Arch. Pharm. Res. Vol.21, No.6, pp. 703-706, 1998

Anticomplementary Activity of Stilbenes from Medicinal Plants

Sei-Ryang Oh, Shi Yong Ryu[1], Si Hyung Park, Keun Young Jung, Im Seon Lee, Kyung Seop An, Jung Joon Lee and Hyeong-Kyu Lee*

Korea Research Institute of Bioscience & Biotechnology, KIST, Yusong P.O. Box 115, Taejon 305-600, Korea and [1]Korea Research Institute of Chemical Technology, Taejon 305-606, Korea

(Received July 30, 1998)

The anticomplementary activity of stilbenes from medicinal plants in Korea was investigated *in vitro*. 3,5-Dihydroxy-4'-methoxystilbene (**3**) was most potent with IC_{50} value of 1.5×10^{-4} M followed by rhapontigenin (**4**), oxyresverastrol (**2**), 2,3,4',5-tetrahydroxystilbene-2-*O*-β-glucoside (**9**), rhaponticin (**8**), resverastrol (**1**), and piceid (**7**). The activity was found to be increased by a methylation on a hydroxy group of C-4' of **1**, but decreased by further methylation on hydroxy groups of C-3 and C-5 and glucosylation on any hydroxy group of **1**. Addition of hydroxy group on C-2' of **1** or C-3' of **3** was little affected on the anticomplementary activity but the activity was increased by *O*-glucosylation on C-2 of **1**.

Key words : Anticomplementary activity, Stilbenes, Resveratrol, Oxyresveratrol, 3,5-Dihydroxy-4-methoxystilbene, Rhapontigenin, Rhaponticin, Piceid, 2,3,4',5-Tetrahydroxy stilbene-2-*O*-β-glucoside, 2,3,4',5-Tetrahydroxystilbene-2-*O*-β-glucosyl-4'-*O*-β-gluco-side

INTRODUCTION

The complement system is a humoral effector of inflammation and consists of over 30 serum proteins which are activated by a cascade mechanism through antigen-antibody mediated process (classical pathway, CP) and/or antibody independent processes (alternative pathway, AP). Activation of the system normally mediates protective process of inflammation against foreign invasive antigens (Kuby, 1994). However, excessive activation of it involves to the pathological reactions in a variety of inflammation which causes to evoke various degenerative diseases (Rother *et al.*, 1985; Alexander *et al.*, 1988; Strunk *et al.*, 1988; Takematsu *et al.*, 1991; Corvetta *et al.*, 1992; Sato *et al.*, 1993; Wortel *et al.*, 1993; Mcgeer *et al.*, 1995) and hyperacute rejection in transplantation (Miyagawa *et al.*, 1988; Deitch *et al.*, 1993). Therefore, specific inhibitors for modulation of complement activation should be useful in therapy of inflammatory diseases.

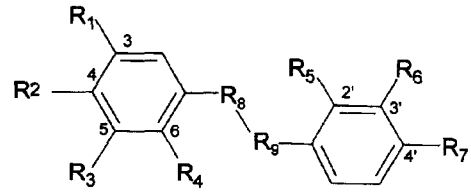
In searching for anticomplementary compounds from medicinal plants, we have found some phenolic compounds such as rosmarinic acid (Oh *et al.*, 1996) from *Agastache rugosa* (Labiatae), protosappanin B, E, brazilin and brazilein (Oh *et al.*, 1998) from the heartwood of *Caesalpinea sappan* L. (Leguminosae) and tiliroside (Jung *et al.*, 1998) from the Magnolia Flos (Magnoliaceae) showed a strong anticomplementary activity on complement system *in vitro*. Subsequently, we examined the anticomplementary activity of stilbenes which have been reported to have various biological activities. This paper deals with the anticomplementary activity of hydroxystilbenes isolated from various medicinal plants in Korea.

Correspondence to: Hyeong-Kyu Lee, Korea Research Institute of Bioscience & Biotechnology, KIST, Yusong P.O. Box 115, Taejon 305-600, Korea

