Saponins Isolated from *Allium chinense* G. DON and Antitumor-promoting Activities of Isoliquiritigenin and Laxogenin from the Same Drug\(^1\)

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Received November 1, 1999; accepted February 2, 2000

Investigation of the Chinese crude drug “Xiebai,” the bulbs of *Allium chinense* G. DON (Liliaceae), led to the isolation of 2 saponins, xiebai-saponin 1 (laxogenin 3-O-β-xylopyranosyl (1→4)-α-arabinopyranosyl (1→6)-β-glucopyranoside) (1) and laxogenin 3-O-α-arabinopyranosyl (1→6)-β-glucopyranoside (2), and the aglycone, laxogenin (3), together with 2 chalcones, isoliquiritigenin (4) and isoliquiritigenin-4-O-glucoside (5), and β-sitosterol glucoside (6). Compounds 1–5 were tested *in vitro* for their inhibitory effect on the 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulated \(^{32}\)P-incorporation into phospholipids of HeLa cells. In addition to this, laxogenin (3) was proven to have an antitumor-promoting activity in a two-stage lung carcinogenesis experiment.

**Key words** *Allium chinense*; xiebai-saponin 1; isoliquiritigenin; antitumor-promoting activity; laxogenin; Liliaceae

*Allium chinense* G. DON has been cultivated since ancient times, and the bulbs, named “Rakkyō” in Japan, are widely used as pickles and spices. The bulbs of both *Allium chinense* G. DON and *A. macrostemum* Bunge are also main sources of a Chinese traditional medicine “Xiebai”, which is used for the treatment for chest pain, stenocardia, and heart asthma. We were the first to report on the chemical constituents of the Chinese crude drug “Xiebai” and the pharmacological activities of 3 kinds of Chinese prescriptions containing “Xiebai” against human platelet aggregation.\(^2\)

Afterwards, Okuyama and Yao et al. isolated a number of spirosstane and fuurostane oligoglycosides and sulfur-containing compounds from *A. chinense* and *A. macrostemum*.\(^3,4\) Along this line, we have examined the antitumor-promoting constituents of *Allium* plants on 12-O-tetradecanoylphorbol-13-acetate (TPA)-enhanced phospholipid metabolism and isolated laxogenin and N-p-coumaroyl tyramine from *A. chinense* as the active principles.\(^5\) In the present study, we tried to isolate the more active constituents of *A. chinense* by biological-active directed fractionation using a \(^{32}\)P-incorporation assay.

The fractions which showed potential activity in the \(^{32}\)P-incorporation assay were purified repeatedly, and they gave a new saponin (1) and a known one (2), laxogenin (3) as their aglycone, and 2 chalcones (4 and 5) as active constituents.

A new compound, xiebai-saponin 1 (1), has the molecular formula C\(_{38}\)H\(_{50}\)O\(_{17}\), as determined by FAB-MS at \(m/z\) 856 and the quasi molecular ion peak at \(m/z\) 857 (M+H\(^+\)) in the chemical ionization mass spectrum (CI-MS). Investigation of the NMR spectra (see Table 1) suggested that 1 and 2 are glycosides of 3. From chemical shifts of C\(_{32–26}\) these compounds were ascribable to the (25R)-spirosstane type. The bands of absorption at 982, 918, 900, and 864 cm\(^{-1}\) in the IR spectrum supported the presence of (25R)-spirosstane. In the NMR spectra of 1, 3 anomic protons and carbons were recognized at \(\delta\) 4.92, 5.02, 5.47, and 102.07, 105.14, 105.73, respectively. All the carbons of the aglycone were assigned in comparison with those of 3 and 2 by distortionless enhance-

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Fig. 1. Structures of Compounds 1–5
tained by acid hydrolysis of chinonoside 1 isolated from *A. chinense* G. DON.6) No antitumor property of this compound was previously reported.

The latter compounds 2—6 were characterized by comparison of their spectral data with those in the literature.6—10) Incorporation of $^{32}$Pi into the phospholipids of HeLa cells was assayed by the same method described previously.11) As noted in Table 2, chalcone 4 exhibited potential activity comparable to that of 5, which is a glycoside of 4. Yamamoto et al. reported the antitumor-promoting effect of 4 in *vitro* and *in vivo*.12) Moreover, various biological activities of compound 4 were also reported, e.g. an anti-platelet aggregation effect, inhibition of the aldose reductase, and c-adenosine 5'-diphosphate (ADP) phosphodiesterase.13) 3 showed remarkable activity at a high dose level, while two saponins, 1 and 2, showed strong cytotoxicity to HeLa cells at the same dose. These results suggest that cytotoxicity might be caused by the structures of the sugar chain in these compounds.

We examined the antitumor-promoting activity of 3 in the mouse lung tumor formation induced by 4-nitroquinoline-1-oxide (4-NQO) as an initiator and by glycerol as a promoter. Polyoxyethylene hydrogenated castor oil (HCO-60), a surface-active agent, was used to dissolve 3, while it did not affect tumorigenesis in this experiment. As shown in Table 3, oral administration of 3 reduced the average number of tumors per mouse. The tumors per mouse in control 2 group were 7.8, and those in the group treated with 3 were 1.9. The inhibitory effect of 3 was 75.6% ($p<0.01$, t-test) compared with that in the control 2 group at 25 weeks of promotion.

