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SYNTHESIS AND HEPATOPROTECTIVE EFFECTS OF SOYASAPOGENOL B DERIVATIVES

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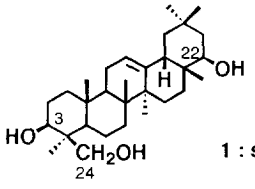
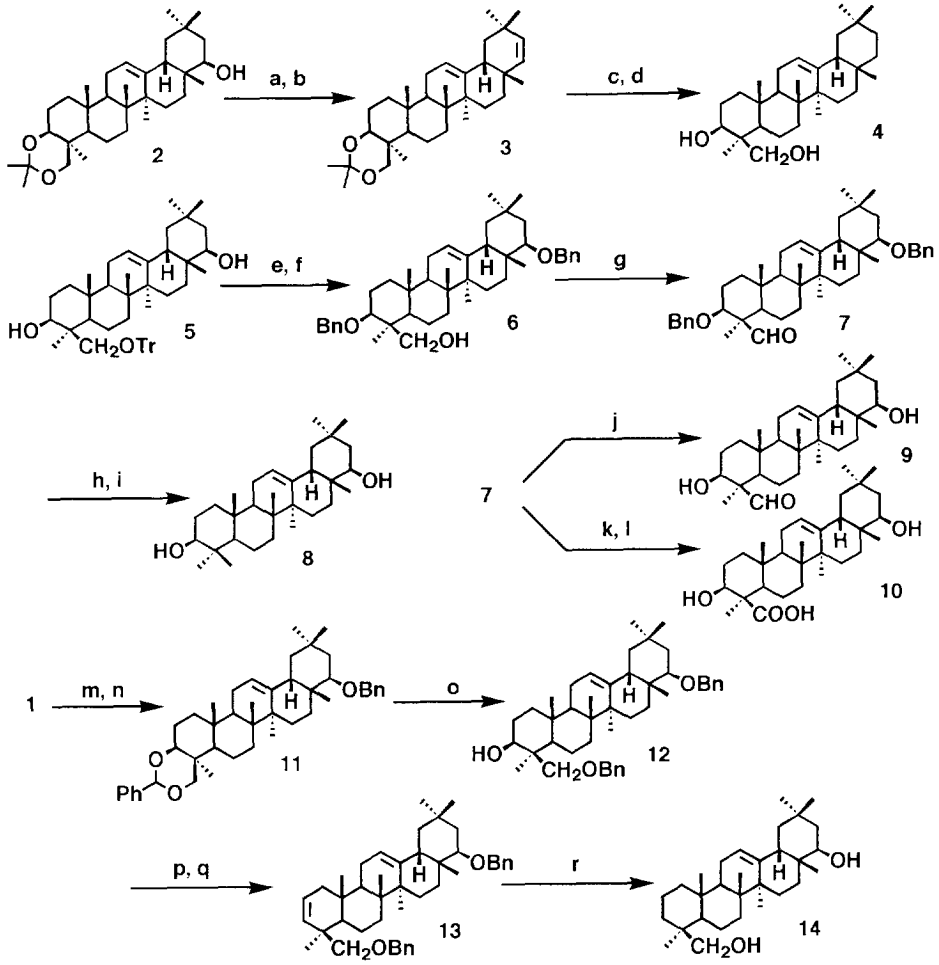
Abstract: Derivatives of soyasapogenol B (**1**), which is the aglycon moiety of soyasaponins from soybean, were synthesized and evaluated for their hepatoprotective effects *in vitro*.

