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Antitumor Triterpenes from Medicinal Plants

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Thirteen kinds of naturally occurring or derivatised triterpenes, reported to have an antitumoral property, were reinvestigated on the basis of their direct cytotoxicity or the inhibitory activity on cell growth against five kinds of cultured human tumor cells, *i. e.*, A-549, SK-OV-3, SK-MEL-2, XF498 and HCT15, *in vitro*. Ursonic acid **III**, betulinic acid **VIII**, betulonic acid **X** and glycyrrhetinic acid **XI** were exhibited a marked inhibition on cell growth.

Key words: Triterpene, Antitumor, Betulinic acid, Betulonic acid, Ursonic acid, Glycyrrhetinic acid.

INTRODUCTION

We have been seeking for potent tumor inhibitors from natural resouces especially from medicinal plants. For this purpose, two approaches were currently attempted. One is the same way as adopted in many other laboratories, just to isolate the active principle(s) which was responsible for the activity, according to the activity-guided fractionation of the crude material which exhibited an activity upon the preliminary test for cytotoxicity. By this way, we have isolated several kinds of components as active principles from various plants (Ryu *et al.*, 1992a, 1994a and 1994b). The other one is by the estimation of the inhibitory activity on the growth of human tumor cell lines, to reinvestigate the antitumor activity of known substances, especially of which activity has been reported.

On the literature survey, a large number of triterpenes and triterpenoidal saponins (Kong *et al.*, 1986; Quetin-Leclercq *et al.*, 1992) were found to exhibit a significant antitumor activity, even though they were evaluated by different bioassay methods or tested with different tumor cell lines. Most of them were comprised in an unusually complexed skeleton such as cucurbitacins (Konopa *et al.*, 1974), limonoids (Polonsky *et al.*, 1978) or dammaranes (Anisimov *et al.*, 1975). One of the interesting thing is that some triterpenes in α- and β-amyrin (Hori *et al.*, 1987; Nozaki *et al.*, 1986; Kong *et al.*, 1986; Inada *et al.*, 1993) and lupan (Sheth *et al.*, 1973; Konoshima *et al.*, 1987), which were most widely abundant in plant kingdom, were also referred so often as a prominent antitumor agent. On present paper, we would like to report the reinvestigation of the antitumor activity of thirteen triterpenes, classified as a typical number of α- or β-amyrin and lupan, on the basis of their inhibition on the growth of human tumor cells, *i.e.*, A-549, SK-OV-3, SK-MEL-2, XF498 and HCT15, *in vitro*.

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MATERIALS AND METHODS

Test Materials

Compounds **I**, **V** and **VIII** were isolated from *Prunella vulgaris*, and fully identified by the direct comparisons with the authentic samples (Ryu *et al.*, 1992b). **II**, **III**, **IV**, **VI** and **IX** were prepared by the derivatization of **I**, **V** and **VIII**, repectively (Ryu *et al.*, 1993). The betulin **VII**, oleanolic acid **XIII** and glycyrrhizin were purchased from Sigma and used without further purification. **XI** and **XII** were prepared separately by the acid hydrolysis and the CrO_3 oxidation of glycyrrhizin (Ryu *et al.*, 1993).

Test for Cytotoxicity *in vitro*

Each tumor cells was obtained from National Cancer Institute (NCI), USA, which was currently used in NCI as standard cell lines for the *in vitro* drug screening on antitumor activity. All exprimental procedures were followed up the NCI's protocol, based on the SRB (sulforhodamine-B)-smear method (Skehan *et al.*, 1990). The maintenance of the stock cell cultures and detailed experimental procedures were mentioned on the

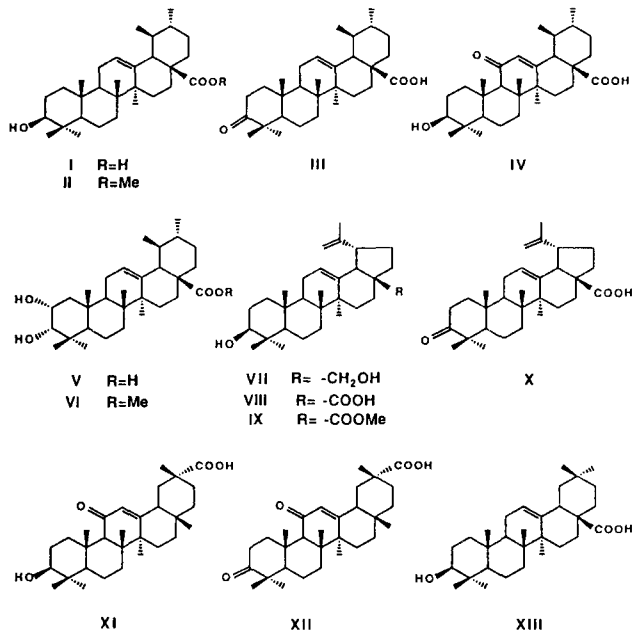

Fig. 1. Triterpenes in α- or β-*amyrin* and *lupane* series.

