Anti-Tumor-Promoting Activities of Afromosin and Soyasaponin I Isolated from Wistaria brachybotrys

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ANTI-TUMOR-PROMOTING ACTIVITIES OF AFROMOSIN AND SOYASAPONIN I ISOLATED FROM *WISTARIA BRACHYBOTRYS*

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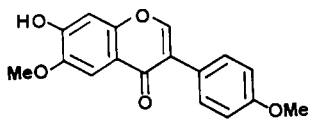
ABSTRACT.—Afromosin [**1**] and soyasaponin I [**2**] isolated from *Wistaria brachybotrys* exhibited remarkable inhibitory effects on mouse skin tumor promotion, and afromosin also exhibited a significant inhibitory effect on pulmonary tumor promotion. The combined effects of these compounds on the two-stage skin carcinogenesis were also examined, and it was concluded that the combination of **1** with **2** enhanced the inhibitory effect.

The knots of *Wistaria brachybotrys* Sieb. et Zucc. (Leguminosae) have been used in Japanese folk medicine for the treatment of gastric cancer (1). The knots are hard swellings or masses formed in the wood.

In a previous paper, we reported that four isoflavonoids and nine triterpenoid saponins were isolated from the knots of *W. brachybotrys*, and the primary screening tests searching for the anti-tumor promoters have been carried out. Some of these compounds exhibited inhibitory effects on the Epstein-Barr virus early antigen (EBV-EA) activation and TPA-stimulated ^{32}P incorporation into phospholipids of HeLa cells (2–4). Particularly, afromosin [**1**] and soyasaponin I [**2**] exhibited most remarkable inhibitory effects on EBV-EA activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). On the other hand, many compounds that inhibit EBV-EA induced by tumor promoters have been shown to act as inhibitors of tumor promotion in vivo (5–11). Therefore, we carried out the two-stage carcinogenesis tests in vivo of afromosin and soyasaponin I on skin tumors and pulmonary tumors.



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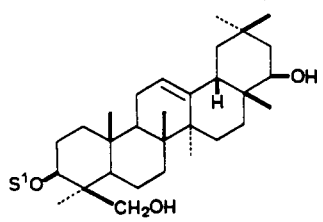

2 S^1=glc acid (2→1) gal (2→1) rha

EXPERIMENTAL

PLANT MATERIAL AND ISOLATION OF AFROMOSIN [**1**].—The knots of *W. brachybotrys* were collected in Shikoku, Japan in 1986. Herbarium specimens have been deposited in the herbarium of Kyoto Pharmaceutical University. Afromosin [**1**] and soyasaponin I [**2**] were isolated from the MeOH extract of the chopped knots of *W. brachybotrys*. The extraction, purification and identification of afromosin and soyasaponin I were carried out as previously described (2–4).

CHEMICALS.—Dimethylbenz[*a*]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were obtained from Sigma Chemical. 4-Nitroquinoline-*N*-oxide (4NQO) and the fine grade of glycerol were purchased from Wako Pure Chemical Industries, Osaka, Japan.

ANIMALS.—Specific-pathogen-free female ICR mice (6 weeks old) were obtained from Nippon SLC Co. (Shizuoka, Japan) and housed in polycarbonate cages in a temperature-controlled room with daily care.

TWO-STAGE CARCINOGENESIS TEST ON SKIN TUMORS.—Each group of 15 mice was housed 5 mice per cage and given H_2O ad libitum. The back of each mouse was shaved with surgical clippers.

The mice were initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA, 100 μg, 394 nmol) in Me_2CO. One week after initiation, they were promoted twice a week by application of TPA (1 μg, 1.6 nmol) in Me_2CO. One hour before each TPA treatment, the mice were treated with sample (85 nmol) in Me_2CO. The incidence of papillomas was observed weekly for 20 weeks (12).

TWO-STAGE CARCINOGENESIS TEST ON PULMONARY TUMORS.—All mice were fed ad libitum with commercial rodent pellets and either tap H_2O or H_2O containing glycerol (8%) (13). The 75 mice were divided into five groups of 15 each. Each experimental group received the following initiation/promotion treatments: (I) drinking water alone, n=15; (II) 8% glycerol solution alone, n=15; (III) 4NQO/H_2O, n=15; (IV) 4NQO/8% glycerol solution, n=15; (V) 4NQO/8% glycerol with afromosin [**1**] (1.25 mg/100 ml) solution, n=15. The total feeding of afromosin was 0.24 mg per mouse per week.

INITIATION.—In olive oil-cholesterol (20:1), 4NQO was dissolved. Dosage of 0.3 mg per mouse was given by a single subcutaneous injection at the starting time (groups III, IV, and V). As a control, the same amount of the oil mixture was injected into mice at the same time (groups I and II).

PROMOTION.—Glycerol was dissolved in H_2O (8%) and given as drinking H_2O ad libitum. Five weeks after initiation, the promoting treatment with 8% glycerol solution (II and IV) or 8% glycerol and afromosin solution (group V) continued for 25 weeks. Other groups (I and III) were given tap H_2O ad libitum. The consumption of the solutions was measured twice a week.