Liver is an important organ which is vital for metabolism and excretion. It is damaged acutely or chronically by virus, drugs and alcohol. Human hepatitis infection affects a worldwide health problem, which can be managed pharmacologically in only a few case. The current hepatotherapeutic drugs provide only low therapeutic efficacy and moreover have severe side effects. Therefore, search for effective and safe hepatoprotective drugs are needed. In the course of our screening for hepatoprotective agents, we have found that oleanene-type triterpene soyasapogenol B (**1**),[1] which was isolated from soybean, shows hepatoprotective effect *in vitro* against aflatoxin B_1-induced Hep G2 cells. Synthetic study of soyasapogenol derivatives and also their biological activity have ever been little reported. Herein, we describe the synthesis of soyasapogenol B derivatives, transformed regioselectively at the 3, 22 and 24-hydroxyl groups, and comparison of their hepatoprotective effects *in vitro*.



1 : soyasapogenol B

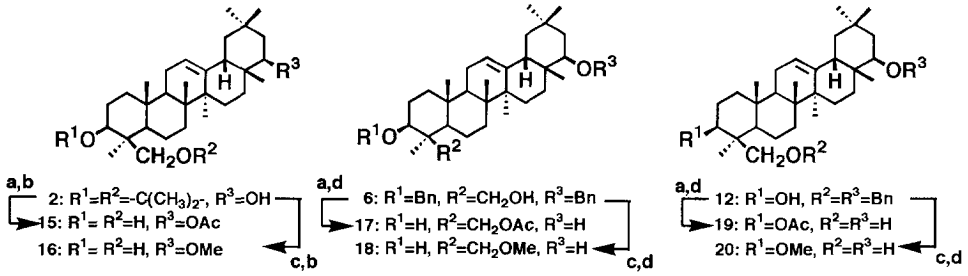
Selective dehydroxylation of each of three hydroxyls of soyasapogenol B (**1**) as well as oxidation of 24-hydroxyl of **1** were carried out as shown in Scheme 1. Thus, monodeoxy derivatives **4**, **8** and **14** were efficiently prepared from **2**, **5**[2] and **1**, respectively. Tosylation of **2**, which was derived from **1**, followed by reduction with Super Hydride[3] in THF at 65°C gave olefin **3** in 93% yield from **1**. Removal of the isopropylidene moiety of **3** and subsequent selective reduction of the 21,22-double bond gave the desired 22-deoxy product **4**.[4] Treatment of **5** with benzyl bromide and sodium hydride afforded dibenzyl ether and then detritylation gave alcohol **6**. Oxidation of the primary alcohol in **6** under Swern conditions[5] gave aldehyde **7**. Huang-Minlon reduction[6] of the resulting aldehyde **7**, followed by removal of the protecting group, gave the desired 24-deoxy product **8**.[2,7] Treatment of **1** with benzaldehyde dimethylacetal in the presence of CSA,



Scheme 1 : (a) TsCl, Py., 4-DMAP, rt; (b) $LiEt_3BH$, THF, 65°C, 93% (over 2 steps) ; (c) 1N HCl, MeOH:CH_2Cl_2 (2:1), rt, 90%; (d) H_2, 10%Pd/C, MeOH:CH_2Cl_2 (2:1), rt, 55%; (e) NaH, BnBr, DMF, 45°C, 65%; (f) conc HCl, MeOH:acetone (5:1), reflux, 72%; (g) Swern oxidation, 82%; (h) $H_2NNH_2.H_2O$, $(HOCH_2CH_2)_2O$, EtOH, 140°C; KOH, reflux, 71%; (i) H_2, 10%Pd/C, MeOH:CH_2Cl_2 (1:1), rt, 80%; (j) H_2, 20%$Pd(OH)_2$/C, rt, 98%; (k) $NaClO_2$,tBuOH, 2-methyl-2-butene, NaH_2PO_4, rt, 80%; (l) H_2, 10%Pd/C, rt, 92%; (m) $PhCH(OMe)_2$, CSA, DMF, 45°C, 83%; (n) NaH, BnBr, DMF, 50°C, 50%; (o) DIBAL, $PhCH_3$, 0°C, 69%; (p) TsCl, Py., 4-DMAP, rt, 47%; (q) DBU, $PhCH_3$, reflux, 74%; (r) H_2,20%$Pd(OH)_2$/C, MeOH:CH_2Cl_2 (1:1), rt, 93%.

