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Effects of Interaction of Tannins with Co-existing Substances. VII.[1] Inhibitory Effects of Tannins and Related Polyphenols on Xanthine Oxidase

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The inhibitory effects of hydrolyzable tannins, condensed tannins and related polyphenols on the activity of xanthine oxidase (XOD), catalyzing uric acid formation from xanthine, were investigated. Marked differences in the strength of the inhibition were observed. Some of the differences among the monomeric hydrolyzable tannins were due to their molecular weights, reflecting the number of phenolic hydroxyl groups in the molecule. However, the inhibitory activity of several oligomeric hydrolyzable tannins seemed particularly low in spite of their large molecular size. It was also observed that differences in location of acyl groups on the carbohydrate cores caused differences in the inhibitory activity among monomeric and oligomeric hydrolyzable tannins. A caffeic acid derivative (caffeetannin), 3,5-di-*O*-caffeoylquinic acid (24), also inhibited this enzyme. Galloylation and the degree of polymerization in proanthocyanidins were also shown to affect remarkably the strength of the inhibition. Among the compounds tested in the present study, valoneic acid dilactone (29), isolated from *Mallotus japonicus*, inhibited the enzyme most effectively. A kinetic study showed that this dilactone inhibited XOD non-competitively. Comparison of the inhibitory effect on XOD, with the binding activity to hemoglobin, for each tannin, suggests that their inhibition of XOD is not based on non-specific binding to the protein. Similar comparison of the inhibitory effect on XOD with the inhibitory effect on the generation of superoxide anion radical ($O_2^{\cdot-}$) from the hypoxanthine–XOD system revealed that the inhibition of $O_2^{\cdot-}$ generation by tannins is due to their radical-scavenging activity, and not due to their inhibitory activity upon the enzyme.

Keywords tannin; galloylglucose; ellagitannin; proanthocyanidin; valoneic acid dilactone; *Mallotus japonicus*; xanthine oxidase; superoxide anion; inhibition

Uric acid, which causes gout, is formed from xanthine in the presence of xanthine oxidase (XOD). Superoxide anion radical ($O_2^{\cdot-}$), generated upon the formation of uric acid, has been found to cause oxidative damage of living tissues.[1] In a previous report, we showed that various types of tannins and related polyphenols inhibited generation of $O_2^{\cdot-}$ in the hypoxanthine–XOD system.[1] The radical-scavenging action of tannins through the formation of stable free radicals, which has been revealed to contribute to the inhibitory effects of tannins on lipid peroxidation,[2] is also considered to participate in the inhibition of the generation of $O_2^{\cdot-}$.[1] On the other hand, various phenolic compounds have been reported to inhibit the formation of uric acid from xanthine by XOD.[3] Thus, we have investigated the inhibitory effects of tannins on the activity of XOD to find effective inhibitors for XOD, and also to examine the correlation of the inhibition of the $O_2^{\cdot-}$ generation and the inhibition of the enzyme activity.

Experimental

Materials Tannins and related polyphenols used in the present experiment (except for **4**, **5**, **13**, **26**—**29**, **32**) were those isolated from the following medicinal plants (given in parentheses)[4]: 1,2,6-tri-*O*-galloyl-β-D-glucose (**1**), rugosin A (**12**) and rugosin D (**21**) (*Rosa rugosa* THUNB.)[4a,b]; 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**2**), tellimagrandin II (**9**), isoterchebin (**14**), cornusiin A (**16**), cornusiin D (**19**), cornusiin E (**22**) and cornusiin C (**23**) (*Cornus officinalis* SIEB. *et* ZUCC.)[4c-e]; 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**3**) (*Euphorbia hirta* L.)[4f]; gemin D (**6**) and coriariin A (**20**) (*Coriaria japonica* A. GRAY)[4g,h]; tellimagrandin I (**7**), pedunculagin (**8**), casuarictin (**10**) and casuarinin (**11**) (*Casuarina stricta* AIT.)[4i]; geraniin (**15**) (*Geranium thunbergii* SIEB. *et* ZUCC.)[4j,k]; oenothein B (**17**) (*Oenothera erythrosepala* BORBAS)[4l]; camptothin B (**18**) (*Camptotheca acuminata* DECNE.)[4d,m]; 3,5-di-*O*-caffeoylquinic acid (**24**) (*Artemisia montana* PAMPAN.)[4n]; rabdosiin (**25**) (*Rabdosia japonica* HARA)[4o]; (−)-epigallocatechin (**30**), (−)-epigallocatechin gallate (**31**) and (−)-epicatechin gallate [=3-*O*-galloylepicatechin, **35**] (*Thea sinensis* L.)[4p]; procyanidin B-2 (**33**) and procyanidin C-1 (**34**) (*Chaenomeles sinensis* KOEH.)[4q]; 3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin (**36**), 3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin (**37**) and 3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin (**38**) (*Saxifraga stolonifera* MEERB.).[4q,r] Penta-*O*-galloyl-β-D-glucose (**4**) and methyl gallate (**27**) were prepared by methanolysis[5] of tannic acid. Corilagin (**5**) and oenothein C (**13**) were prepared from geraniin (**15**)[4j] and cornusiin B,[4d] respectively. Valoneic acid dilactone (**29**)[6] was isolated from *Mallotus japonicus* MUELL. ARG. as described below. Other reagents were purchased from the following companies: XOD from cow's milk (Boehringer Mannheim), xanthine (Wako), gallic acid (**26**) (Tokyo Kasei), ellagic acid (**28**) and (−)-epicatechin (**32**) (Sigma), and allopurinol (Aldrich).

