Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 4900–4911
Bioorganic & Medicinal Chemistry

Preventive effects of soyasapogenol B derivatives on liver injury in a concanavalin A-induced hepatitis model

Kazue Sasaki, Nobuto Minowa,* Hiroyuki Kuzuhara and Shoji Nishiyama

Medicinal Chemistry Research Laboratory, Pharmaceutical Research Department, Meiji Seika Kaisha, Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan

Received 28 March 2005; accepted 27 April 2005
Available online 25 May 2005

Abstract—To shed light on the structure–activity relationship, various soyasapogenol B derivatives were synthesized and evaluated for preventive effects on liver injury in the concanavalin A (Con A)-induced hepatitis model in mice. Con A injection into mice induces some pathophysiology of human liver disease such as autoimmune or viral hepatitis. Two hydroxyl groups on the A ring of soyasapogenol B are required for amelioration of liver damage. Modification of the C-22 hydroxyl moiety with an acyloxy or alkyloxy group, or removal of the hydroxyl group, resulted in a greatly enhanced percentage of alleviation. Among the series of soyasapogenol B derivatives examined, six compounds exhibited preventive effects on liver damage.

1. Introduction

Infectious hepatitis is a global health problem.[1] Hepatitis B virus (HBV) as well as hepatitis C virus (HCV) infections are leading causes of chronic hepatitis, which often results in liver cirrhosis and hepatocellular carcinoma.[2] Current recommended therapies for HBV and HCV infections treated with interferon-α,[3] lamivudine[4], and ribavirin[5] provide sustained efficacy only in a portion of the treated patients and often result in severe side effects.[6] Therefore, more effective and reliable therapies are required. The development of new drugs depends primarily on the availability of suitable animal models. Although the methods of xenobiotics have been widely recognized, those methods hardly reflect the clinical condition in human beings since it is well known that HBV and HCV appear to be mainly mediated by the immune response against virus.[7] Concanavalin A (Con A) injection into mice induces T cell-dependent liver failure.[8] In the Con A-induced hepatitis model, liver injury in mice results from T-cell stimulation and the release of tumor necrosis factor-α, interferon-γ, and interleukin-2 in vivo.[9] More recent studies suggest a key pathogenic role for the STAT1/T-bet signaling pathway for T-cell activation in the Con A model.[10] There is evidence that these mechanisms are also relevant for the events during the pathophysiology of human liver disease like autoimmune or viral hepatitis.[11]

We focus on triterpenes as anti-hepatitic agents since some triterpenes are major components of several traditional medicinal herbs and display a variety of biological effects, such as hepatoprotective and anti-inflammatory effects.[12] In the course of screening triterpenes for novel agents showing preventive effects in liver, we have found that soyasapogenol B (**1**), which is one of the oleanene-type triterpene aglycons of soyasaponins, and its derivatives show potent preventive effects in vitro against aflatoxin B_1-induced cell damage.[13] On the other hand, interestingly, soyasapogenol A (**2**), which is another aglycon of soyasaponins, markedly reduced the elevated plasma alanine aminotransferase activity and liver cell necrosis in the Con A-induced hepatitis model in mice.[9] These findings led us to investigate the activities of soyasapogenol B and its derivatives on liver injury mediated by the immune response in the Con A-induced hepatitis model (Fig. 1).

In this paper, we report the synthesis of various soyasapogenol B derivatives, modified mainly at hydroxyl groups attached to the A and E rings, and their preventive effects on liver injury in the Con A model.

Keywords: Hepatitis; Soyasapogenol B; Triterpene; Concanavalin A.
* Corresponding author. Tel.: +81 45 545 2521; fax: +81 45 543 9771; e-mail: nobuto_minowa@meiji.co.jp

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.04.074

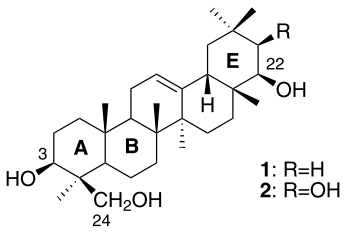
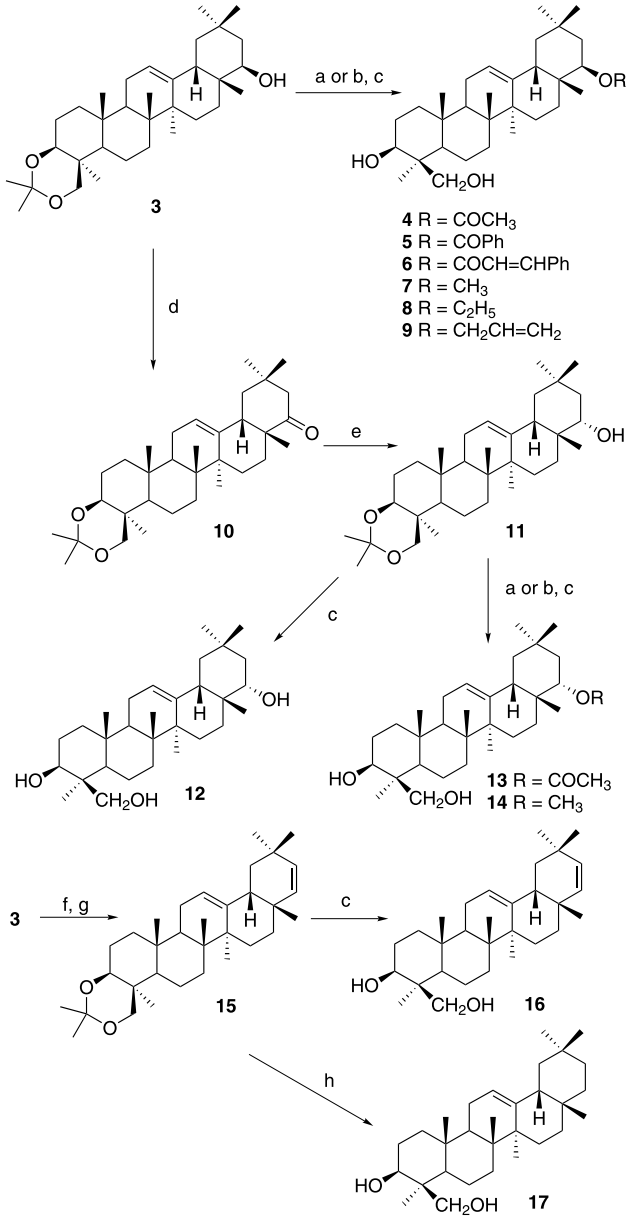


Figure 1. Structures of soyasapogenol A (**2**) and B (**1**).

2. Preparations of derivatives

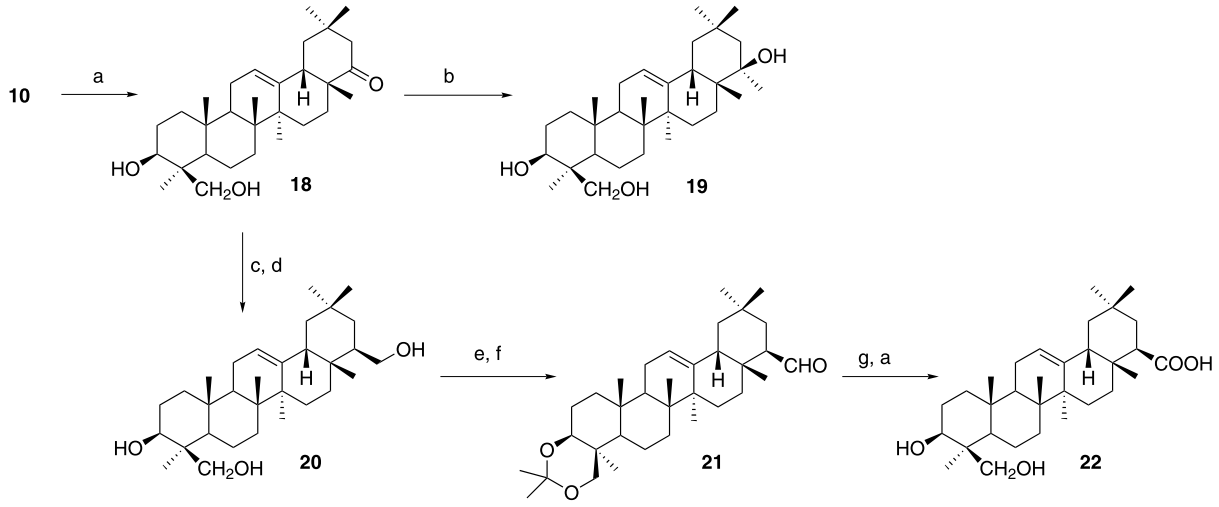
Modifications of the C-22 substituent of soyasapogenol B (**1**) were carried out as shown in Scheme 1. Thus, treatment of **3**, which was prepared by acetonidation of **1** with acetic anhydride or acyl chloride, followed by deprotection of the isopropylidene group, gave **4**, **5**, and **6**, whereas alkylation of **3** with alkyl iodide and sodium hydride followed by deprotection afforded **7**, **8**, and **9**. Oxidation of **3** (to **10**) and subsequent reduction with lithium aluminum hydride (LAH) gave the 22α-ol **11** and 22β-ol **3** in 34% and 66% yields, while reduction of the ketone **10** with sodium and *tert*-butyl alcohol in refluxing toluene proceeded stereoselectively to afford the 22α-ol **11** and 22β-ol **3** in 75% and 6% yields, respectively. Removal of the isopropylidene group of **11** gave 22-*epi*-soyasapogenol B **12**, while acetylation or methylation of **11** and subsequent deprotection of the isopropylidene moiety furnished **13** or **14**. Tosylation of **3** followed by reduction with Super Hydride[14] in THF at 65 °C provided the olefin **15** in 79% yield from **3**. Deprotection of **15** under acidic conditions gave **16**, which is known as soyasapogenol C.[15] Selective hydrogenation over palladium-charcoal of the 21,22-double bond of **15** afforded the desired 22-deoxy compound **17**[16] with concomitant removal of the isopropylidene group.