Table I. Inhibition of various triterpenes of plant origin against the growth of human tumor cells, *in vitro*

Compound	ED_{50}(μg/ml)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
I	11.6	29.2	9.3	11.9	15.6
II	81.8	>100	>100	>100	>100
III	3.5	14.6	10.1	11.8	2.1
IV	22.2	59.3	12.5	32.0	27.7
V	15.1	54.7	14.6	21.1	20.8
VI	12.7	30.0	6.4	27.1	34.6
VII	50.1	>100	50.5	>100	>100
VIII	2.0	3.8	2.5	4.8	5.3
IX	15.2	11.4	17.5	16.2	24.1
X	1.1	2.3	2.6	2.5	0.8
XI	2.7	11.2	3.1	5.0	3.3
XII	18.4	35.2	6.9	19.6	30.2
XIII	38.8	>100	>100	>100	>100

[a] ED_{50} value of compound against each cancer cell line, which was defined as concentration (μg/ml) that caused 50% inhibition of cell growth *in vitro*

previous paper (Ryu *et al.*, 1992b).

Each test materials was dissolved in dimethylsulfoxide (DMSO) and then, diluted with the medium solution so that the final concentration of DMSO in medium did not exceed 0.5%.

RESULTS AND DISCUSSION

Thirteen kinds of triterpenes in α- and β-*amyrin* or lupan series (Fig. 1) were reinvestigated for the antitumor activity by the examination of their inhibitory activity on the growth of five cultured human tumor cells, *in vitro*. Among them, the ursonic acid (3-oxo-ursolic acid, **III**), which was a rare number of α-amyrin found in nature, but was easily produced by the chemical modification of the ursolic acid **I** (Ryu *et al.*, 1993), and the betulinc acid **VIII**, betulonic acid **X** in *lupan* and the glycyrrhetinic acid **XI** in β-*amyrin* series were found to exhibit a significant activity (ED_{50} value agsinst A549 were below 4 μg/ml/, Table I). Concerned with the antitumor activity of the α-*amyrin* triterpenes, Yamagishi *et al* (1988a) has reported that the ursolic acid **I** and sevevn kinds of its derivatives including a 3-O-acetylursolic acid, ursolic acid methyl ester **II**, *etc.* showed significant cytotoxicity against the growth of lymphocytic leukemia cells P-388 and L-1210 as well as the human lung carcinoma cell A-549, respectively. They mentioned in other article (1988b) that 2α-hydroxyursolic acid, the 3-epimer of **V** (2α, 3α-*dihydroxy-urs*-12-*en*-28-*oic acid*), showed also a significant activity against human colon HCT-8 and other human or murine tumor cells. However, present work revealed that the activity of **I**, **II**, **IV**, **V** and **VI** against the A549 and other human tumor cells was not so prominent as the ursonic acid (3-oxo-ursolic acid, **III**), which showed a significant activity against A549. In β-*amyrin* series, the glycyrrhetinic acid **IX**, aglycone of glycyrrhizin, exhibited a marked activity, whereas the glycyrrhizin, 3-oxo-glycyrrhetinic acid **XII** and the oleanolic acid **XIII**, a typical number of β-*amyrin*, were exhibited a poor activity against each tumor cell lines. This result was in agreement with the conclusion of recent paper reporting the inhibition of tumor by some saponins against murine B-16 melanoma cells (Quetin-Leclercq *et al.*, 1992). The most distinct one in this experiment was the activity of lupan triterpenes, which has been reported frequently (Sheth *et al.*, 1973 and Konoshima *et al.*, 1987 and Bae *et al.*, 1992). The betulinic acid **VIII** and betulonic acid **X** showed a marked inhibition against all tested tumor cell lines, but the activity was remarkably reduced, while the 28-carboxylic group of **VIII** was reduced to the hydroxymethyl group (betulin, **VII**) or blocked by the esterification (**IX**).

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REFERENCES CITED

Anisimov, M. M., Shentsova, E. B., Shcheglov, V. V., Strigina, L. I., Uvarova, N. I., Levina, E. V., Oshitok, G. I. and Elyakov, G. B., A comparative study of the cytotoxic effect of dammaran triterpenoids and

betulin on early embryogenesis of the sea urchin. *Toxicon.*, 13(abstr.), 86, (1975).