MATERIALS AND METHODS

Stilbenes

Resveratrol (**1**) and picied (**7**) were isolated from the rhizome of *Polygonum cuspidatum* (Polygonaceae) and rhapontigenin (**2**), 3,5-dihydroxy-4'-methoxystilbene (**3**), rhaponticin (**8**) were isolated from the rhizome of *Rheum undulatum* (Polygonaceae). 3,4'-Dimethoxy-5-hydroxystilbene (**5**) was prepared from **7** and 3,4',5-trimethoxy-resveratrol (**6**) was prepared from compound **1**. The isolation procedures of them were described on the previous paper (Ryu *et al.*, 1994). Dimethoxoxy-veratrol (**4**), 2,3,4',5-tetrahydroxystilbene-2-*O*-β-glucoside (**9**) and 2,3,4',5-tetrahydroxystilbene-2-*O*-β-glucosyl-4'-*O*-β-glucoside (**10**) were isolated from *Polygonum multiflorum* (Polygonaceae) and the isolation procedure of them were desescribed on the previous paper (Ryu *et al.*, 1988). Diethylstilbestrol (**11**) was purchased from



compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8-R_9
1	OH	H	OH	H	H	H	OH	CH=CH
2	OH	H	OH	H	OH	H	OH	CH=CH
3	OH	H	OH	H	H	H	OCH_3	CH=CH
4	OH	H	OH	H	H	OH	OCH_3	CH=CH
5	OH	H	OCH_3	H	H	H	OCH_3	CH=CH
6	OCH_3	H	OCH_3	H	H	H	OCH_3	CH=CH
7	O-Glc	H	OH	H	H	OH	OH	CH=CH
8	O-Glc	H	OH	H	H	OH	OCH_3	CH=CH
9	OH	H	OH	O-Glc	H	H	OH	CH=CH
10	O-Glc	H	OH	H	H	H	O-Glc	CH=CH
11	H	OH	H	H	H	H	OH	$CH(C_2H_5)$-$CH(C_2H_5)$

Fig. 1. Structures of stilbenes

Sigma (D-4628).

Complement titration

Complement activity was assessed by the procedure described previously (Oh *et al.*, 1996). Each compound was dissolved in DMSO and the solution was diluted to 2.5% in GVB^{++} (1.8 mM sodium barbital, 3.1 mM barbituric acid, 0.1% gelatin, 0.141 M NaCl, 0.5 M $MgCl_2$, 0.15 M $CaCl_2$ and 0.3% sodium azide, pH 7.3). Normal human serum (NHS) from a healthy volunteer in this laboratory was used as the complement source. Serum concentration for CH_{50} was evaluated just before sample treatment. The optimal dilution range was 1/95 to 1/110 for CP assay and 1/5 to 1/7.5 for AP assay in each buffer. Sheep erythrocyte suspension (4.0×10^8 cells/ml) was sensitized by incubation with an equal volume of 1/75 to 1/120 diluted hemolysin (S-1389, Sigma) at 37°C for 30 min. Afterwards, sensitized erythrocytes (EA) were kept at 4°C and restored to formal concentration prior to use. For CP assay, 80 µl of optimal dilution of NHS were pre-incubated with 80 µl of sample solution at 37°C for 30 min, then 40 µl of EA were added to them and incubated in the same conditions. The reaction mixture was centrifuged immediately and 100 µl of the supernatant were transferred to a flat-buttomed microplate and optical density measured at 405 nm by using a microplate reader. For AP, rabbit erythrocytes (3.0×10^8 cells/ml) and Mg^{2+}-EGTA-GVB [1.8 mM sodium barbital, 3.1 mM barbituric acid, 0.1% gelatin, 0.141 M NaCl, 8 mM $MgCl_2$, 4 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N', N'-tetraacetic acid and 0.3% sodium azide, pH 7.3] were used for optimal hemolysis of the control (APH_{50}). The overall procedure was identical with that of CP assay.

Anticomplementary activity

The % hemolysis was calculated by $(A_i/A_{max}\times100\%)$, where A_i and A_{max} indicated OD_{405nm} values of a given titration and maximum lysis by H_2O from the corresponding background. The anticomplementary activity was determined as a mean of triplicate tests per concentration and expressed as percent inhibition from complement-dependent hemolysis of the control.

RESULTS AND DISCUSSION

The anticomplementary activity of the isolated compounds was strong on the CP, but very weak on the AP of the complement system (Table I). The difference of activity between CP and AP was thought to be caused by the sensitivity of the assay system. It was generally found that the anticomplementary compounds from medicinal plants showed strong activity on the CP (Cimanga *et al.*, 1995; Knous *et al.*, 1996; Cimanga *et al.*, 1997; Kim *et al.*, 1997; Oh *et al.*, 1998).

