# Inhibition of Tumor-Promoter-Enhanced H-Choline Incorporation into Cellular Phospholipids by Phloroglucinol Derivatives from Mallotus japonicus

Munehisa Arisawa, Akio Fujita, Naokata Morita, Toru Okuyama, and Hoyoku Nishino

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# INHIBITION OF TUMOR-PROMOTER-ENHANCED $^3$H-CHOLINE INCORPORATION INTO CELLULAR PHOSPHOLIPIDS BY PHLOROGLUCINOL DERIVATIVES FROM *MALLOTUS JAPONICUS*

MUNEHISA ARISAWA,* AKIO FUJITA, NAOKATA MORITA,

*Department of Medicinal Resources, Faculty of Pharmaceutical Sciences, Toyama Medical & Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan*

TORU OKUYAMA,

*Department of Pharmacognosy & Phytochemistry, Meiji College of Pharmacy, 1-35-23 Nozawa, Setagaya-ku, Tokyo 154, Japan*

and HOYOKU NISHINO*

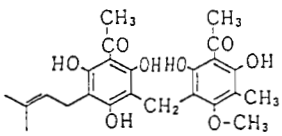
*Department of Biochemistry, Kyoto Prefectural University of Medicine, Hirokoji Kawaramachi-douri, Kamigyoj-ku, Kyoto 602, Japan*

ABSTRACT.—The MeOH and $CHCl_3$ extracts of the pericarps of *Mallotus japonicus* showed potential anti-tumor-promoter activity. Seven constituents of the $CHCl_3$ extract and two derivatives from the most abundant constituent, mallotojaponin [**1**], markedly inhibited the incorporation of $^3$H-choline into phospholipids of C3H10T1/2 cells enhanced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter.

Many research efforts on anti-tumor agents from natural resources have been attempted in order to find useful drugs for medical treatment of cancer, and potential anti-tumor-promoter agents from plant sources also have been examined for the purpose of chemoprevention of cancer.

Potential anti-tumor-promoter activity of flavonoids widely distributed in the plant kingdom was described in two-stage carcinogenesis on mouse skin (1–4). The studies on inhibition of tumor promotion by triterpenoids, such as glycyrrhetic acid, the aglycone of glycyrrhizin, were also carried out extensively (5–8). The anti-tumor-promoter activity of berberine sulfate, an isoquinoline alkaloid (9), coumarin derivatives (10), curcumin, a constituent of *Curucuma longa* L. (11), and an extract of garlic (genus *Allium*) (12), were also reported.

As part of a program for survey of biologically active constituents from natural resources, we have been studying constituents of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) and have reported the isolation, identification, and structural elucidation of several new phloroglucinol derivatives and the cytotoxic, anti-tumor, and antiherpetic activities of the isolated compounds (13–20). During the continuation of our research, we found that the MeOH and $CHCl_3$ extracts of pericarps of *M. japonicus* showed potent anti-tumor-promoter activity; i.e., the extracts markedly inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-enhanced $^3$H-choline incorporation into phospholipids of C3H10T1/2 cells. It has been known that tumor promoters induce primarily a change of phospholipid metabolism in vitro and in vivo (21,22), and it is suggested that enhancement of phospholipid metabolism plays an important role in tumor promotion in vivo. Furthermore, various kinds of chemicals that modulate cellular phospholipid metabolism were proved to have potential anti-tumor-promoter activity in vivo, and the evaluation of the inhibitory potency for the TPA-enhanced phospholipid metabolism has been shown to be valuable for the screening of new potential anti-tumor-promoters. Accordingly, we attempted to evaluate the anti-tumor-promoter activity of some phloroglucinol derivatives purified from the



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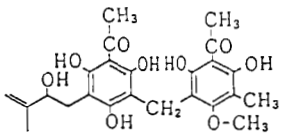

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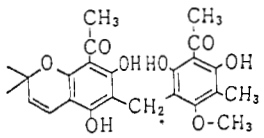

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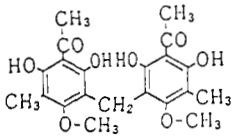

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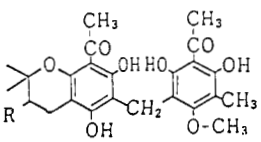
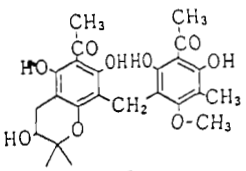


5 R=OH
8 R=H



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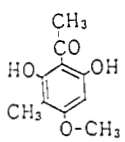

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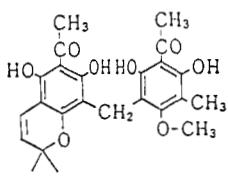

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$CHCl_3$ extract by an in vitro screening test to assay the inhibitory potency on tumor-promoter-enhanced phosphatidylcholine synthesis.

## RESULTS AND DISCUSSION

The MeOH and $CHCl_3$ extracts proved to have potent anti-tumor-promoter activity in the screening test in vitro; i.e., both extracts showed 100% inhibitory effect on TPA-enhanced $^3$H-choline incorporation into phospholipids of C3H10T1/2 cells in vitro at 50 μg/ml. Seven isolated phloroglucinol derivatives from the $CHCl_3$ extract of *M. japonicus*, mallotojaponin [**1**], mallotolerin [**2**], mallotochromene [**3**], mallotophenone [**4**], mallotochromanol [**5**], isomallotochromanol [**6**], and 2,6-dihydroxy-3-methyl-4-methoxyacetophenone [**7**] and two phloroglucinols derived from mallotojaponin [**1**], namely, mallotochroman [**8**] and isomallotochromene [**9**], were tested for potential anti-tumor-promoter activity. All of them markedly inhibited tumor-promoter-stimulated $^3$H-choline incorporation into phospholipids of C3H10T1/2 cells (Table 1).

