Communications to the Editor

Alkane-6,8-diol: Inhibitor of Tumor Promotion in Two-Stage Carcinogenesis in Mouse Skin

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Cancer prevention is now most urgently required for public health. It would be particularly useful to develop a method of prevention at the promotion stage of carcinogenesis, as such a method may be applicable even after exposure to tumor-initiating agents, which seem, in many cases, to be unavoidable in human life. Our earlier studies have demonstrated that some extracts from edible plants and crude drugs inhibited 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced inflammatory ear edema, and some active compounds were separated from the extracts of edible plants and crude drugs.[1–4] Alkane-6,8-diols were isolated from *Carthamus tinctorius* (safflower).[5] These compounds inhibited TPA-induced inflammatory ear edema and tumor promotion in mouse skin (K. Yasukawa et al., unpublished results).[6]

Homologs **4a–c** in the alkane-6,8-diol series were prepared as shown in Scheme 1. The carbanion derived from methyl *p*-tolyl sulfoxide (**1**) with slight excess of lithium diisopropylamide (LDA) was reacted with 1-hexanal to afford a diastereomeric mixture of the 1,2-adduct **2** in 95% yield.[7] In order to obtain 7-(*p*-tolylsulfinyl)-alkane-6,8-diol **3**, the carbanion derived from 1-(*p*-tolylsulfinyl)heptan-2-ol (**2**) with 2 equiv of *n*-BuLi was reacted with aldehydes (RCHO, R = **a–c**) to afford the 1,2-adducts **3a–c** in moderate yields. The aldehydes (R = **b,c**) were derived from oxidation of the corresponding alcohols with 2,2,6,6-tetramethylpiperidin-1-yloxy and sodium bromite in good yield.[8] The sulfinyl group of **3a–c** was reduced with Raney Ni in EtOH at room temperature to give alkane-6,8-diols **4a–c** in moderate yields.

Female ICR mice were housed in an air-conditioned specific pathogene free room (22–23 °C) lit from 08:00 to 20:00. Food and water were available ad libitum. TPA (1 nM) dissolved in acetone (20 μL) was applied to the right ear of each mouse with a micropipette. A volume of 10 μL was delivered to both the inner and outer surfaces of the ear. Twenty microliters of the sample solution, its vehicle, and chloroform–methanol (1:1), as a control, was applied topically ca. 30 min before each TPA treatment. For ear thickness determinations, a pocket thickness gauge (Mitsutoyo Co., Ltd., Tokyo, Japan) with a range of 0–9 mm, graduated at 0.01-mm intervals and modified so that the contact surface area was increased, thus reducing the tension, was applied to the tip of the ear. Ear thickness was determined before treatment (a). Edema was measured at 6 h after TPA treatment (b, TPA alone; b′, TPA plus sample). The following values were then calculated: edema A, edema induced by TPA alone (b − a), and edema B, edema induced by TPA plus sample (b − a).

$$\text{inhibition ratio (\%)} = \frac{\text{edema A} - \text{edema B}}{\text{edema A}} \times 100$$

Each value was the mean of individual determinations from five mice. The 50% inhibitory dose (ID_{50}) values were determined by probit-graphic interpolation for four dose levels.

Of the synthetic alkane-6,8-diols **4a–c** assayed, C_{27},C_{29}-alkane-6,8-diols **4b,c** inhibited TPA-induced inflammatory ear edema in mice, whereas C_{15}-alkane-6,8-diol **4a** proved to have no effect. As Table 1 shows, the ID_{50}s of these compounds on TPA-induced inflammation were 0.5 and 0.4 mg/ear, respectively. In comparison with standard drugs, these compounds inhibited at a level corresponding to that of indomethacin. These compounds were less effective inhibitors than hydrocortisone but more effective than quercetin, a known inhibitor of tumor promotion.