### MATERIALS AND METHODS

**Plant Material** The Chinese crude drug “Xiebai,” the bulbs of *Allium chinense* G. DON, were purchased from Mikuni Co., Ltd. (Osaka, Japan).

**Extraction and Isolation** The materials (20 kg) were extracted twice with hot water. A small volume of the aqueous solution was lyophilized to yield a water-extract (12 kg). The aqueous solution was partitioned into AcOEt and then *n*-butanol-soluble fractions, which were evaporated in vacuo, and gave corresponding portions (198 g, 1.1 kg), respectively. The AcOEt-soluble part (45.5 g) was chromatographed subsequently on silica gel to give 10 fractions (fr. 1—10). Each fraction was monitored on the TPA-stimulated $^{32}$Pi-incorporation into the phospholipids of HeLa cells. Those fractions which showed potential activity in this assay were purified repeatedly to give a new (1) and a known (2) saponin, laxogenin (3) as their aglycone, and 2 chalcones (4 and 5) as the active constituents. Fraction 8 (24.6 g) was separated by silica gel column chromatography into 11 fractions (fr. 12—22). Then fraction 18 was subjected to a Sephadex LH-20
column (MeOH, detection of eluates by TLC), which gave two saponins, 1 (463 mg) and 2 (229 mg). Moreover, separation of fraction 16 by the same condition gave 3 (695 mg) as their aglycone. Compounds 1 and 2 were recrystallized from MeOH. 3 was purified by HPLC (silica gel (20×250 mm), n-hexane: AcOEt = 1: 4, 8.5 ml/min, t_{R} 14 min).

Fraction 5 (11.6 g) was followed by silica gel column chromatography and a Sephadex LH-20 column (MeOH, detection by TLC), and that chalcone 5 (126 mg). 5 was recrystallized from MeOH–water. 4 was isolated from fraction 2 (9.1 g) by a silica gel column.

Fraction 9 (6.8 g) was separated by a silica gel column, which gave β-sitosterol glucoside (6, 112 mg).

Compounds 1 (Xiebai-saponin I) (1): Colorless needles; mp 265.0—266.0 °C; FAB-MS at m/z 879 (M⁺+Na), 857 (M⁺+H), 431 (aglycone-H); EI-MS at m/z 857 (M⁺+H), IR ν cm⁻¹: 3440 (OH), 2948, 1708 (C=O), 982, 918, 900, 864. ¹H-NMR: δ 4.91 (1H, d, J=7.59 Hz, anomic-H), 5.02 (1H, d, J=7.59 Hz, anomic-H), 5.47 (1H, d, J=7.92 Hz, anomic-H). ¹³C-NMR spectrum was shown in Table 1.

Compounds 2 (2): Colorless needles; mp 265.0—266.0 °C; FAB-MS (Pos.) at m/z 725 (M⁺+1). IR ν cm⁻¹: 3440 (OH), 2948, 1708 (C=O), 982, 918, 900, 864. ¹H-NMR: δ 4.93 (1H, d, J=7.59 Hz, anomic-H), 4.94 (1H, d, J=7.76 Hz, anomic-H). 2 was identified as laxogenin-3-O-α-arabinopyranosyl(1→6)-β-glucopyranoside isolated from Smilax sieboldii (Litaceae) by comparison of its physical and spectroscopic data.

Compounds 3 (3) (laxogenin): Colorless needles; mp 214.0—216.5 °C; EI-MS spectrum m/z 430 (M⁺). 2 was identified as laxogenin isolated from the same material by comparison of its physical and spectroscopic data.

Compounds 4 (4): Yellow powder; mp 202.0—203.0 °C; 4 afforded the molecular weight at m/z 256 in EI-MS spectrum. ¹H and ¹³C-NMR spectra of 4 suggested a chalcone skeleton (see Table 1). In comparing the spectral data with those values reported, 4 was identified as isoliquiritigenin.

Compounds 5 (5): Yellow powder; mp 140.0—142.0 °C. On acetic acid hydrolysis, 5 gave 4 as an aglycone and glucose. 5 was identified as isoholoside by comparison of its physical and spectroscopic data with the reported values. 5

Compounds 6 (6): White powder; mp 284.0—286.0 °C; EI-MS spectrum m/z 576 (M⁺), 414 (aglycone). 6 was identified as β-sitosterol glucoside by comparison of its physical and spectroscopic data with the previous report.

TPA-Enhanced ³²P- Incorporation into the Phospholipid of Cultured Cells Incorporation of ³²P into phospholipids of HeLa cells was assayed by the same method described previously. ¹¹

Two-Stage Mouse Lung Carcinogenesis Experiments This experiment was performed by the same method as indicated in our previous papers. ¹² 1.25 mg of laxogenin (3: 2.91 μmol) was dissolved in 5% 100 ml of glycerol solution with HCO-60. Mice in the control group were given 5% glycerol without (control 1) or with (control 2) HCO-60 as a surface-active agent. Each experimental group consisted of 15 mice.

REFERENCES AND NOTES