On the CP, the activity of **1** was far lower than that of rosmarinic acid, a well-known inhibitor against complement activation. However, the anticomplementary activity was drastically changed by methylation of hydroxyls of **1**. That is, the activity of **2** was more potent than that of **1** and, in case of **3**, it was most potent among them even similar with that of rosmarinic acid. But further methylation decreased the activity. Thus **5** and **6**, the methylated compounds on the A ring of **3**, showed very weak or no activity. These results suggested that the anticomplementary activity of resveratrol derivatives was affected by the presence of free hydroxy groups and the hydrophobic group of the structure, at least, masking of 4'-OH seemed to be important for the potency. This activity

Table I. Anticomplementary activities of stilbenes

compound	Classical Pathway IC_{50}	Alternative pathway % inhibition[a]
1	7.2×10^{-4} M	-6.7±2.5[b]
2	$6.9\times^{-4}$ M	8.4±10.5
3	$1.5\times^{-4}$ M	9.9±1.0
4	$3.7\times^{-4}$ M	-14.1±1.6[b]
5	$2.6\times^{-4}$ M	1.1±5.1
6	-10.8±4.1[a,b]	17.7±2.0
7	$8.3\times^{-4}$ M	-1.4±21.1[b]
8	$7.0\times^{-4}$ M	26.7±2.3[b]
9	$5.0\times^{-4}$ M	-6.4±3.0[b]
10	$8.1\times^{-4}$ M	-23.6±11.4[b]
rosmarinic acid[c]	$1.8\times^{-4}$ M	86.0±1.7

[a]It was measured at the concentration of 250 μg/ml
[b]Negative value denoted enhancement of hemolysis from standard condition.
[c]This compound was used as a positive control.

pattern was similar with the inhibition effect against prostaglandin synthesis (Goda *et al.*, 1987) in that the potent stilbenes possessed an aromatic hydroxyls in addition to a hydrophobic group. Compounds **7** and **8**, monodesmosides at C-3, and **10**, bisdesmoside at C-3 and C-4', also showed lower activities than that of each aglycon. Methylation or glucosylation on the hydroxy group of stilbenes were commonly found to decrease the activity such as antitumor effect (Ryu *et al.*, 1994), inhibition of monoamine oxidase-A (Ryu *et al.*, 1988), antioxidant effect (Han *et al.*, 1991) and coronary vasodilatory effect (Inamori *et al.*, 1984a). The anticomplementary activity of **9** was somewhat higher than that of **1**, thus the possibility of potency increasement by the position of sugar was not excluded. From the result that **11** did not show the inhibition of hemolysis any more, the trans-olefinic moiety of the stilbenes also seemed to contribute to the potency. It was consistent to the result that the trans-olefinic moiety of 3,3',4,5'-tetrahydroxystilbenes was necessary for hypotensive effect and vasodilatory effect (Inamori *et al.*, 1984a).

Resveratrol was reported to have inhibition effect on the various stages of carcinogenesis, *in vitro* and *in vivo* (Nettleton, 1996) and various hydroxystilbenes were also known to have hypotensive, ichthyotoxic (Inamori *et al.*, 1985), antileukaemic (Mannila *et al.*, 1992), antifungal (Inamori *et al.*, 1984b), antineoplantic activity (Petit *et al.*, 1987). This is the first report that hydroxystilbenes have a anticomplementary activity.

REFERENCES CITED

Alexander, E. L., Provost, T. T., Sanders, M. E., Frank M. M. and Joiner, K. A., Serum complement activation in central nervous system disease in Sjögren's syndrome. *Am. J. Med.*, 85, 513-518 (1988).

Cimanga, K., de Bruyne, T., Lasure, A., Poel, B. V., Pieters, L., Berghe D. V. and Vlietinck, A., *In vitro* anticomplementary activity of constituents from *Morinda morindoides*. *J. Nat. Prod.*, 58, 372-378 (1995).

Cimanga, K., de Bruyne, T., Poel, B. V., Ma, Y., Claeys, M., Pieters, L., Kambu, K., Tona. L., Bakana, P., Berghe, D. V. and Vlietinck, A. J., Complement-modulating properties of a kaempferol 7-*O*-rhamnosyl-sophroside from the leaves of *Morinda morindoides*. *Planta Medica*, 63, 220-223 (1997).

Corvetta, A., Pomponio, G., Rinaldi, N., Luchetti, M. M., di-Loreto, C. and Stramazzotti, D., Terminal complement complex in synovial tissue from patients affected by rheumatoid arthritis, osteoarthritis and acute joint truma. *Clin. Exp. Rheumatol.*, 10, 433-438 (1992).

Deitch, E. A. and Mancini, M. C., Complement receptors in shock and transplantation. *Arch. Surg.*, 128, 1222-1226 (1993).

Goda, Y., Shibuya, M. and Sankawa, U., Inhibitors of the arachidonate cascade from Alliunm chinense and their effect on *in vitro* platelet aggregation. *Chem. Pharm. Bull.*, 35, 2668-2674 (1987).