C_{29}-Alkane-6,8-diol **4c** inhibited tumor promotion by TPA following initiation with 7,12-dimethylbenz[*a*]-anthracene (DMBA). Two groups of 15 mice underwent initiation by application of 50 μg (195 nmol) of DMBA in acetone (100 μL) to the dorsal skin. Promotion with 1 μg (1.6 nmol) of TPA in acetone (100 μL), applied twice

Scheme 1

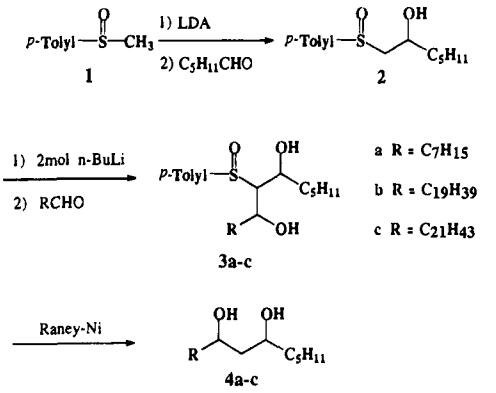

Table 1. Inhibitory Effect of Synthetic Alkane-6,8-diols and Reference Compounds on TPA-Induced Inflammation in Mice

compound	ID_{50} (mg/ear)[b]	IR[c]
C_{15}-alkane-6,8-diol		23
C_{27}-alkane-6,8-diol	0.5	82*
C_{29}-alkane-6,8-diol	0.4	85*
quercetin[d]	1.6	40*
indomethacin[d]	0.3	96*
hydrocorisone	0.03	99*

[a] Compounds were applied 30 min before TPA (1 mg). Ear thickness was determined 6 h after TPA treatment. [b] ID_{50}: 50% inhibitory dose. [c] Inhibition ratio at 1.0 mg/ear. * $p < 0.01$ by Student's *t*-test as compared to the control group. [d] Data taken from ref 2.

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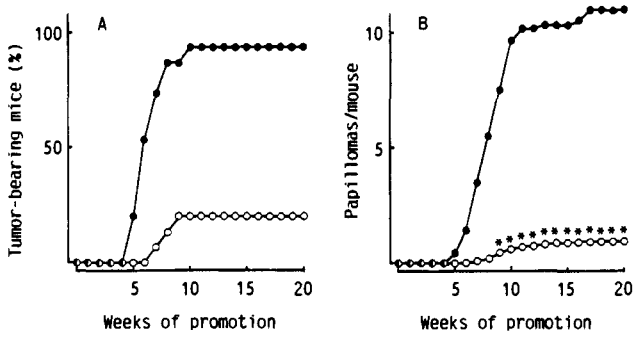

Figure 1. Inhibitory effect of synthetic C_{29}-alkane-6,8-diol on the promotion of skin papillomas produced by TPA following DMBA initiation in mice. From 1 week after initiation by a single topical application of 50 μg of DMBA, 1 μg of TPA was applied twice weekly. Topical application of synthetic C_{29}-alkane-6,8-diol (2 μmol/mouse; ○) and vehicle (●) was performed 30 min before each TPA treatment. Data are expressed as percentages of mice bearing papillomas (A) and as average numbers of papillomas per mouse (B). $^*p < 0.05$ by Student's t-test, compared with the control group.

a week, was begun 7 days after the initiation. C_{29}-Alkane-6,8-diol **4c** (2 μmol/mouse) and its vehicle, acetone–dimethyl sulfoxide (9:1, 100 μL), were applied topically 30 min before TPA treatment. These treatments were continued for 20 weeks. Figure 1A illustrates the time course of skin tumor formation in the groups treated with DMBA plus TPA, with or without synthetic C_{29}-alkane-6,8-diol **4c**. The first tumor appeared at week 5 in the group treated with DMBA plus TPA. In the group treated with DMBA plus TPA and 2 μmol of synthetic C_{29}-alkane-6,8-diol **4c**, the first tumor appeared during week 7. The percentage of tumor-bearing mice treated with DMBA plus TPA was 93% at week 20, whereas that in the group treated with DMBA plus TPA and 2 μmol of synthetic C_{29}-alkane-6,8-diol **4c** was 20%. Figure 1B shows the average number of tumors per mouse. The group treated with DMBA plus TPA produced 11.1 tumors/mouse at week 20, whereas the group treated with DMBA plus TPA and 2 μmol of synthetic C_{29}-alkane-6,8-diol **4c** had 0.9 tumor/mouse. The treatment with 2 μmol of synthetic C_{29}-alkane-6,8-diol **4c** caused a 92% reduction in the average number of tumors per mouse at week 20.

