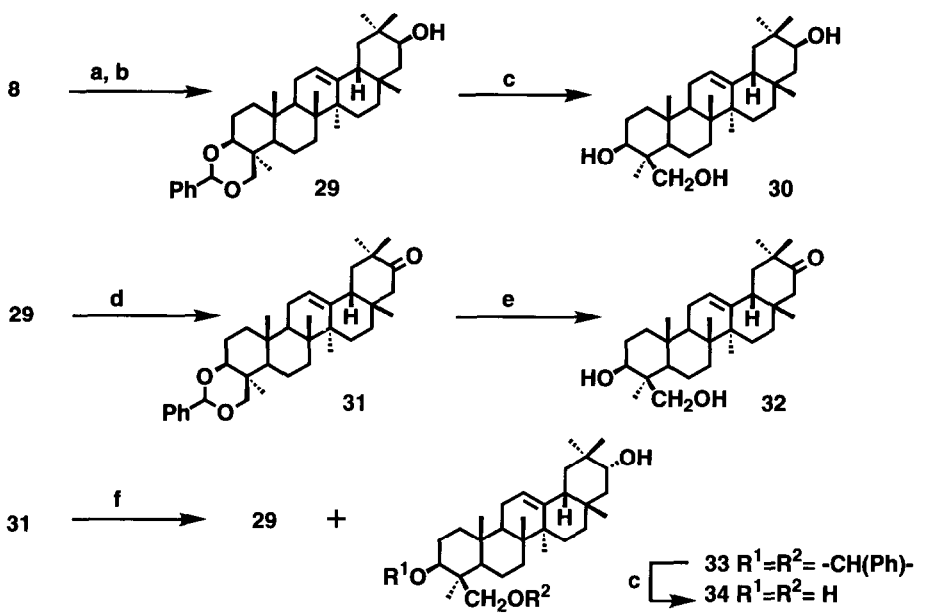


Figure 1. ORTEP drawing of **18**.

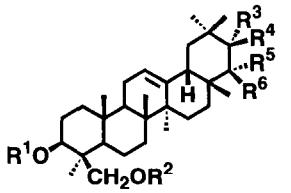
Finally, 21β-ol **30**[8] and 21α-ol **34**[9] have been prepared as shown in Scheme 3. Mesylation of **8** followed by reduction of the resulting 22-mesylate with Super Hydride in THF at room temperature provided **29** in 70% yield from **8**, which was then deprotected to afford 21β-ol **30**. Compound **30** is the 21-isomer of soyasapogenol B (**2**). Oxidation of **29** under Swern conditions, followed by removal of the protecting group, gave the 21-keto derivative **32**. Reduction of the ketone **31** with LAH afforded **29** in 68% yield as well as the desired 21α-ol **33** in 20% yield. Deprotection of **33** gave 3β, 21α, 24(4β) -triol **34**.



Scheme 3: (a) MsCl, Py., 4-DMAP, rt; (b) $LiEt_3BH$, THF, rt; (c) H_2, 10%Pd/C, MeOH:CH_2Cl_2 (1:1), rt; (d) Swern oxidation; (e) H_2, 20%$Pd(OH)_2$/C, MeOH:CH_2Cl_2 (1:1), rt; (f) $LiAlH_4$, THF, 0°C.

Hepatoprotective effects of soyasapogenol A derivatives thus prepared have been evaluated in aflatoxin B_1-induced Hep G2 cells[10] and the results are summarized in Table 1. Glycyrrhizin (GL),[11] the positive control, has been used for the treatment of chronic hepatitis. Among the preparations examined the 21, 22-diketo derivative **19** and the 22-keto derivative **20** were found to be most active. Soyasapogenol A (**1**),the diacetates (compounds **4** and **9**) and the dimethyl ether (compound **14**) showed no activity. In contrast, 21β-acetoxy and 22β-methoxy derivatives (**10** and **16**) showed improved activity compared to parent **1**. Among the 22-deoxy derivatives, 21β-ol **30** was found to be more active than 21α-ol **34** and 21-keto **32**. The diastereoisomers (compounds **24** and **25**) were not improved in the activity. It is noteworthy to mention here that morphological changes in the cultured Hep G2 cells treated with above-mentioned hepatoprotective compounds (**10**, **19**, **20** and **30**) were significantly less than those in the cells treated with **2**.