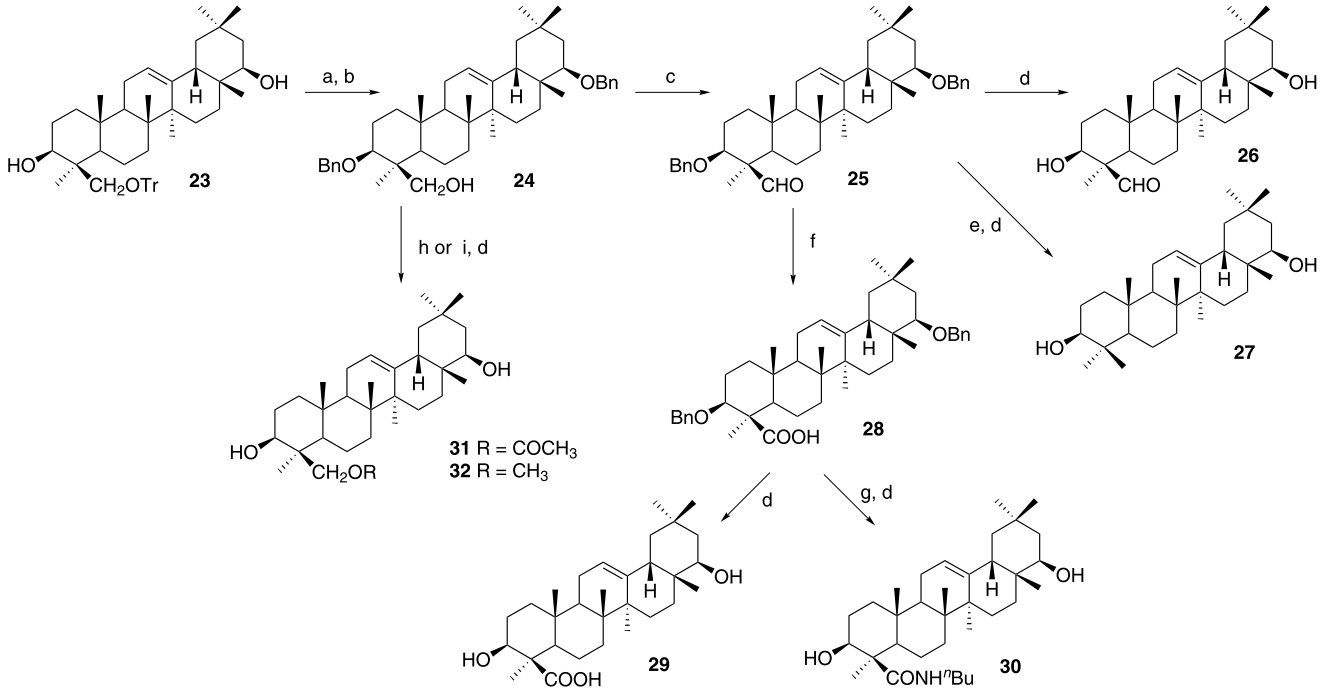
Scheme 1. Reagents: (a) Ac_2O, Py, or RCOX, 4-DMAP, CH_2Cl_2; (b) NaH, RX, THF, or DMF; (c) 1 N HCl, MeOH/CH_2Cl_2 (2:1); (d) PCC, CH_2Cl_2; (e) Na, ^{t}BuOH, toluene; (f) TsCl, Py, 4-DMAP; (g) $LiEt_3BH$, THF; (h) H_2, 10% Pd/C, MeOH/CH_2Cl_2 (2:1).

Next, homologations of soyasapogenol B (**1**) at C-22 were conducted as shown in Scheme 2. After removal of the isopropylidene moiety, **18** thus formed was treated with methyllithium to provide the desired 22α-methyl-22β-hydroxyl derivative **19** as a single stereoisomer. On the other hand, treatment of **18** with Nysted[17] reagent in the presence of $TiCl_4$ afforded an *exo*-methylene derivative. Hydroboration of the *exo*-methylene with diborane followed by hydrogen peroxide oxidation gave the 22β-hydroxymethyl derivative **20** in 79% yield with high diastereoselectivity (22α:22β = 1:7). In contrast, the stereoselectivity of the hydroboration using the more bulky 9-BBN was significantly lost.[18] The 3β,24-diol of **20** was protected with an isopropylidene group and then subjected to Swern oxidation to afford the 22β-formyl derivative **21**. Oxidation of the formyl moiety with sodium chlorite[19] and subsequent removal of the isopropylidene group gave the desired C-22 carboxylic acid **22**. The structures of the 3,24-diacetate derivative of **19** and the triol **20** were confirmed by single-crystal X-ray analyses.

Next, modifications of the 4β-hydroxymethyl moiety of soyasapogenol B (**1**) were carried out as shown in Scheme 3. Treatment of **23**,[20] which was prepared by tritylation of **1**, with benzyl bromide and sodium hydride afforded a dibenzyl ether, and detritylation gave an alcohol **24**. Oxidation of the primary alcohol (at C-4) in **24** under Swern conditions, followed by removal of the benzyl protecting groups, provided an aldehyde **26**. Huang–Minlon reduction[21] of the aldehyde **25** and subsequent removal of the protecting benzyl groups gave the desired 24-deoxy analog of soyasapogenol B **27**, which is known by the name of sophoradiol.[22] On the other hand, oxidation of the aldehyde **25** with sodium chlorite at room temperature proceeded smoothly to provide a carboxylic acid **28**, and subsequent removal of the dibenzyl protecting groups afforded a carboxyl derivative **29**. Condensation of the carboxylic acid **28** and *n*-butylamine in the presence of 1*H*-benzotriazol-



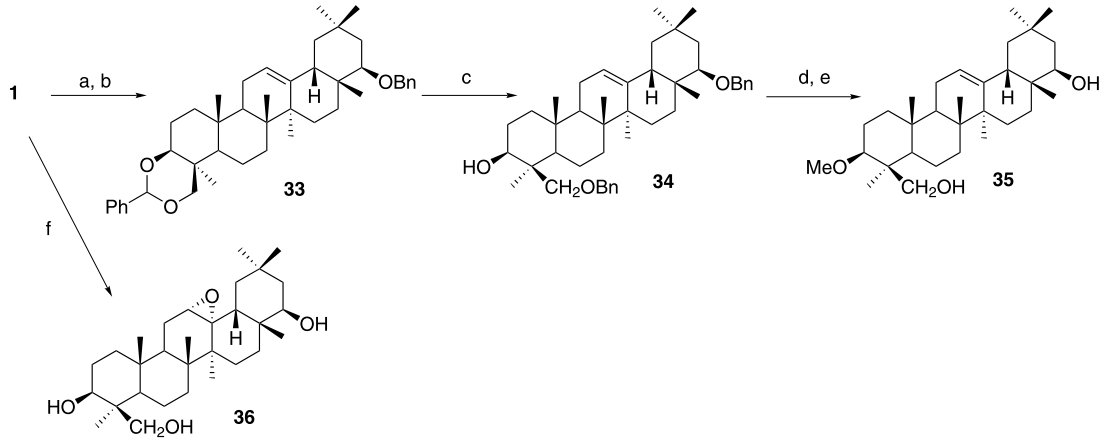
Scheme 2. Reagents: (a) 1 N HCl, MeOH/CH_2Cl_2 (2:1); (b) CH_3Li, THF; (c) Nysted reagent, $TiCl_4$, THF; (d) BH_3·THF, then 10% NaOH, 30% H_2O_2; (e) $Me_2C(OMe)_2$, CSA, acetone; (f) Swern oxidation; (g) $NaClO_2$, ^{t}BuOH, 2-methyl-2-butene, NaH_2PO_4.



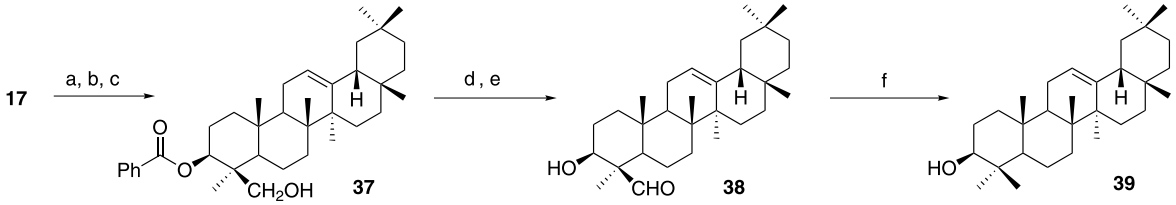
Scheme 3. Reagents: (a) NaH, BnBr, DMF; (b) concd HCl, MeOH/acetone (5:1); (c) Swern oxidation; (d) H_2, 10% Pd/C or 20% $Pd(OH)_2$/C, MeOH/CH_2Cl_2 (1:1); (e) H_2NNH_2·H_2O, $(HOCH_2CH_2)_2O$, EtOH, KOH; (f) $NaClO_2$, ^{t}BuOH, 2-methyl-2-butene, NaH_2PO_4; (g) BOP, ^{n}BuNH$_2$, DMF; (h) Ac_2O, Py; (i) NaH, CH_3I, DMF.

1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) followed by deprotection of the benzyl groups gave an *n*-butylamide **30**. Acetylation or methylation of the 24-hydroxyl group in **24**, followed by removal of the benzyl protecting groups, provided **31** or **32**[23] without difficulty.