Bae, K. H., Min, B. H., Do, D. S., Kim, N. S., Yang, G. J. and Ahn, B. Z., Screening on cytotoxicity of medicinal plants against L1210 cell. *Yakhak Hoeji, 36*(5), 491-495 (1992).

Hori, H., Pang, G. M., Harimaya, K., Iitaka, Y. and Inayama, S., Isolation and structure of regelin and regelinol, new antitumor ursane-type triterpenoids from *Tripterygium regelli. Chem. Pharm. Bull., 35*(2), 2125-2128 (1987).

Inada, A., Somekawa, M., Murata, H., Nakanishi, T., Tokuda, H., Nishino, H., Iwashima, A., Darnaedi, D. and Murata, J., Phytochemical studies on Meliaceous plants. VIII. Structures and inhibitory effects on Epstein-Barr virus activation of triterpenoids from leaves of *Chisocheton macrophyllus King. Chem. Pharm. Bull., 43*(3), 617-619 (1993).

Konopa, J., Matuszkiewicsz, A., Hrabowska, M. and Onoszaka, K., Cucurbitacines, cytotoxic and antitumor substances from *Bryonia alba L. Arzeim. Forsch., 24*(11), 1741-1743 (1974).

Konoshima, T., Takasaki, M. and Kozuka, M., Studies on inhibitors of skin-tumor promotion, I. Inhibitory effects of triterpenes from Euptelea polyandra on *Epstein-Barr virus activation. J. nat. Prods., 50*(6), 1167-1170 (1987).

Kong, F., Zhu, D., Xu, R., Fu, Z., Zhou, L., Iwashita, T. and Komura, H., Structural study of tubeimoside 1, a constituent of Tu-bei-mu. *Tetrahedron Lett., 27* (47), 5765-5768 (1986).

Lee, K. H., Lin, Y. M., Wu, T. S., Zhang, D. C., Yamagishi, T., Hayashi, T., Hall, I. H., Chang, J. J., Wu, R. Y. and Yang, T. H., The cytotoxic principles of *Prunella vulgaris, Psychotria serpens,* and *Hyptis capitata*: Ursolic acid and related derivatives. *Planta Med.*, 308-311 (1988b).

Nozaki, H., Suzuki, H., Hirayama, T., Kasai, R., Wu, R. Y. and Lee, K. H., Antitumor triterpenes of *Maytenus diversifolia. Phytochemistry, 25*(2), 476-485 (1986).

Polonsky, J., Arnoux, B., Pascard, C., Pettit, G. R. and Schumidt, J. M., Isolation and structure of amoorastatin. *J. Am. Chem. Soc., 100*(24), 7731-7733 (1978).

Quetin-Leclercq, J., Elias, R., Balansard, G., Bassleer, R. and Angenot, L., Cytotoxic activity of some triterpenoid saponins. *Planta Med., 58*, 279-281 (1992).

Ryu, S. Y., Choi, S. U., Lee, C. O. and Zee, O. P., Antitumor activity of *Psoralea corylifolia. Arch. Pharm. Res., 15*(4), 356-359 (1992a).

Ryu, S. Y., Lee, C. K., Lee, C. O., Kim, H. S. and Zee, O. P., Antiviral triterpenes from *Prunella vulgaris. Arch. Pharm. Res., 15*(3), 242-245 (1992b).

Ryu, S. Y., Lee, C. K., Ahn, J. W., Lee, S. H. and Zee, O. P., Antiviral activity of triterpenoid derivatives. *Arch. Pharm. Res., 16*(4), 339-342 (1993).

Ryu, S. Y., Choi, S. U., Lee, S. H., Ahn, J. W. and Zee, O. P., Antitumor activity of some phenolic components in plants. *Arch. Pharm. Res., 17*(1), 42-44 (1994a).

Ryu, S. Y., Lee, S. H., Choi, S. U., Lee, C. O., Noh, J. S. and Ahn, J. W., Antitumor activity of *Trichosanthes kirilowii. Arch. Pharm. Res., 17*, in press (1994b).

Sheth, K., Bianchi, R., Wiedhopf, R. and Cole, J. R., Antitumor agents from Alnus oregona (Betulaceae). *J. Pharm. Sci., 62*(1), 139-140 (1973).

Skehan, P., Streng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst., 82*, 1107-1112 (1990).

Yamagishi, T., Zhang, D. C., Chang, J. J., McPhail, D. R., McPhail, A. T. and Lee, K. H., The cytotoxic principles of *Hyptis capitata* and the structures of the new triterpenes Hyptic acid-A and B. *Phytochemistry, 27*(10), 3213-3216 (1988a).