Isolation of Valoneic Acid Dilactone (29) from Leaves of *Mallotus japonicus* Dried leaves (500 g) of *Mallotus japonicus* were homogenized in 70% acetone, and the concentrated filtrate obtained from the homogenate was extracted successively with diethyl ether and ethyl acetate. A portion (3 g) of the ethyl acetate extract (22 g) was chromatographed over Sephadex LH-20 with EtOH as a developer, to give valoneic acid dilactone (35 mg), which was identified by co-chromatography on HPLC (high-performance liquid chromatography) with an authentic sample, using three systems of column and solvent: i) Merck Superspher Si60 column (4×125 mm), *n*-hexane–MeOH–tetrahydrofuran–formic acid (55:33:11:1) containing oxalic acid (450 mg/l); ii) Merck LiChrospher RP-18 column (4×250 mm), 0.01 M H_3PO_4–0.01 M KH_2PO_4–EtOH–ethyl acetate (11:11:2:1); iii) Merck LiChrospher RP-18 column, 0.01 M H_3PO_4–0.01 M KH_2PO_4–acetonitrile (11:11:3). The identification was also based on the spectral data: ultraviolet (UV) spectrum, λ_{max}^{MeOH} nm (log ε) 257 (4.42), 344 (sh) and 358 (3.75); proton nuclear magnetic resonance (^{1}H-NMR) spectrum (90 MHz, in acetone-d_6), δ 7.59 (1H, s) and 7.22 (2H, s).

Estimation of Inhibitory Effects of Tannins and Related Polyphenols on the Activity of Xanthine Oxidase Inhibitory effects were estimated by the reported method[3a] with a slight modification. A mixture consisting of XOD solution (0.04 U/ml; 40 μl) in 0.1 M phosphate buffer (pH 7.5, 360 μl), and a tannin (or related compound) solution in 12% dimethyl sulfoxide in water (200 μl) was preincubated for 10 min at 37 °C. Then, an aqueous solution of xanthine (0.1 mM, 600 μl) was added to the mixture, and the resulting solution was incubated for 30 min at 37 °C. The enzyme reaction was terminated by adding 1 N HCl (200 μl), and the absorbance of the reaction mixture at 295 nm was measured. The concentration of uric acid formed in the mixture was calculated from the absorbance, after subtracting the absorbance of the blank solution which was prepared in the same way as that described above, except that the XOD solution was added

after the addition of HCl. The value of inhibition (%) of the formation of uric acid was obtained as the mean of triplicate experiments.

Results and Discussion

The inhibitory activity of each tannin or related compound is expressed in Tables I and II in terms of IC_{50}, the concentration of the compound required for 50% inhibition of the uric acid formation. These tables show a remarkable variation of the inhibitory activity, due to differences in the chemical structures of tannins.