followed by benzylation of 22-hydroxyl gave **11**. Regioselective reductive cleavage of the benzylidene acetal **11** with DIBAL[8] gave secondary alcohol **12** in 69% yield and its regio isomer **6** in 6 % yield after silica gel chromatography. Tosylation of **12** followed by elimination of the tosyl group with DBU in toluene at reflux gave olefin **13**. Selective reduction of the 2,3-double bond in **13**, with concomitant removal of the protecting group, provided the desired 3-deoxy product **14**. On the other hand, aldehyde **9** and carboxylic acid **10** were readily prepared from **7**. Thus, the dibenzyl ether moieties of **7** were removed by catalytic hydrogenation to give **9**. Oxidation of the aldehyde **7** with sodium chlorite[9] at room temperature proceeded smoothly to provide carboxylic

acid, subsequent removal of the dibenzyl ether moieties afforded **10** in 74% yield from **7**.

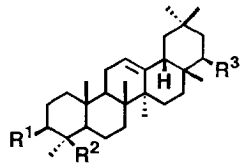


Scheme 2 : (a) Ac_2O, Py., rt; (b) 1N HCl, MeOH:CH_2Cl_2 (2:1) , rt; (c) NaH, CH_3I, DMF, rt~50°C; (d) H_2, 20%$Pd(OH)_2$/C, MeOH:CH_2Cl_2 (1:1) , rt.

Regioselective acetylation and methylation of three hydroxyl groups on **1** effected by using the intermediates **2**, **6** and **12** as shown in Scheme 2. Compounds **2**, **6** and **12** were treated with acetic anhydride and the resulting acetates were deprotected to give **15**, **17** and **19**, respectively. Methylation of **2**, **6** and **12** with methyl iodide and sodium hydride followed by deprotection gave **16**,[10] **18** and **20**, respectively.

Notably, intermediates **2**, **6** and **12** would be useful for synthesis of various triterpene derivatives.

Table 1. Effect of soyasapogenol B (**1**) and its derivatives (10μg/ml) on the cell growth and lesions in Hep G2 cells treated with aflatoxin B_1 (10^{-5}M)[11]



Compound	R^1	R^2	R^3	Protection (%)
1	OH	CH_2OH	OH	14
4	OH	CH_2OH	H	33
8	OH	CH_3	OH	19
9	OH	CHO	OH	32
10	OH	COOH	OH	44
14	H	CH_2OH	OH	0
15	OH	CH_2OH	OAc	11
16	OH	CH_2OH	OMe	9
17	OH	CH_2OAc	OH	45
18	OH	CH_2OMe	OH	48
19	OAc	CH_2OH	OH	0
20	OMe	CH_2OH	OH	2
GL [a]				15 [b]

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

Hepatoprotective effects of soyasapogenol B derivatives were evaluated in aflatoxin B_1-induced Hep G2 cells and the screening results are summarized in Table 1. Soyasapogenol B (**1**) was more active when compared to glycyrrhizic acid (GL) which has been successfully used to treat chronic hepatitis. Among the deoxy-derivatives, 22-deoxy **4** was more active than **1**, while 3-deoxy **14** was not improved in the activity. Oxidation of 24-hydroxyl group resulted in enhancement of the activity (compounds **9** and **10**). On the other hand, the 24-acetylated and 24-methylated derivatives **17** and **18** were found more active than **1**, but derivativation the 3-hydroxyl group as in **19** and **20** lost the activity. Morphological changes in cultured Hep G2 cells treated with **4**, **9**, **10**, **17** and **18** were apparently less than those in cells treated with **1**. These results let us to presume that the hydroxyl group at the 3-position and oxygen atom at the 24-position are essential to the activity.

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11. The test compound (10μg/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

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