Methylation of the 3β-hydroxyl group of soyasapogenol B (**1**) and epoxidation of the olefin moiety were effected as shown in Scheme 4. Thus, treatment of **1** with benzaldehyde dimethylacetal in the presence of camphorsulfonic acid (CSA) and subsequent benzylation of the 22β-hydroxyl moiety gave **33**. Reductive cleavage of the benzylidene acetal in **33** with diisobutylaluminum hydride (DIBAL) proceeded with unusual regioselectivity[24] to give the 22,24-dibenzyl ether **34** in 69% yield and the 3,22-dibenzyl ether **24**, a regio-isomer of **34**, concomitantly formed in 6% yield, the two isomers being effectively separated by silica gel column chromatography. Methylation of **34** followed by removal of two benzyl groups by hydrogenation furnished the desired 3-*O*-methylsoyasapogenol B (**35**). Treatment of **1** with 3-chloroperoxybenzoic acid (*m*CPBA) provided an α-epoxidized product **36** as a single isomer in 81% yield.[25] It is presumed that epoxidation of the 12,13-double bond in **1** occurs from less hindered equatorial direction to give the α-epoxide **36**. The structure of **36** was confirmed by X-ray crystallographic analysis.



Scheme 4. Reagents: (a) $PhCH(OMe)_2$, CSA, DMF; (b) NaH, BnBr, DMF; (c) DIBAL, $PhCH_3$; (d) NaH, CH_3I, DMF; (e) H_2, 20% $Pd(OH)_2/C$, $MeOH/CH_2Cl_2$ (1:1); (f) *m*CPBA, $CH_2Cl_2/CHCl_3$ (10:3).



Scheme 5. Reagents: (a) TrCl, Py; (b) PhCOCl, 4-DMAP, CH_2Cl_2; (c) concd HCl, MeOH/acetone (5:1); (d) PCC, CH_2Cl_2; (e) 1 N NaOH; (f) $H_2NNH_2{\cdot}H_2O$, $(HOCH_2CH_2)_2O$, EtOH, KOH.

Finally, the 22,24-dideoxy derivative of soyasapogenol B (**1**) was prepared as shown in Scheme 5. Tritylation of 22-deoxysoyasapogenol B (**17**) gave the 24-monotrityl ether, which was then treated with benzoyl chloride, followed by detritylation to afford 3-*O*-benzoyl-22-deoxysoyasapogenol B (**37**). Oxidation of **37** with pyridinium chlorochromate (PCC) followed by hydrolysis gave the 4β-formyl analog (i.e., 22-deoxy-24-oxosoyasapogenol B) **38**. Huang-Minlon reduction of **38** provided **39**, which is known as β-amyrin,[26] thus confirming the parent carbon skeleton of soyasapogenol B (**1**).

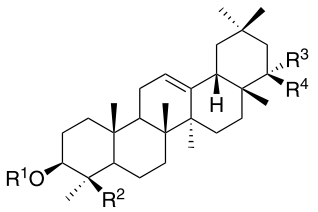
3. Results and discussion

Various soyasapogenol B derivatives, in which the hydroxyl groups at C-3, C-22, and C-24 and the double bond at C-12,13 were modified, were evaluated for preventive effects on Con A-induced liver injury. The percentages of alleviation of liver damage are shown in Tables 1–3.

Modification of the hydroxyl groups at C-3 and C-24 of soyasapogenol B (**1**) decreased the preventive effect, as shown in Table 1. The C-24 modified derivatives, 24-formyl **26**, 24-amide **30**, and 24-acetoxymethyl **31**, exhibited no preventive activity, and the 24-deoxy **27**, 24-carboxyl **29**, 24-methoxymethyl **32**, and 3-methoxyl **35** analogs were less active than soyasapogenol B (**1**) at the dose of 80 mg/kg. These results suggested that modification at C-3 and C-24 has a detrimental effect on the amelioration of liver injury. Hydroxyl groups on the A ring of soyasapogenol B (**1**) seem to be essential for the activity.

As shown in Table 2, modification of the C-22 hydroxyl group yielded derivatives with greatly enhanced preventive effects. The acyloxy and alkyloxy derivatives (**4**, **5**, **7**, and **8**) showed potent protective activities at doses of 8.0 and 80 mg/kg, respectively. However, replacement of the acyl substituent with a cinnamoyl group led to a dramatic decrease in activity (compounds **4** and **5** vs **6**). Similarly, replacement of the alkyl substituent with an allyl group reduced the activity (compounds **7** and **8** vs **9**). These data indicate that steric properties of the C-22 substituent have a significant influence on the activity. 22-*epi*-Soyasapogenol B (**12**) was shown to exhibit a slightly higher activity than soyasapogenol B (**1**) in this assay system. Further, methylation and acylation of **12** (compounds **13** and **14**) led to an increase in activity as compared with **12**. In particular, it is noteworthy that the acetoxy derivative **13** at a dose of 8.0 mg/kg showed a preventive effect similar to 22-*O*-acetylsoyasapogenol B (**4**) at a dose of 8.0 mg/kg. The 21,22-dehydro analog **16** showed slightly increased activity as compared with **1**. Interestingly, the 22-deoxy derivative **17** exhibited a significant increase in preventive effects, while the 22,24-dideoxy derivative **39** displayed slightly lower activity. Homologation of soyasapogenol B (**1**) at C-22 (compounds **19**, **20**, and **22**) resulted in significantly decreased activity at a dose of 80 mg/kg, as shown in Table 3. These findings suggested that the substituent attached

Table 1. Preventive effects of soyasapogenol B derivatives modified at the A ring in the concanavalin A-induced hepatitis model[a]



Compound	R^1	R^2	R^3	R^4	Alleviation (%)[b] 80 (mg/kg)
27	H	Me	H	OH	47*
26	H	CHO	H	OH	0
29	H	COOH	H	OH	46
30	H	CONH^{n}Bu	H	OH	0
31	H	CH_2OAc	H	OH	0
32	H	CH_2OMe	H	OH	42
35	Me	CH_2OH	H	OH	50*
1	H	CH_2OH	H	OH	70*

[a] For details of the assay, see Section 5.

[b]

$$\text{Alleviation}(\%) = \frac{\text{concanavalin A} - (\text{concanavalin A} + \text{compound})}{\text{concanavalin A} - \text{control}}$$

where concanavalin A: ALT level in the concanavalin A alone group ($n = 8$); control: ALT level in the control group ($n = 3$); concanavalin A + compound: ALT level in the concanavalin A + compound group ($n = 8$).

* Significantly different from the concanavalin A alone group. $p < 0.05$ (Student's t-test).

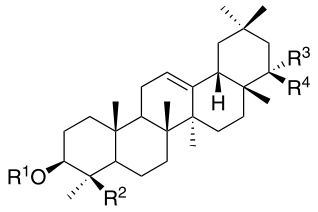
to the C-22 hydroxyl group is important for amelioration of liver injury. In summary, the methoxy and acetoxy derivatives of both configurations at C-22 (**4**, **5**, **7**, **8**, **13**, and **14**) and the C-22 deoxy derivative (**17**) tended to show increased activity. The 12α,13α-epoxide **36**, in which the C ring is modified, showed similar activity to the starting soyasapogenol B (**1**).

In our previous study, we have shown that soyasapogenol A (**2**) directly prevents apoptosis of hepatocytes and secondly inhibits the increase in plasma tumor necrosis factor-α and interferon-γ levels, which eventually resulted in prevention of liver damage in the Con A-induced hepatitis model.[9] It is presumed that soyasapogenol B derivatives, which have structures analogous to soyasapogenol A, exhibit potent preventive effects on liver injury through similar mechanisms.

4. Conclusion

Soyasapogenol B derivatives modified in the A, C, and E rings were prepared and their preventive effects on liver injury were evaluated in the Con A-induced hepatitis model in mice. Two hydroxyl groups in the A ring and modification with an acyloxy and alkyloxy group at C-22 are necessary for amelioration of liver damage. Interestingly, the stereochemistry of the C-22 substituent did not significantly affect the activity, and the C-22 deoxy derivative showed potent activity. In the course of these studies, compounds **4**, **5**, **7**, **8**, **13**, and **17**, which are

Table 2. Preventive effects of soyasapogenol B derivatives modified at the A, C, and E rings in the concanavalin A-induced hepatitis model[a]



Compound	R^1	R^2	R^3	R^4	Alleviation (%)[b]	
					8.0	80 (mg/kg)
4	H	CH_2OH	H	OAc	97*	98*
5	H	CH_2OH	H	OCOPh	80*	93*
6	H	CH_2OH	H	OCOCH=CHPh	13	27
7	H	CH_2OH	H	OMe	86*	97*
8	H	CH_2OH	H	OEt	98*	82*
9	H	CH_2OH	H	$OCH_2CH=CH_2$	0	80*
12	H	CH_2OH	OH	H	29	78*
13	H	CH_2OH	OAc	H	94*	100*
14	H	CH_2OH	OMe	H	56	100*
16[c]	H	CH_2OH			0	94*
17	H	CH_2OH	H	H	86*	96*
39	H	Me	H	H	54*	98*
36[d]	H	CH_2OH	H	OH	14	74*
1	H	CH_2OH	H	OH	0	70*

[a] For details of the assay, see Section 5.

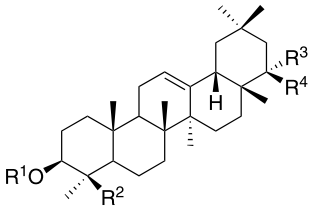
[b] The values were calculated as described in Table 1.

[c] The 21,22-dehydro analog of **17**.

[d] The 12α,13α-epoxide of **1**.

* Significantly different from the concanavalin A alone group. $p < 0.05$ (Student's t-test).

Table 3. Preventive effects of soyasapogenol B derivatives modified at the E ring in the concanavalin A-induced hepatitis model[a]



Compound	R^1	R^2	R^3	R^4	Alleviation (%)[b] 80 (mg/kg)
19	H	CH_2OH	Me	OH	52
20	H	CH_2OH	H	CH_2OH	12
22	H	CH_2OH	H	COOH	28
1	H	CH_2OH	H	OH	70*

[a] For details of the assay, see Section 5.