Han, Y. N., Ryu, S. Y. and Han Y. H., Antioxidant activity of resveratrol closely correlates with its monoamine oxidase-A inhibitiory activity, *Arch. Pharm. Res.*, 13, 132-135 (1990).

Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M., The coronary vasodilatory and hypotensive effects of 3,3',4,5'-tetrahydroxystilbene and its derivatives. *Yakugaku Zasshi*, 104, 819-821 (1984a).

Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M., The antifungal activity of stilbene derivative. *Chem. Pharm. Bull.*, 32, 801-804 (1984b).

Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M., The biological activities of 3,4-*O*-isopropyllidene-3,3',4,5'-tetrahydroxystilbene. *Chem. Pharm. Bull.*, 33, 2904-2909 (1985).

Jung, K. Y., Oh, S. R., Park, S. H., Lee, I. S., Ahn, K. S. and Lee, H.-K., Anticomplementary activity of tiliroside from the flower buds of *Magnolia fargesii*. *Biol. Pharm. Bull.*, in press.

Kim, D. S., Baek, N. I., Oh, S. R., Jung, K. Y., Lee, I. S., Kim, J. H., and Lee, H.-K., Anticomplementary activity of ergosterol peroxide from *Naematoloma fasciculare* and reassignment of NMR data. *Arch. Pharm. Res.*, 20, 201-205 (1997).

Knaus, U. and Wagner, H., Effects of boswellic acid of *Boswellia serrata* and other triterpenic acids on the complement system, *Phytomed.*, 3, 77-81 (1996).

Kuby, J., Complement in 'Immunology' 2nd ed., W. H. Freeman Company, New York, pp. 336-355, 1994.

Mannila, E. and Talvitie, A., Stilbenes from *Picea abies*

bark. *Phytochemistry,* 31, 3288-3289 (1992).

Mcgeer, D. L. and Mcgeer, E. G., Alzheimer's disease, arthritis of the brain? *Drug News & Perspectives,* 8, 80-83 (1995).

Miyagawa, S., Hirose, H., Shirakura, R., Naka, Y. Nakata, S., Kawashima, Y., Seya, T., Matsumoto, M., Uenaka, A. and Kitamura, H., The mechanism of discordant xenograft rejection. *Transplantation,* 46, 825-830 (1988).

Nettleton, Jr. D. E., Biotechnology and natural products. *Drug News & Perspectives,* 9, 485-491 (1996).

Oh, S. R., Jung, K. Y. and Lee, H. K., Micro-screening method for the anticomplement substances from natural resources. *Agr. Chem. Biotech.,* 39, 147-152 (1996).

Oh, S. R., Kim, D. S., Lee, I. S., Jung, K. Y., Lee, J. J. and Lee, H.-K., Anticomplementary activity of constituents from the heartwood of *Caesalpinia sappan, Planta Medica,* 64, 456-458 (1998).

Petit, G. R., Singh, S. B., Niven, M. L., Hamel, E. and Schmidt, J. M., Isolation, structure and synthesis of combretastatins A-1 and B-1, potent new inhibitors of microtubule assembly, derived from *combretum caffrum. J. Nat. Prod.,* 50, 119-131 (1987).

Rother, K., Rother, U. and Hansch, G., The role of complement in inflammation. *Path. Res. Pract.,* 180, 117-124 (1985).

Ryu, S. Y., Han, Y. N. and Han, B. H., Monoamine oxidase A inhibitors from medicinal plants. *Arch. Pharm. Res.,* 11, 230-239 (1988).

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

Sato, Y., Sato, R., Watanabe, H., Kogure, A., Watanabe, K., Nishimaki, T., Kasukawa, R., Kuraya, M. and Fujita, T., Complement activating properties of monoreactive and polyreactive IgM factors. *Ann. Rheum. Dis.,* 52, 795-800 (1993).

Strunk, R. C., Eidlen, D. M. and Mason, R. J., Pulmonary alveolar type II epithelial cells synthesize and secrete proteins of the classical and alternative complement pathways. *J. Clin. Invest.,* 81, 1419-1426 (1988).

Takematsu, H. and Tagami, H., Complement fragment C4d and Bb levels in inflammatory skin diseases (eg. SLE, atopic dermatitis, erythroderma and pustulosis palmaris et plantaris) for assessment of complement activation. *Tohoku. J. Exp. Med.,* 163, 263-268 (1991).

Wortel, C. H. and Doerschuk, C. M., Neutrophils and neutrophil-endothelial cell adhesion in adult respiratory distress syndrome. *New. Horiz.,* 1, 631-637 (1993).