Table 1. Effect of soyasapogenol A (**1**) and its derivatives at a dose of 10 μg/ml in comparison with soyasapogenol B (**2**) and glycyrrhizin (GL) on the cell growth and lesions in Hep G2 cells treated with aflatoxin B_1 (10^{-5}M)[10]



Compound	R^1	R^2	R^3	R^4	R^5	R^6	Protection (%)
1	H	H	H	OH	H	OH	0
2	H	H	H	H	H	OH	14
4	Ac	Ac	H	OH	H	OH	0
5	Me	Me	H	OH	H	OH	7
9	H	H	H	OAc	H	OAc	0
10	H	H	H	OAc	H	OH	25
14	H	H	H	OMe	H	OMe	0
15	H	H	H	OMe	H	OH	8
16	H	H	H	OH	H	OMe	20
19	H	H	=O		=O		131
20	H	H	H	OH	=O		107
24	H	H	OH	H	H	OH	0
25	H	H	H	OH	OH	H	0
28	H	H	OH	H	OH	H	8
30	H	H	H	OH	H	H	25
34	H	H	OH	H	H	H	0
32	H	H	=O		H	H	15
GL [a]							15 [b]

[a] glycyrrhizin. [b] at a dose of 20 μg/ml.

Acknowledgment: We gratefully thank Professor Isao Kitagawa of Kinki University for helpful comments. We also thank Drs. Y. Kodama and N. Watanabe of this Research Center for the X-ray crystallographic data.

References and Notes:

1. (a) Kitagawa, I.; Yoshikawa, M.; Yosioka, I. *Chem. Pharm. Bull.* **1976**, *24*, 121. (b) Kitagawa, I.; Yoshikawa, M.; Wang, H. K.; Saito, M.; Tosirisuk, V.; Fujiwara, T.; Tomita, K. *Chem. Pharm. Bull.* **1982**, *30*, 2294.
2. Sasaki, K.; Minowa, N.; Kuzuhara, H.; Nishiyama, S.; Omoto, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 85.
3. The soyasapogenol A derivatives prepared in this paper gave satisfactory physicochemical and chemical analytical data.
4. Kitagawa, I.; Wang, H. K.; Taniyama, T.; Yoshikawa, M. *Chem. Pharm. Bull.* **1988**, *36*, 153.
5. To be reported elsewhere in due course.
6. Compound **19**: ^{1}H NMR (400 Hz, $CDCl_3$) δ 0.90 (s, 3H), 0.94 (s, 3H), 1.12 (s, 3H), 1.14 (s, 3H), 1.15 (s, 3H), 1.17 (s, 3H), 1.25 (s, 3H), 0.80-2.60 (m, 21H), 3.35 (d, J= 11.0 Hz, 1H), 3.45 (dd, J= 4.4, 11.8 Hz, 1H), 4.21 (d, J= 11.0 Hz, 1H), 5.39 (t, J= 3.6 Hz, 1H).
7. Compound **20**: ^{1}H NMR (400 Hz, $CDCl_3$) δ 0.69 (s, 3H), 0.90 (s, 3H), 0.93 (s, 3H), 1.05 (s, 3H), 1.11 (s, 3H), 1.25 (s, 3H), 1.26 (s, 3H), 0.85-2.75 (m, 21H), 3.35 (d, J= 11.2 Hz, 1H), 3.45 (td, J= 4.4, 11.8 Hz, 1H), 3.65 (d, J= 4.1 Hz, 1H), 4.18 (d, J= 4.1 Hz, 1H), 4.22 (d, J= 11.2 Hz, 1H), 5.30 (t, J= 3.6 Hz, 1H).
8. A triterpene which is known as kudzusapogenol C: Kinjo, J.; Miyamoto, I.; Murakami, K.; Kida, K.; Tomimatsu, T.; Yamasaki, M.; Nohara, T. *Chem. Pharm. Bull.* **1985**, *33*, 1293.
9. A triterpene which is known as yunganogenin C: Ohtani, K.; Ogawa, K.; Kasai, R.; Yang, C. -R.; Yamasaki, K.; Zhou, J.; Tanaka, O. *Phytochemistry* **1992**, *31*, 1747.
10. The test compound (10 μg/ml) was added to fresh culture medium in the presence of 10^{-5} M aflatoxin B_1, and the Hep G2 cells were incubated for 2 days. The morphological examination of cultured cells were carried out by use of phase-contrast microscope, and viable cell numbers were stained with 0.1% of crystal violet and determined with monocellator (Olympus Co. Ltd.).

    The percent of protection was expressed according to the formula:

$$\text{Percent of protection} = \frac{B - A}{100 - A} \times 100$$

    A: lesions value due to aflatoxin B_1

    B: lesions value due to aflatoxin B_1 and test compound
11. (a) Kiso, Y.; Tohkin, M.; Hikino, H. *Planta Med.* **1983**, *49*, 222. (b) Kiso, Y.; Tohkin, M.; Hikino, H.; Hattori, M.; Sakamoto, T.; Namba, T. *Planta Med.* **1984**, *50*, 298. (c) Nose, M.; Ito, M.; Kamimura, K.; Shimizu, M.; Ogihara, Y. *Planta Med.* **1993**, *59*, 136.