[b] The values were calculated as described in Table 1.

* Significantly different from the concanavalin A alone group. $p < 0.05$ (Student's *t*-test).

soyasapogenol B derivatives modified at C-22, were shown to exhibit potent amelioration of liver damage in this model. These results indicate that soyasapogenol B derivatives are promising candidates for a new class of drugs in the treatment of chronic immunoinflammatory liver disease in patients.

5. Experimental

5.1. Chemistry

5.1.1. General. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-GSX 400 NMR spectrometer. Chemical shifts are given in δ value (ppm) with tetramethylsilane (TMS) as the internal standard. Optical rotations were recorded on a JOEL DIP-370 polarimeter. Elemental analyses were within $\pm$0.4% of the theoretical value for the elements indicated unless otherwise noted. High-resolution mass spectra (HRMS) data were obtained on a JEOL JMS-700. Soyasapogenol B was prepared according to Kitagawa's procedure.[27]

5.1.2. 22β-Acetoxyolean-12-ene-3β,24-diol (4). A solution of **3**[20] (150 mg, 0.30 mmol) in pyridine (2 ml) was treated with acetic anhydride (2 ml) at room temperature. After stirring for 20 h, the mixture was diluted with EtOAc and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 15:1) to afford 22β-acetoxy-3β,24-isopropylidenedioxyolean-12-ene (119 mg, 73%).

A solution of 22β-acetoxy-3β,24-isopropylidenedioxyolean-12-ene (119 mg, 0.22 mmol) in CH_2Cl_2/MeOH (3 ml, 1:2) was treated with 1 N HCl and stirred at room temperature for 0.5 h. The mixture was diluted with CH_2Cl_2 (20 ml) and washed with aqueous saturated $NaHCO_3$ (5 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by preparative TLC (*n*-hexane/THF, 2:1) to give **4** (83 mg, 75%) as a white solid. Compound **4**: mp 252–254 °C; $[\alpha]_D^{23}$ +71.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s), 0.89 (6H, s), 0.95 (3H, s), 1.00 (3H, s), 1.14 (3H, s), 1.25 (3H, s), 2.03 (3H, s), 0.84–2.24 (21H, m), 2.44 (1H, t, J = 4.2 Hz), 2.73 (1H, dd, J = 2.6 and 8.7 Hz), 3.35 (1H, t, J = 11.0 Hz), 3.41–3.49 (1H, m), 4.20 (1H, dd, J = 2.6 and 11.0 Hz), 4.64 (1H, t, J = 3.6 Hz), 5.25 (1H, t, J = 3.6 Hz); Anal. Calcd for $C_{32}H_{52}O_4$: C, 76.75; H 10.47. Found: C, 76.91; H, 10.31.

5.1.3. 22β-Benzoyloxyolean-12-ene-3β,24-diol (5). A solution of **3** (50 mg, 0.1 mmol) and 4-DMAP (18 mg, 0.15 mmol) in CH_2Cl_2 (5 ml) was treated with benzoyl chloride (17 μl, 0.15 mmol). After stirring overnight at reflux, the reaction mixture was diluted with CH_2Cl_2 (25 ml) and washed with water (10 ml). The organic layer was dried over $MgSO_4$, filtered, concentrated, and the residue was purified by preparative TLC (*n*-hexane/EtOAc, 3:1) to give 22β-benzoyloxy-3β,24-isopropylidenedioxyolean-12-ene (24 mg, 40%).

22β-Benzoyloxy-3β,24-isopropylidenedioxyolean-12-ene thus obtained was subjected to the deprotection reaction through the same procedure as for **4**, to furnish **5** (19 mg, 83%) as a white solid. Compound **5**: mp 159–160 °C; $[\alpha]_D^{23}$ +72.1 (*c* 0.98, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, s), 0.91 (3H, s), 0.93 (3H, s), 0.95 (3H, s), 1.05 (3H, s), 1.18 (3H, s), 1.26 (3H, s), 0.85–2.73 (23H, m), 3.35 (1H, t, J = 11.0 Hz), 3.43–3.49 (1H, m), 4.21 (1H, d, J = 11.0 Hz), 4.93 (1H, t, J = 3.8 Hz), 5.32 (1H, t, J = 3.6 Hz), 7.44 (2H, t, J = 7.2 Hz), 7.55 (1H, t, J = 7.2 Hz), 8.05 (2H, d, J = 7.2 Hz); Anal. Calcd for $C_{37}H_{54}O_4 \cdot 1/4H_2O$: C, 78.33; H, 9.68. Found: C, 78.30; H, 9.67.

5.1.4. 22β-Cinnamoyloxyolean-12-ene-3β,24-diol (6). Compound **6** was prepared through the procedure as described for **5**, except using cinnamoyl chloride instead of benzoyl chloride, in 53% yield from **3**. Compound **6**: mp 252–254 °C; $[\alpha]_D^{23}$ +49.4 (*c* 0.97, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, s), 0.89 (3H, s), 0.91 (3H, s), 0.95 (3H, s), 1.04 (3H, s), 1.16 (3H, s), 1.25 (3H, s), 0.85–2.70 (23H, m), 3.35 (1H, t, J = 11.0 Hz), 3.42–3.49 (1H, m), 4.21 (1H, d, J = 11.0 Hz), 4.79 (1H, t, J = 3.6 Hz), 5.29 (1H, t, J = 3.5 Hz), 6.42 (1H, d, J = 15.9 Hz), 7.36–7.40 (3H, m), 7.51–7.55 (2H, m), 7.65 (1H, d, J = 15.9 Hz); Anal. Calcd for $C_{39}H_{56}O_4$: C, 79.55; H, 9.59. Found: C, 79.39; H, 9.54.

5.1.5. 22β-Methoxylolean-12-ene-3β,24-diol (7). A solution of **3** (200 mg, 0.4 mmol) in THF (2 ml) was treated with 60% sodium hydride (48 mg, 1.2 mmol). After stirring for 1 h at room temperature, the mixture was treated with methyl iodide (75 μl, 1.2 mmol). After stirring further for 20 h at 50 °C, the mixture was diluted with EtOAc (20 ml) and washed with water (5 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 30:1)

to afford 22β-methoxy-3β,24-isopropylidenedioxyolean-12-ene (173 mg, 85%) as a white solid.

22β-Methoxy-3β,24-isopropylidenedioxyolean-12-ene was subjected to the deprotection reaction using the same procedure as for **4**, to give **7** (140 mg, 88%) as a white solid. Compound **7**: mp 238–239 °C; $[\alpha]_D^{23}$ +81.4 (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.85 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.94 (3H, s), 1.00 (3H, s), 1.11 (3H, s), 1.25 (3H, s), 0.83–2.11 (21H, m), 2.38 (1H, d, *J* = 4.2 Hz), 2.70 (1H, dd, *J* = 2.5 and 8.9 Hz), 2.81 (1H, dd, *J* = 2.8 and 6.7 Hz), 3.28 (3H, s), 3.36 (1H, t, *J* = 11.0 Hz), 3.41–3.48 (1H, m), 4.22 (1H, dd, *J* = 2.6 and 11.0 Hz), 5.22 (1H, t, *J* = 3.6 Hz); Anal. Calcd for $C_{31}H_{52}O_3$: C, 78.76; H 11.09. Found: C, 78.54; H, 11.11.

5.1.6. 22β-Ethoxylolean-12-ene-3β,24-diol (8). Compound **8** was prepared through the procedure as described for **7**, except for using ethyl iodide in place of methyl iodide, in 67% yield from **3**. Compound **8**: mp 231–233 °C; $[\alpha]_D^{23}$ +84.0 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.95 (3H, s), 1.01 (3H, s), 1.12 (3H, s), 1.14 (3H, t, *J* = 7.2 Hz), 1.25 (3H, s), 0.84–2.13 (21H, m), 2.40 (1H, d, *J* = 4.2 Hz), 2.70 (1H, d, *J* = 8.8 Hz), 2.89 (1H, dd, *J* = 2.8 and 6.4 Hz), 3.22–3.30 (1H, m), 3.35 (1H, t, *J* = 9.7 Hz), 3.42–3.47 (1H, m), 3.52–3.60 (1H, m), 4.21 (1H, d, *J* = 9.7 Hz), 5.21 (1H, t, *J* = 3.6 Hz); Anal. Calcd for $C_{32}H_{54}O_3$: C, 78.96; H 11.18. Found: C, 78.84; H, 11.10.

5.1.7. 22β-Allyloxylolean-12-ene-3β,24-diol (9). Compound **9** was prepared as described for **7**, except for using allyl iodide in place of methyl iodide, in 57% yield from **3**. Compound **9**: mp 210–211 °C; $[\alpha]_D^{23}$ +76.9 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.94 (3H, s), 1.01 (3H, s), 1.11 (3H, s), 1.25 (3H, s), 0.84–2.15 (21H, m), 2.41 (1H, br s), 2.71 (1H, br s), 2.97 (1H, dd, *J* = 2.8 and 6.4 Hz), 3.34 (1H, d, *J* = 11.0 Hz), 3.42–3.48 (1H, m), 3.79 (1H, tdd, *J* = 1.5, 5.4, and 13.1 Hz), 4.05 (1H, tdd, *J* = 1.5, 5.4 and 13.1 Hz), 4.20 (1H, d, *J* = 11.0 Hz), 5.11 (1H, dd, *J* = 2.1 and 10.5 Hz), 5.21–5.28 (2H, m), 5.84–5.95 (1H, m); Anal. Calcd for $C_{33}H_{54}O_3$: C, 79.46; H 10.91. Found: C, 79.24; H, 10.64.