TREATMENT OF ANIMALS.—Each experimental group was killed by cervical dislocation after 25 weeks. Each pulmonary lobe was separated, and the number of induced tumors was counted under a dissecting microscope. The lungs were embedded in paraffin, sectioned, and stained with hematoxylin eosin by conventional methods to study the pulmonary tumors histologically.

RESULTS AND DISCUSSION

On the basis of our reported results in vitro (2–4), the effects of afromosin [**1**] and soyasaponin I [**2**] on two-stage carcinogenesis test of skin tumors using DMBA as an initiator and TPA as a promoter were investigated. The activities, evaluated by both rate (%) of papilloma-bearing mice and average number of papillomas per mouse, were compared with those of a positive control. As shown in Figure 1, both afromosin and soyasaponin I, when applied continuously before each TPA treatment, delayed the formation of papillomas in mouse skin as compared with the control experiment with only TPA, and they

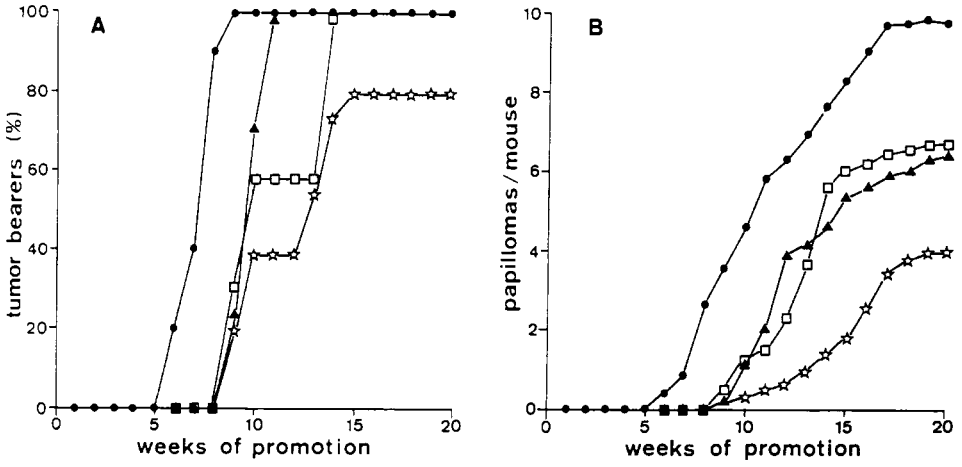

FIGURE 1. Inhibition of TPA-induced tumor promotion by multiple application of afromosin [**1**] (85 nmol), soyasaponin I [**2**] (85 nmol) and combination of **1** (42.5 nmol) with **2** (42.5 nmol). All mice were initiated with DMBA (394 nmol) and promoted with TPA (1.6 nmol) given twice weekly starting 1 week after initiation. A: Percentage of mice with papillomas. B: Average number of papillomas per mouse. ●, control TPA alone; □, TPA+85 nmol of afromosin; ▲, TPA+85 nmol of soyasaponin I; ☆, TPA+42.5 nmol of afromosin+42.5 nmol of soyasaponin I.

TABLE 1. Incidence of Pulmonary Tumors in Mice Treated with Afromosin [**1**].

Group		Total number of tumors	Number of tumors per mouse	Mice with tumor (%)
I	H_2O alone	0	0	0
II	8% glycerol alone	0	0	0
III	4NQO[a]+H_2O	3	0.2	13.3
IV	4NQO+8% glycerol	48	3.2	100
V	4NQO+8% glycerol+afromosin (1.25 mg/100 ml)	14	0.93	40

[a]4NQO=4-nitroquinoline-*N*-oxide (10 mg/kg, hypodermic injection, as an initiator).

reduced the number of papillomas per mouse (about 40% reduction even at 20 weeks). Furthermore, the combined application of afromosin with soyasaponin I enhanced the inhibitory effects both on the rate of papilloma-bearing mice (20% reduction even at 20 weeks) and on the average number of papillomas per mouse (60% reduction even at 20 weeks). In the knots of *W. brachybotrys*, afromosin and soyasaponin I are present in nearly equivalent molar amounts.

These results suggest that afromosin [**1**] and soyasaponin I [**2**] might be valuable anti-tumor-promoters in carcinogenesis. Furthermore, the enhancement of anti-tumor-promoting activity by the combined application of afromosin with soyasaponin I supports the concept of synergistic effects of plural constituents in crude drugs.

The two-stage carcinogenesis test of afromosin on pulmonary tumors was also carried out using 4NQO as an initiator and glycerol as a promoter. As shown in Table 1, both the total number of tumors in 15 mice and percentage of mice with pulmonary tumors were remarkably reduced (60% reduction after 25 weeks on the percentages of mice with tumors) by taking afromosin together with the promoter (group V) compared with the positive control group (group IV). Body weight increase of tested mice was not affected by afromosin treatment.

These results strongly suggest that afromosin and soyasaponin I might be valuable as anti-tumor-promoters in chemical carcinogenesis. The inhibitory mechanisms of these compounds on the tumor promotion are now in progress.

ACKNOWLEDGMENTS

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