Previous reports described cytotoxicity of these compounds toward KB cells in culture (10,11,15). Compounds **1–4** and **9** were cytotoxic; compound **1** especially showed intense cytotoxic activity for KB cells with $ED_{50}$ 0.58 μg/ml. Compound **1** was further examined for anticancer effects on Ehrlich solid tumor and ascites carcinoma in mice (14).

Recently, the cytotoxicity toward five cultured tumor cell lines, Hep-2 and PC-13 carcinomas, B-16 melanoma, and L5178Y and P-388 leukemias, by 13 phloroglucinols from *M. japonicus* and anticancer effects of compound **1** on mouse L5178Y leukemia in vivo and combined treatment of compound **1** and OK-432 on mouse L5178Y leukemia and Ehrlich ascites carcinoma were de-

TABLE 1. Inhibition Effect of Some Phloroglucinol Derivatives on Tumor-promoter-enhanced $^3$H-choline Incorporation into Phospholipids of C3H10T1/2 Cells in vitro (TPA 50 nM).[a]

| Concentration | Compound | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** |
| 50 μg/ml | 100 | 100 | 100 | 99.1 | 100 | 100 | 100 | 74.3 | 100 |
| 10 μg/ml | 100 | 100 | 66.7 | 97.0 | 29.2 | 95.5 | 100 | 68.7 | 100 |
| 2 μg/ml | 85.8 | 78.8 | — | 57.1 | 2.9 | 0 | 49.5 | 9.3 | 97.9 |

[a]Table entries are inhibition percent. — indicates compound was not tested at this level.

scribed (20). Compound **1** showed significant cytotoxicity for all of the five tumor cell lines in vitro, and potent antileukemic activity in vitro was observed. The combined treatment gave facorable results in a 60 day experiment.

In the present study, compound **1** was found apparently to inhibit TPA-enhanced phosphatidylcholine synthesis. However, it is not still assured that it is really an anti-TPA effect. In other words, since the compound is cytotoxic as mentioned above, $^3$H-choline incorporation into the phospholipid fraction could be effected secondary to the cytotoxicity, and the effect may not be specific. To distinguish among these possibilities, we further examined the effect of compound **1** on the incorporation of $^3$H-choline into phospholipids of C3H10T1/2 cells in the absence of TPA as well as in the presence of TPA. As shown in Figure 1, up to the concentration of 1.5 μg/ml, compound **1** scarcely affected basal incorporation of $^3$H-choline and specifically suppressed the TPA-enhanced portion of $^3$H-choline incorporation. $ID_{50}$ is calculated to be about 1 μg/ml. Thus, compound **1** was proved to have specific potential anti-tumor-promoter acitivity, at least at the $ID_{50}$ concentration.

Together with these results, it is of interest to investigate the mechanism of action of compound **1**, which has a wide spectrum of antineoplastic activities (i.e., potential anti-tumor-promoter activity, cytotoxicity, antileukemic activity) and the therapeutic activity of combined treatment for leukemia and Ehrlich carcinoma.

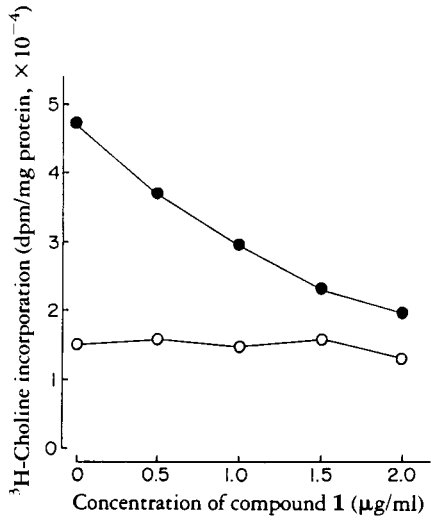


FIGURE 1. Effect of compound **1** on tumor-promoter-enhanced $^3$H-choline incorporation into phospholipids of C3H10T1/2 cells. C3H10T1/2 cells were incubated with various concentrations of compound **1**. After 1 h, $^3$H-choline (9.25 kbq/culture) was added with (closed circle) or without (open circle) TPA (50 nM). Incubation was continued for 4 h, and the radioactivity incorporated into phospholipid fraction was assayed. Data are mean values of duplicate experiments.

Because various kinds of phloroglucinol derivatives besides compound **1** showed potential anti-tumor-promoter activity, more extensive study on structure-activity relationship is also of interest. Among the phloroglucinol derivatives tested in this study, compound **9**

showed the strongest potential anti-tumor-promoter activity. Thus, it is of particular interest to examine the anti-tumor-promoter activity in vivo of compound **9**.

## EXPERIMENTAL

MATERIALS.—The extracts were prepared from the pericarps as described previously (13). Mallotojaponin [**1**], mallotolerin [**2**], mallotochromene [**3**], mallotophenone [**4**], mallotochromanol [**5**], isomallotochromanol [**6**], and 2,6-dihydroxy-3-methyl-4-methoxyacetophenone [**7**] were isolated from *M. japonicus* (13,14), and mallotochroman [**8**] and isomallotochromene [**9**] were derived from mallotojaponin [**1**] (15) as described previously. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was obtained from Pharmacia PL Biochemicals. [Methyl-$^3$H]-choline chloride was purchased from Amersham International.

$^3$H-CHOLINE INCORPORATION INTO PHOSPHOLIPIDS OF CULTURED CELLS.—Incorporation of $^3$H-choline chloride into phospholipids of C3H10T1/2 cells, established by Reznikoff *et al.* (23), was assayed by the method described previously (24).

## ACKNOWLEDGMENTS

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