5.1.8. Olean-12-ene-3β,22α,24-triol (12). A slurry of PCC (0.97 g, 4.5 mmol) in dry CH_2Cl_2 (15 ml) was added to a solution of **3** (1.5 g, 3.0 mmol) in dry CH_2Cl_2 (15 ml) at room temperature, and the whole mixture then turned dark. After stirring for 1.5 h, the reaction mixture was diluted with EtOAc and filtered through a short column of silica gel. The filtrate was then concentrated in vacuo and purified by column chromatography on silica gel (*n*-hexane/EtOAc, 15:1) to furnish the ketone **10** (1.0 g, 68%) as a white powder.

A solution of sodium (70 mg, 3.0 mmol) in toluene (3 ml) was heated to reflux. After vigorous stirring for 40 min, a solution of **10** (500 mg, 1.0 mmol) in ^{t}BuOH (5 ml) and toluene (3 ml) was added, and the whole mixture was stirred under reflux. After 5 h, additional sodium (130 mg, 5.7 mmol) was added, and the reaction mixture was refluxed for an additional 22 h. The mixture was cooled to 0 °C, carefully diluted with ice water (5 ml), and extracted with EtOAc (50 ml). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to afford the 22α-ol **11** (378 mg, 75%) and 22β-ol **3** (31 mg, 6%).

Compound **11** was submitted to the deprotection reaction using the same procedure as for **4**, to give **12** (190 mg, 98%) as a white solid: **12**. mp 243–245 °C; $[\alpha]_D^{23}$ +75.1 (*c* 0.92, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 0.98 (3H, s), 1.15 (3H, s), 1.25 (3H, s), 0.83–2.02 (22H, m), 2.47 (1H, d, *J* = 4.4 Hz), 2.74 (1H, dd, *J* = 2.6 and 8.7 Hz), 3.32–3.38 (1H, m), 3.42–3.47 (1H, m), 3.51–3.57 (1H, m), 4.21 (1H, dd, *J* = 2.6 and 11.3 Hz), 5.20 (1H, t, *J* = 3.6 Hz); HRMS (FAB) Calcd for $C_{30}H_{50}O_3Na$ $[M+Na]^+$ 481.3658. Found 481.3654.

5.1.9. 22α-Acetoxyolean-12-ene-3β,24-diol (13). Compound **13** was prepared through the procedure for **4** from **3** in 89% yield from **11**. Compound **13**: mp 215–216 °C; $[\alpha]_D^{23}$ +70.2 (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (3H, s), 0.89 (3H, s), 0.91 (3H, s), 0.93 (3H, s), 0.99 (3H, s), 1.16 (3H, s), 1.25 (3H, s), 2.02 (3H, s), 0.82–2.08 (21H, m), 2.55 (1H, br s), 2.81 (1H, br s), 3.35 (1H, t, *J* = 11.0 Hz), 3.43–3.48 (1H, m), 4.20 (1H, d, *J* = 11.0 Hz), 4.81 (1H, dd, *J* = 5.9 and 11.3 Hz), 5.22 (1H, t, *J* = 3.6 Hz); Anal. Calcd for $C_{32}H_{52}O_4 \cdot 2/5H_2O$: C, 75.66; H, 10.48. Found: C, 75.74; H, 10.49.

5.1.10. 22α-Methoxyolean-12-ene-3β,24-diol (14). Compound **14** was prepared as described for **7** from **3** in 69% yield from **11**. Compound **14**: mp 214–215 °C; $[\alpha]_D^{23}$ +83.1 (*c* 0.96, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 0.96 (3H, s), 1.14 (3H, s), 1.25 (3H, s), 0.83–1.99 (21H, m), 2.49 (1H, br s), 2.76 (1H, d, *J* = 7.2 Hz), 2.96 (1H, dd, *J* = 4.5 and 12.2 Hz), 3.33 (3H, s), 3.33–3.37 (1H, m), 3.42–3.47 (1H, m), 4.20 (1H, d, *J* = 11.3 Hz), 5.19 (1H, t, *J* = 3.6 Hz); Anal. Calcd for $C_{31}H_{52}O_3 \cdot 1/2H_2O$: C, 77.29; H, 11.09. Found: C, 77.42; H, 11.08.

5.1.11. Soyasapogenol C (16). A solution of **3** (500 mg, 1.0 mmol) in pyridine (5 ml) was treated with *p*-toluenesulfonyl chloride (287 mg, 1.5 mmol) and a small amount of 4-DMAP at room temperature. After stirring overnight, the mixture was cooled to 0 °C, diluted with water, and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated in vacuo to furnish the desired intermediary tosylate as an oil (654 mg, 100%).

The crude tosylate (65 mg) was treated with 1.0 M THF solution of lithium triethylborohydride (2 ml) at 65 °C for 1 h. After cooling to room temperature, the mixture was added with water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to afford the olefin **15** (38 mg, 79%) as a white solid.

The olefin **15** thus obtained was subjected to the deprotection reaction using the same procedure as for **4** from **3** to furnish **16** (36 mg, 82%) as a white solid. Compound **16**: mp 234–235 °C (lit.[15] mp 239.5–240 °C); $[\alpha]_D^{23}$ +65.7 (*c* 0.99, $CHCl_3$) {lit.[15] $[\alpha]_D$ + 65 (c 0.93, $CHCl_3$)}. The ^1H NMR spectroscopy and mass spectrometry data for synthetic **16** were identical to the data reported for natural material.[15]

5.1.12. Olean-12-ene-3β,24-diol (17). A solution of **15** (30 mg, 0.062 mmol) in CH_2Cl_2/MeOH (3 ml, 1:2) was treated with 10% Pd/C (5 mg) and hydrogenated under atmospheric pressure at room temperature overnight. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give **17** (26 mg, 93%) as a white solid. Compound **17**: mp 243–245 °C (lit.[16] mp 251–253 °C); $[\alpha]_D^{23}$ +92.7 (*c* 0.96, $CHCl_3$) {lit.[16] $[\alpha]_D^{23}$ +84.7 (*c* 0.46, $CHCl_3$)}. The ^1H NMR spectroscopy and mass spectrometry data for synthetic **17** were identical to the data reported for natural material.[16]

5.1.13. 22α-Methyllolean-12-ene-3β,22β,24-triol (19). A solution of **18** (1.3 g, 2.85 mmol) in dry THF (70 ml) was treated dropwise with MeLi (1.08 M in Et_2O, 15.8 ml, 17.1 mmol) at −78 °C, then the whole mixture was allowed to warm to 0 °C. After stirring further for 1 h, the mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/THF, 3:1) to afford **19** (808 mg, 60%) as a white solid. Compound **19**: mp 244–245 °C; $[\alpha]_D^{23}$ +80.7 (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.86 (3H, s), 0.89 (6H, s), 0.96 (3H, s), 1.07 (3H, s), 1.11 (3H, s), 1.16 (3H, s), 1.25 (3H, s), 0.84–1.89 (21H, m), 2.32 (1H, d, J = 10.8 Hz), 2.59 (1H, d, J = 4.4 Hz), 2.84 (1H, dd J = 2.6 and 8.7 Hz), 3.35 (1H, t, J = 11.0 Hz), 3.41–3.48 (1H, m), 4.21 (1H, dd, J = 2.6 and 11.0 Hz), 5.23 (1H, t, J = 3.6 Hz); Anal. Calcd for $C_{31}H_{52}O_3$: C, 78.76; H 11.09. Found: C, 78.91; H, 11.06.

5.1.14. 22β-Hydroxymethyllolean-12-ene-3β,24-diol (20). A slurry of Nysted reagent (20% suspension in THF, 12 ml, 6.2 mmol) and **18** (1.0 g, 2.2 mmol) at −78 °C was added with a 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (5 ml, 5 mmol) dropwise over 3 min. The reaction mixture was cooled to 0 °C, added with 1 N HCl (6 ml), and the whole mixture was extracted with $CHCl_3$ (100 ml ×2). The combined organic layers were dried, filtered, concentrated, and subjected to flash chromatography on silica gel to furnish the 22-*exo*-methylene (518 mg, 52%).

A stirred solution of the 22-*exo*-methylene (300 mg, 0.66 mmol) in dry THF (7 ml) was treated with a borane–THF complex (1.0 M borane solution in THF, 3.3 ml, 3.3 mmol) at room temperature. After standing for an additional 20 h, the reaction mixture was cooled to 0 °C and was treated cautiously with 10% aqueous NaOH (3 ml) and then with 30% H_2O_2 (3 ml) within 5 min. After stirring at 0 °C for another 45 min, the whole mixture was extracted with EtOAc (30 ml ×2) and the combined organic layers were washed with brine (10 ml) and dried over $MgSO_4$. The solution was filtered, and the solvent was evaporated to yield a crude product, which was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 1:1) to give **20** (245 mg, 79%) as a white solid. Compound **20**: mp 281 °C dec; $[\alpha]_D^{23}$ +68.2 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.70 (3H, s), 0.90 (3H, s), 0.91 (3H, s), 0.92 (3H, s), 0.96 (3H, s), 1.05 (3H, s), 1.25 (3H, s), 0.84–1.87 (23H, m), 2.47 (1H, br s), 2.76 (1H, br s), 3.28–3.35 (2H, m), 3.45 (1H, dd, J = 4.2 and 11.7 Hz), 3.68 (1H, dd, J = 4.7 and 10.5 Hz), 4.20 (1H, d, J = 11.0 Hz), 5.25 (1H, t, J = 3.6 Hz); Anal. Calcd for $C_{31}H_{52}O_3 \cdot 1/3H_2O$: C, 77.77; H, 11.09. Found: C, 77.65; H, 11.00.