To sort out new inhibitors as chemopreventive agents, we intentionally chose nontoxic compounds, safflower and its oil are added to foods. We have already reported the inhibition of tumor promotion by glycyrrhizin (licorice root),[9] caffeine (coffee and tea),[9] monascorubrin (red malted rice),[10] and ergosterol (edible mushroom).[2] These food additives are likely to be of importance for the chemoprevention of cancer.

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Supporting Information Available: Experimental procedures, NMR spectra, and analytical data for final products (3 pages). Ordering information is given on any current masthead page.

References

(1) Yasukawa, K.; Yamaguchi, A.; Arita, J.; Sakurai, S.; Ikeda, A.; Takido, M. Inhibitory effect of edible plant extracts on 12-O-tetradecanoylphorbol-13-acetate-induced ear oedema in mice. *Phytother. Res.* **1993**, *7*, 185–189.
(2) Yasukawa, K.; Aoki, T.; Takido, M.; Ikekawa, T.; Saito, H.; Matsuzawa, T. Inhibitory effect of ergosterol isolated from edible mushroom *Hypsizigus marmoreus* on TPA-induced ear oedema and tumor promotion in mice. *Phytother. Res.* **1994**, *8*, 10–13.
(3) Yu, S. Y.; Yasukawa, K.; Takido, M. Atractylodis Rhizoma extract and its component, atractylon, inhibit tumor promotion in mouse skin two-stage carcinogenesis. *Phytomedicine* **1994**, *8*, 55–58.
(4) Kasahara, Y.; Kumaki, K.; Katagiri, S.; Yasukawa, K.; Yamanouchi, S.; Takido, M.; Akihisa, T.; Tamura, T. Carthami Flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytother. Res.* **1994**, *8*, 327–331.
(5) Akihisa, T.; Oinuma, T.; Tamura, T.; Kasahara, Y.; Kumaki, K.; Yasukawa, K.; Takido, M. erythro-Hentriacontane-6,8-diol and 11 other alkane-6,8-diols from *Carthamus tinctorius*. *Phytochemistry* **1994**, *36*, 105–108.
(6) Yasukawa, K.; Akihisa, T.; Kasahara, Y.; Kaminaga, T.; Kanno, H.; Kumaki, K.; Tamura, T.; Takido, M. Inhibitory effect of alkane-6,8-diols, the components of safflower, on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Oncology*, submitted for publication.
(7) Trost, B. M., Fleming, I., Eds. *Comprehensive Organic Synthesis*; Pergamon Press: Oxford, U.K., 1991; Vol. 1, pp 505–539.
(8) Inokuchi, T.; Matsumoto, S.; Nishiyama, T.; Torii, S. A Selective and Efficient Method for Alcohol Oxidations Mediated by N-Oxoammonium Salts in Combination with Sodium Bromite. *J. Org. Chem.* **1990**, *55*, 462–466.
(9) Yasukawa, K.; Takido, M.; Takeuchi, M.; Nakagawa, S. Inhibitory effect of glycyrrhizin and caffeine on two-stage carcinogenesis in mice. *Yakugaku Zasshi* **1988**, *108*, 794–796.
(10) Yasukawa, K.; Takahashi, M.; Natori, S.; Kawai, K.; Yamazaki, M.; Takido, M. Azaphilones inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Oncology* **1994**, *51*, 108–112.

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