5.1.15. 3β,24-Dihydroxyolean-12-ene-22β-carboxylic acid (22). A solution of **20** (200 mg, 0.42 mmol) in acetone (13 ml) was treated with 2,2-dimethoxypropane (1 ml, 8.2 mmol) and DL-10-camphorsulfonic acid (3 mg, 0.01 mmol). After stirring for 20 h at 37 °C, the reaction mixture was concentrated and diluted with EtOAc (50 ml). The whole mixture was washed with saturated $NaHCO_3$ (5 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in EtOAc (10 ml) and the solution was added with silica gel (5 g). After stirring at room temperature for 2 days, the mixture was filtered, concentrated in vacuo, and the product was purified by flash chromatography (*n*-hexane/EtOAc, 5:1) to give 22β-hydroxymethyl-3β,24-isopropylidenedioxyolean-12-ene (105 mg, 48%).

A solution of oxalyl chloride (0.15 ml, 1.7 mmol) in dry CH_2Cl_2 (4 ml) was cooled to −78 °C. The cooled solution was added with dry DMSO (0.23 ml, 3.2 mmol) in dry CH_2Cl_2 (1 ml). The whole mixture was stirred for 10 min and was added dropwise within 3 min with the solution of above-obtained alcohol (105 mg, 0.21 mmol) in dry CH_2Cl_2 (2 ml). The whole solution was stirred at −78 °C for 15 min, then treated with triethylamine (0.7 ml, 4.8 mmol), and the reaction mixture was stirred for 5 min and allowed to warm to 0–5 °C. Water (5 ml) was added and the aqueous layer was extracted with CH_2Cl_2 (20 ml ×3). The combined organic layers were washed with brine (10 ml), dried over $MgSO_4$, filtered, concentrated in vacuo, and subjected to flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to give **21** (91 mg, 87%).

A solution of the aldehyde **21** (20 mg, 0.039 mmol) in ^{t}BuOH (1 ml) and 2-methyl-2-butene (0.2 ml, 1.8 mmol) was treated with a solution of sodium chlorite (35 mg, 0.39 mmol) and sodium dihydrogenphosphate (61 mg, 0.39 mmol) in water (0.4 ml). After stirring at room temperature overnight, the reaction mixture was concentrated in vacuo, diluted with EtOAc (10 ml), and washed with water (2 ml). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to give 3β,24-isopropylidenedioxyolean-12-ene-22β-carboxylic acid (21 mg, 99%).

3β,24-Isopropylidenedioxyolean-12-ene-22β-carboxylic acid was subjected to the deprotection reaction through the same procedure as for **4** from **3** to give **22** (12 mg,

64%) as a white solid. Compound **22**: mp 280–281 °C; $[\alpha]_D^{23}$ +84.2 (*c* 1.02, MeOH); ^{1}H NMR ($CDCl_3$-CD_3OD) δ 0.81 (3H, s), 0.90 (9H, s), 0.98 (3H, s), 1.06 (3H, s), 1.23 (3H, s), 0.89–1.90 (21H, m), 2.20 (1H, d, J = 13.6 Hz), 3.32 (1H, d, J = 11.1 Hz), 3.40 (1H, dd, J = 4.3 and 11.2 Hz), 4.20 (1H, d, J = 11.1 Hz), 5.28 (1H, t, J = 3.4 Hz); Anal. Calcd for $C_{31}H_{50}O_4 \cdot 1/2H_2O$: C, 75.11; H, 10.37. Found: C, 75.25; H, 10.23.

5.1.16. 24-Oxolean-12-ene-3β,22β-diol (26). A solution of **23** (95 mg, 0.14 mmol) in DMF (5 ml) at room temperature was treated with 60% sodium hydride (83 mg, 2.1 mmol) and stirred for 1.5 h. To the solution was added benzyl bromide (75 mg, 0.44 mmol) at room temperature. The mixture was stirred at 40 °C for 5 h, diluted with EtOAc, and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (*n*-hexane/EtOAc, 10:1) to afford 3β,22β-dibenzyloxy-24-trityloxyolean-12-ene (118 mg, 65%) as a white solid.

A solution of 3β,22β-dibenzyloxy-24-trityloxyolean-12-ene (440 mg, 0.50 mmol) in MeOH/acetone (12 ml, 5:1) was treated with concd HCl (0.4 ml). The mixture was heated to reflux and stirred for 0.5 h. The solution was neutralized with 1 N KOH and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to afford **24** (231 mg, 72%) as a white solid.

A solution of oxalyl chloride (0.15 ml, 1.6 mmol) in dry CH_2Cl_2 (4 ml) was cooled to −78 °C and was treated with dry DMSO (0.23 ml, 3.2 mmol) in dry CH_2Cl_2 (1 ml). The whole mixture was stirred for 10 min and was added dropwise within 3 min with a solution of the alcohol **24** (128 mg, 0.2 mmol) in dry CH_2Cl_2 (2 ml). The reaction mixture was stirred at −78 °C for 15 min and then was added with triethylamine (0.7 ml, 4.8 mmol). After stirring for 5 min, the reaction mixture was allowed to warm to 0–5 °C. Water (5 ml) was added and the aqueous layer was extracted with CH_2Cl_2 (20 ml ×3). The combined organic layers were washed with brine (10 ml), dried over $MgSO_4$, filtered, concentrated in vacuo, and the product was subjected to flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to give **25** (105 mg, 82%).

A solution of **25** (30 mg, 0.047 mmol) in CH_2Cl_2/MeOH (2 ml, 1:1) was treated with 20% $Pd(OH)_2$/C (30 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give **26** (21 mg, 98%) as a white solid. Compound **26**: mp 202–204 °C; $[\alpha]_D^{23}$ +84.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (6 H, s), 0.92 (3H, s), 1.00 (3H, s), 1.04 (3H, s), 1.13 (3H, s), 1.30 (3H, s), 0.88–2.14 (23H, m), 3.18–3.24 (1H, m), 3.43 (1H, t, J = 5.2 Hz), 5.27 (1H, t, J = 3.6 Hz), 9.76 (1H, d, J = 2.7 Hz); Anal. Calcd for $C_{30}H_{48}O_3 \cdot 4/5H_2O$: C, 76.48; H, 10.61. Found: C, 76.36; H, 10.65.

5.1.17. Sophoradiol (27). A solution of **25** (25 mg, 0.039 mmol) in diethylene glycol (2.5 ml) and EtOH (3 ml) was treated with $NH_2NH_2 \cdot H_2O$ (1 ml). The mixture was heated to reflux and stirred for 1 h. After the solution was cooled to room temperature, EtOH was removed in vacuo. The residue was treated with KOH (250 mg) and the resulting mixture was heated to reflux and stirred for 2 h. The mixture was poured into water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by preparative TLC (*n*-hexane/EtOAc, 15:1) to give 3β,22β-dibenzyloxyolean-12-ene (18 mg, 71%).

A solution of 3β,22β-dibenzyloxyolean-12-ene (15 mg, 0.024 mmol) in CH_2Cl_2/MeOH (2 ml, 1:1) was treated with 20% $Pd(OH)_2$/C (15 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give **27** (10 mg, 93%) as a white solid. Compound **27**: mp 224–225 °C (lit.[22] mp 221–222 °C); $[\alpha]_D^{23}$ +90.1 (*c* 0.94, $CHCl_3$) {lit.[22] $[\alpha]_D^{20}$ +88 ($CHCl_3$)}. The ^{1}H NMR spectroscopy and mass spectrometry data for synthetic **27** were identical to the data reported for natural material.[22]

5.1.18. 3β,22β-Dihydroxyolean-12-en-24-oic acid (29). A solution of the aldehyde **25** (20 mg, 0.03 mmol) and 2-methyl-2-butene (0.2 ml, 1.8 mmol) in ^{t}BuOH (1 ml) was treated with a solution of sodium chlorite (35 mg, 0.39 mmol) and sodium dihydrogenphosphate (61 mg, 0.39 mmol) in water (0.4 ml). After stirring at room temperature overnight, the reaction mixture was concentrated in vacuo, diluted with EtOAc (10 ml), and washed with water (2 ml). The organic layer was dried over $MgSO_4$, filtered, concentrated, and subjected to preparative TLC (*n*-hexane/EtOAc, 3/1) to give **28** (7 mg, 34%).

A solution of **28** (5 mg, 7.7×10^{-3} mmol) in CH_2Cl_2/MeOH (1 ml, 1:1) was treated with 10% Pd/C (5 mg) and hydrogenated under atmospheric pressure at room temperature for 45 min. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give **29** (3 mg, 92%) as a white solid. Compound **29**: mp >300 °C; $[\alpha]_D^{23}$ +122.7 (*c* 0.55, MeOH); ^{1}H NMR ($CDCl_3$–CD_3OD) δ 0.86 (3H, s), 0.92 (6H, s), 1.00 (3H, s), 1.03 (3H, s), 1.11 (3H, s), 1.43 (3H, s), 0.90–2.10 (21H, m), 3.11 (1H, dd, J = 4.1 and 12.1 Hz), 3.43 (1H, dd, J = 3.9 and 7.2 Hz), 5.26 (1H, t, J = 3.5 Hz); Anal. Calcd for $C_{30}H_{48}O_4$: C, 76.23; H 10.24. Found: C, 76.07; H, 10.07.

5.1.19. *N*-*n*-Butyl-3β,22β-dihydroxyolean-12-ene-24-amide (30). A solution of **28** (20 mg, 0.03 mmol) in dry DMF (1 ml) was added with BOP (16 mg, 0.037 mmol). After stirring at room temperature for 2.5 h, the mixture was added with *n*-butylamine (0.1 ml, 1.0 mmol). After further stirring for 1 h at room temperature, the reaction mixture was diluted with EtOAc (10 ml) and washed with water (5 ml ×2). The organic layer was dried over $MgSO_4$, filtered, concentrated, and subjected to preparative TLC (*n*-hexane/EtOAc, 5:1) to give

N-*n*-butyl-3β,22β-dibenzyloxyolean-12-ene-24-amide (16 mg, 73%).

A solution of *N*-*n*-butyl-3β,22β-dibenzyloxyolean-12-ene-24-amide (13 mg, 0.018 mmol) in CH_2Cl_2/MeOH (2 ml, 1:1) was treated with 10% Pd/C (13 mg) and hydrogenated under atmospheric pressure at room temperature for 2 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give **30** (10 mg, 100%) as a white solid. Compound **30**: mp 223–225 °C; $[\alpha]_D^{23}$ +113.1 (*c* 0.96, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.94 (3H, t, J = 7.4 Hz), 1.02 (3H, s), 1.04 (3H, s), 1.12 (3H, s), 1.38 (3H, s), 0.88–2.25 (26H, m), 3.10–3.17 (1H, m), 3.19–3.25 (2H, m), 3.44 (1H, t, J = 4.8 Hz), 3.80 (1H, d, J = 9.5 Hz), 5.27 (1H, t, J = 3.6 Hz), 6.00 (1H, t, J = 4.8 Hz); Anal. Calcd for $C_{34}H_{57}NO_3 \cdot 1/4H_2O$: C, 76.71; H, 10.88; N, 2.63. Found: C, 76.75; H, 10.63; N, 2.60.

5.1.20. 24-Acetoxyolean-12-ene-3β,22β-diol (31). A solution of **24** (30 mg, 0.047 mmol) in pyridine (1 ml) was treated with acetic anhydride (1 ml) at room temperature. After stirring for 20 h, the mixture was diluted with EtOAc, washed with water, and the organic layer was dried over $MgSO_4$, filtered, concentrated, and subjected to preparative TLC (*n*-hexane/EtOAc, 5:1) to furnish 24-acetoxy-3β,22β-dibenzyloxyolean-12-ene (24 mg, 76%).

A solution of 24-acetoxy-3β,22β-dibenzyloxyolean-12-ene (24 mg, 0.035 mmol) in CH_2Cl_2/MeOH (3 ml, 1:2) was treated with 20% $Pd(OH)_2$/C (18 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give **31** (12 mg, 69%) as a white solid. Compound **31**: mp 198–200 °C; $[\alpha]_D^{23}$ +103.4 (*c* 0.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, s), 0.91 (3H, s), 0.94 (3H, s), 0.96 (3H, s), 1.04 (3H, s), 1.12 (3H, s), 1.16 (3H, s), 2.07 (3H, s), 0.86–2.12 (23H, m), 3.27–3.31 (1H, m), 3.44 (1H, t, J = 5.3 Hz), 4.15 (1H, d, J = 11.7 Hz), 4.35 (1H, d, J = 11.7 Hz), 5.25 (1H, t, J = 3.6 Hz); Anal. Calcd for $C_{32}H_{52}O_4 \cdot CH_3OH$: C, 74.39; H, 10.59. Found: C, 74.53; H, 10.57.

5.1.21. 24-Methoxyolean-12-ene-3β,22β-diol (32). A solution of **24** (800 mg, 1.3 mmol) in DMF (10 ml) was treated with 60% sodium hydride (100 mg, 2.5 mmol) at room temperature. After stirring for 1 h, methyl iodide (355 mg, 2.5 mmol) was added to the resulting suspension. After stirring at room temperature for 2 h, the reaction mixture was added with additional 60% sodium hydride (100 mg, 2.5 mmol) and methyl iodide (355 mg, 2.5 mmol), and stirred for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 30:1) to afford 3β,22β-dibenzyloxy-24-methoxyolean-12-ene (689 mg, 84%) as a white solid.

A solution of 3β,22β-dibenzyloxy-24-methoxyolean-12-ene (689 mg, 1.1 mmol) in EtOAc (12 ml) and EtOH (4 ml) was treated with 20% $Pd(OH)_2$/C (50 mg) and hydrogenated under atmospheric pressure at room temperature for 5 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to afford **32** (250 mg, 50%) as a white solid. Compound **32**: mp 189–191 °C (lit.[23] mp 190–191 °C); $[\alpha]_D^{23}$ +99.6 (*c* 0.95, $CHCl_3$) {lit.[23] $[\alpha]_D^{20}$ +50.1 (*c* 1.0, $CHCl_3$)}. The ^{1}H NMR spectroscopy and mass spectrometry data for synthetic **32** were identical to the data reported for authentic sample.[23]

5.1.22. 3β-Methoxyolean-12-ene-22β,24-diol (35). A solution of **1** (1.0 g, 2.18 mmol) in dry DMF (10 ml) was added with benzaldehyde dimethyl acetal (365 mg, 2.40 mmol) and DL-camphorsulfonic acid (15 mg, 0.06 mmol). After stirring at 45 °C for 4 h, the reaction mixture was added with additional benzaldehyde dimethyl acetal (532 mg, 3.50 mmol). The reaction mixture was stirred at 45 °C overnight, diluted with saturated $NaHCO_3$ and water, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 5:1) to give the benzylidene acetal (658 mg, 83%) as a white solid.

The solution of benzylidene acetal (28.7 g, 53.56 mmol) in dry DMF (280 ml) was added with 60% sodium hydride (4.2 g, 105.1 mmol) and benzyl bromide (12.5 ml, 105.1 mmol). The mixture was stirred at 45 °C for 1.5 h and treated with additional 60% sodium hydride (4.2 g, 105.1 mmol) and benzyl bromide (12.5 ml, 105.1 mmol). After 2 h, the reaction mixture was diluted with water and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 15:1) to give **33** (18.8 g, 56%) as a white solid.

A solution of **33** (400 mg, 0.63 mmol) in toluene (2 ml) was treated with a solution of DIBAL (1.0 M in toluene, 3.77 ml, 3.77 mmol) in toluene (6 ml) at 0 °C. After stirring at 0 °C for 2 h, the reaction was quenched with water, and the whole was extracted with EtOAc, the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by preparative TLC (*n*-hexane/EtOAc, 5:1) to give the 24-benzyl ether **34** (275 mg, 69%) and 3-benzyl ether **24** (25 mg, 6%).

A solution of **34** (100 mg, 0.16 mmol) in dry DMF (1 ml) was treated with 60% sodium hydride (31 mg, 0.80 mmol). After stirring for 1 h at room temperature, methyl iodide (50 ml, 0.80 mmol) was added. After further stirring for 20 h, the whole mixture was diluted with EtOAc, washed with water, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by preparative TLC (*n*-hexane/EtOAc, 5:1) to give 22β,24-dibenzyloxy-3β-methoxyolean-12-ene (49 mg, 47%) as a white solid.

A solution of 22β,24-dibenzyloxy-3β-methoxyolean-12-ene (48 mg, 0.073 mmol) in CH_2Cl_2/MeOH (3 ml, 1:1)

was treated with 20% $Pd(OH)_2$/C (10 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was purified by preparative TLC (CH_2Cl_2/MeOH, 30:1) to give **35** (21 mg, 59%) as a white solid. Compound **35**: mp 283–284 °C; $[\alpha]_D^{23}$ +91.9 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (3H, s), 0.88 (3H, s), 0.91 (3H, s), 0.95 (3H, s), 1.04 (3H, s), 1.11 (3H, s), 1.20 (3H, s), 0.84–2.14 (22H, m), 2.93 (1H, dd, J = 4.4 and 11.8 Hz), 3.18–3.26 (2H, m), 3.36 (3H, s), 3.41–3.45 (1H, m), 4.09–4.14 (1H, m), 5.25 (1H, t, J = 3.6 Hz); Anal. Calcd for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.66; H, 10.80.

5.1.23. 12α,13α-Epoxyolean-3β,22β,24-triol (36). A solution of **1** (230 mg, 0.50 mmol) in CH_2Cl_2 (10 ml) and $CHCl_3$ (3 ml) was treated with 50–60% *m*CPBA (216 mg). After stirring at room temperature overnight, the mixture was diluted with CH_2Cl_2 and washed with 5% $Na_2S_2O_3$, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 1:1) to afford the epoxide **36** (193 mg, 81%) as a white solid. Compound **36**: mp 249–251 °C; $[\alpha]_D^{23}$ +36.2 (*c* 0.94, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (3H, s), 0.90 (3H, s), 0.97 (3H, s), 0.98 (3H, s), 0.99 (3H, s), 1.04 (3H, s), 1.22 (3H, s), 0.74–1.87 (22H, m), 2.36 (1H, d, J = 4.2 Hz), 2.76 (1H, dd, J = 2.5 and 9.2 Hz), 3.05 (1H, s), 3.29 (1H, t, J = 11.1 Hz), 3.40–3.45 (1H, m), 3.55–3.60 (1H, m), 4.17 (1H, dd, J = 2.5 and 11.1 Hz); Anal. Calcd for $C_{30}H_{50}O_4 \cdot 1/2H_2O$: C, 74.49; H, 10.63. Found: C, 74.50; H, 10.36.

5.1.24. β-Amyrin (39). A solution of **17** (1.00 g, 2.26 mmol) in pyridine (10 ml) was treated with trityl chloride (881 mg, 3.16 mmol). The mixture was heated to reflux and stirred for 5 h. After the solution was cooled to room temperature, the solvent was removed in vacuo and water was added to the residue. The mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to give 24-trityloxyolean-12-en-3β-ol. Without further purification, 24-trityloxyolean-12-en-3β-ol was dissolved in CH_2Cl_2 (20 ml), and 4-DMAP (690 mg, 5.65 mmol) and benzoyl chloride (474 mg, 3.39 mmol) were added. The solution was stirred at room temperature for 2 h. The mixture was poured into water and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 30:1) to afford 3β-benzoyloxy-24-trityloxyolean-12-ene (1.70 g, 95%).

A solution of 3β-benzoyloxy-24-trityloxyolean-12-ene (1.70 g, 2.16 mmol) in acetone (50 ml) and MeOH (20 ml) was treated with concd HCl (0.5 ml). The mixture was warmed to 60 °C and stirred for 2 h. The solution was neutralized with $NaHCO_3$. The solvent was removed in vacuo and water was added to the residue. The mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 10:1) to afford **37** (818 mg, 69%) as a white solid.

A solution of **37** (150 mg, 0.275 mmol) in CH_2Cl_2 (5 ml) was treated with PCC (71 mg, 0.33 mmol). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and filtered through a short pack of silica gel. The filtrate was concentrated in vacuo and purified by column chromatography on silica gel (*n*-hexane/EtOAc, 20:1) to give 3β-benzoyloxy-24-oxoolean-12-ene (142 mg, 95%) as a white solid.

A solution of 3β-benzoyloxy-24-oxoolean-12-ene (121 mg, 0.222 mmol) in MeOH (3 ml) and THF (4 ml) was treated with 1 N NaOH (0.5 ml). The mixture was stirred at room temperature for 3 h. The solution was neutralized with 1 N HCl. The solvent was removed in vacuo and water was added to the residue. The mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 12:1) to afford **38** (75.4 mg, 77%) as a white solid.

A solution of **38** (50.0 mg, 0.114 mmol) in diethylene glycol (5 ml) and EtOH (15 ml) was treated with $NH_2NH_2 \cdot H_2O$ (2.5 ml). The mixture was heated to reflux and stirred for 1 h. After the solution was cooled to room temperature, EtOH was removed in vacuo. The residue was treated with KOH (300 mg) and the resulting mixture was heated to reflux and stirred for 2 h. The mixture was poured into water and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (*n*-hexane/THF, 4.5:1) to afford **39** (37.3 mg, 77%) as a white solid. Compound **39**: mp 196–197 °C (lit.[26] mp 194–196 °C); $[\alpha]_D^{23}$ +89.0 (*c* 0.89, $CHCl_3$) {lit.[26] $[\alpha]_D^{23}$ +83.6 (*c* 0.4, $CHCl_3$)}. The ^1H NMR spectroscopy and mass spectrometry data for synthetic **39** were identical to the data reported for authentic sample.[26]

5.2. X-ray crystallographic analysis

X-ray data of the 3,24-diacetate derivative of **19** and the triols **20** and **36** have been deposited in the Cambridge Crystallographic Data Centre as deposition numbers CCDC 255979, CCDC 241828, and CCDC 241826, respectively. These data can be obtained free of charge by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: data_request@ccdc.cam.ac.uk].

5.3. Biological method

5.3.1. Concanavalin A-induced hepatitis. Male BALB/c mice were purchased from Charles River Laboratory (Yokohama, Japan). All the experiments were conducted in accordance with local institutional guidelines for the care and use of laboratory animals. Control mice were given solvent (100% polyethylene glycol #400 (PEG-400): 100% dimethylsulfoxide (DMSO): 0.5% carboxymethyl cellulose sodium salt (CMC) = 1:1:2)

administered subcutaneously (sc) into the back at 2 and 14 h prior to an intravenous injection of sterilized phosphate-buffered saline (control group). Concanavalin A at a dose of 20 mg/kg body weight was intravenously injected (concanavalin A alone group). A suspended solution containing compound at the doses shown in the legend was injected sc into the back of the animals at 2 and 14 h prior to an intravenous injection of concanavalin A (concanavalin A + compound group). The animals were not starved overnight before being killed and were quickly decapitated under ether anesthesia between 09.00 and 10.00 h.

Plasma was obtained 24 h after concanavalin A injection. Alanine aminotransferase levels were determined at 340 nm according to the standard method, using an automatic analyzer. The data evaluated were based on Student's *t*-test.

Acknowledgments

We gratefully thank Dr. Isao Kitagawa (Professor Emeritus, Osaka University) for helpful comments. We also thank Drs. Y. Kodama and N. Watanabe for the X-ray crystallographic analysis, and Ms. S. Miki for the mass spectral analyses.

References and notes

1. Cohen, J. *Science* **1999**, *285*, 26–30.
2. Di Bisceglie, A. M. *Hepatology* **1997**, *26*, 34s–38s.
3. Lin, R.; Roach, E.; Zimmerman, M.; Strasser, S.; Farrell, G. C. *J. Hepatol.* **1995**, *23*, 487–496.
4. Dienstag, J. L.; Schiff, E. R.; Wright, T. L.; Perrillo, R. P.; Hann, H. L.; Goodman, Z.; Crowther, L.; Condreay, L. D.; Woessner, M.; Rubin, M.; Brown, N. A. *N. Engl. J. Med.* **1999**, *341*, 1256–1263.
5. Dusheiko, G.; Main, J.; Thomas, H.; Reichard, O.; Lee, C.; Dhillon, A.; Rassam, S.; Fryden, A.; Reesink, H.; Bassendine, M.; Norkrans, G.; Cuypers, T.; Lelie, N.; Telfer, P.; Watson, J.; Weegink, C.; Sillikens, P.; Weiland, O. *J. Hepatol.* **1996**, *25*, 591–598.
6. (a) Davis, G. L.; Esteban-Mur, R.; Rustgi, V.; Hoefs, J.; Gordon, S. C.; Trepo, C.; Shiffman, M. L.; Zeuzem, S.; Craxi, A.; Ling, M.; Albrecht, J. *N. Engl. J. Med.* **1998**, *339*, 1493–1499; (b) McHutchison, J. G.; Gordon, S. C.; Schiff, E. R.; Shiffman, M. L.; Lee, W. M.; Rustgi, V. K.; Goodman, Z. D.; Ling, M.; Cort, S.; Albrecht, J. K. *N. Engl. J. Med.* **1998**, *339*, 1485–1492.
7. Liaw, Y.; Lee, C.; Tsai, S.; Liaw, B.; Chen, T.; Sheen, I.; Chu, C. *Hepatology* **1995**, *22*, 1368–1373.
8. Tiegs, G.; Hentschel, J.; Wendel, A. *J. Clin. Invest.* **1992**, *90*, 196–203.
9. Kuzuhara, H.; Nishiyama, S.; Minowa, N.; Sasaki, K.; Omoto, S. *Eur. J. Pharmacol.* **2000**, *391*, 175–181.
10. Siebler, J.; Wirtz, S.; Klein, S.; Protschka, M.; Blessing, M.; Galle, P. R.; Neurath, M. F. *Hepatology* **2003**, *38*, 1573–1580.
11. Takeda, K.; Hayakawa, Y.; Van Kaer, L.; Matsuda, H.; Yagita, H.; Okumura, K. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5498–5503.
12. (a) Seger, C.; Sturm, S.; Haslinger, E.; Stuppner, H. *Org. Lett.* **2004**, *6*, 633–636; (b) Yoshikawa, M.; Morikawa, T.; Kashima, Y.; Ninomiya, K.; Matsuda, H. *J. Nat. Prod.* **2003**, *66*, 922–927; (c) Adnyana, I. K.; Tezuka, Y.; Banskota, A. H.; Tran, K. Q.; Kadota, S. *J. Nat. Prod.* **2001**, *64*, 360–363.
13. Sasaki, K.; Minowa, N.; Kuzuhara, H.; Nishiyama, S.; Omoto, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 85–88.
14. (a) Krishnamurthy, S.; Brown, H. C. *J. Org. Chem.* **1976**, *41*, 3064–3066; (b) Holder, R. W.; Matturro, M. G. *J. Org. Chem.* **1977**, *42*, 2166–2168.
15. (a) Meyer, A.; Jeger, O.; Ruzicka, L. *Helv. Chim. Acta* **1950**, *33*, 672–687; (b) Heftmann, E.; Lundin, R. E.; Haddon, W. F.; Peri, I.; Mor, U.; Bondi, A. *J. Nat. Prod.* **1979**, *42*, 410–416; (c) Nes, W. D.; Benson, M.; Heftmann, E. *Phytochemistry* **1981**, *20*, 2299–2300.
16. (a) Ogihara, K.; Higa, M.; Hokama, K.; Suga, T. *Phytochemistry* **1987**, *26*, 783–785; (b) Tanaka, R.; Tabuse, M.; Matsunaga, S. *Phytochemistry* **1988**, *27*, 3563–3567.
17. Watson, A. T.; Park, K.; Wiemer, D. F.; Scott, W. J. *J. Org. Chem.* **1995**, *60*, 5102–5106.
18. Sasaki, K.; Minowa, N.; Kuzuhara, H.; Nishiyama, S.; Omoto, S. *Tetrahedron Lett.* **1997**, *38*, 8335–8336.
19. (a) Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888–890; (b) Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175–1196.
20. Kitagawa, I.; Wang, H. K.; Taniyama, T.; Yoshikawa, M. *Chem. Pharm. Bull.* **1988**, *36*, 153–161.
21. Huang-Minlon, J. *J. Am. Chem. Soc.* **1946**, *68*, 2487.
22. (a) Kariyone, T.; Ishimasa, S.; Shiomi, T. *Yakugaku Zasshi* **1956**, *76*, 1210–1211; (b) Chiang, T. C.; Chang, H. M. *Planta Med.* **1982**, *46*, 52–55; (c) Fish, P. V.; Johnson, W. S. *J. Org. Chem.* **1994**, *59*, 2324–2335.
23. Yoshikawa, M.; Wang, H. K.; Kayakiri, H.; Taniyama, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1985**, *33*, 4267–4274.
24. Mikami, T.; Asano, H.; Mitsunobu, O. *Chem. Lett.* **1987**, 2033–2036.
25. Boar, R. B.; Joukhadar, L.; Luque, M.; McGhie, J. F.; Barton, D. H. R.; Arigoni, D.; Brunner, H. G.; Giger, R. *J. Chem. Soc., Perkin Trans.* 1 **1977**, 2104–2109.
26. Corey, E. J.; Lee, J. *J. Am. Chem. Soc.* **1993**, *115*, 8873–8874.
27. Kitagawa, I.; Yoshikawa, M.; Yosioka, I. *Chem. Pharm. Bull.* **1976**, *24*, 121–129.