Structure–Activity Relationship The inhibitory activity among monomeric hydrolyzable tannins increased with the increase of molecular weight, which is accompanied by an increase in the number of phenolic hydroxyl groups in the molecule (Chart 1): 1,2,6-Tri-*O*-galloyl-β-D-glucose (**1**) < 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**2**) < penta-*O*-galloyl-β-D-glucose (**4**); gemin D (**6**) < tellimagrandin I (**7**) < tellimagrandin II (**9**). However, oligomeric hydrolyzable tannins did not show increased inhibitory activity, and in fact a decrease of the activity was observed for some dimeric hydrolyzable tannins: tellimagrandin II (**9**, monomer) > coriariin A (**20**, dimer); tellimagrandin I (**7**, monomer) ≫ cornusiin A (**16**, dimer), oenothein B (**17**, dimer).

Recent investigations showed that oligomeric hydrolyzable tannins including **16**, **17**, **20** and **21**, had characteristic host-mediated antitumor activity,[7] which was not exhibited by monomeric hydrolyzable tannins. Some dimeric hydrolyzable tannins such as **17** and **20** also specifically inhibited the proliferation of HIV (human immunodeficiency virus).[8] Several dimeric hydrolyzable tannins inhibited reverse transcriptase of an RNA (ribonucleic acid) tumor virus more potently than monomeric hydrolyzable tannins.[9] The inhibitory activity of tannins on XOD, observed in the present study, is therefore a rare example among tannins of monomers being more potent than oligomers.

However, the inhibitory effect of a trimeric hydrolyzable tannin, cornusiin C (**23**), on XOD was somewhat stronger than that of the corresponding dimer, cornusiin A (**16**).

There were also differences in inhibitory activity among isomers consisting of the same, or analogous structural units: 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**2**) < 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**3**); corilagin (**5**, IC_{50} 5.4×10^{-5} M) < gemin D (**6**); coriariin A (**20**) < rugosin D (**21**). In particular, the substitution of a hexahydroxydiphenoyl (HHDP) group for the two galloyl groups at O-2 and O-3 on the glucopyranose core in the following examples significantly decreased the inhibitory activity: pedunculagin (**8**) ≪ tellimagrandin I (**7**); casuarictin (**10**) ≪ tellimagrandin II (**9**). The decrease of the inhibitory activity is attributable to the increase of the rigidity of the tannin molecule owing to the substitution. Casuarinin (**11**) is also an example of an increment of rigidity apparently causing a decrease of the inhibitory activity; this tannin, having two HHDP groups, of which one takes part in a C-glucosidic linkage, exhibited weaker inhibition than that of pedunculagin (**8**).

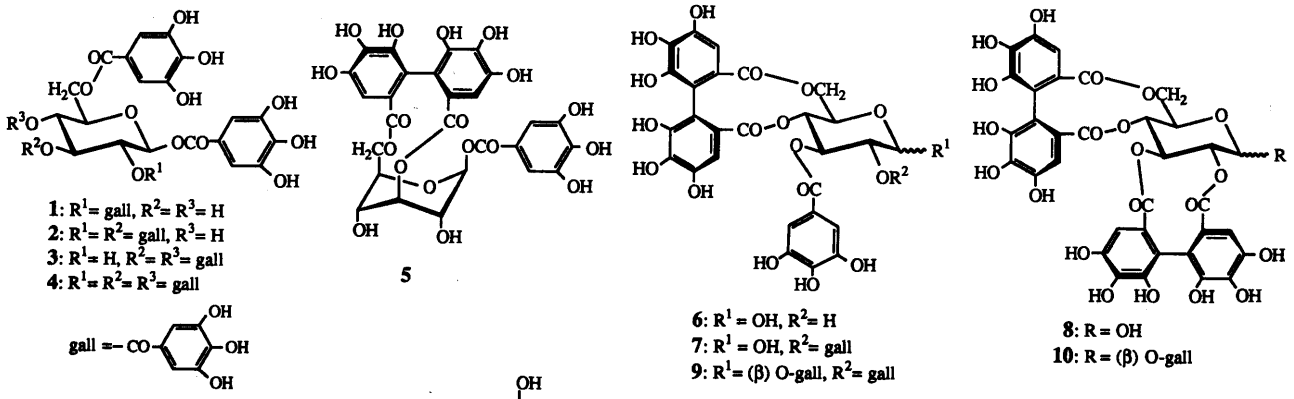
However, the inhibitory activity of tellimagrandin II (**9**) having an HHDP group at O-4—O-6 of the glucose core was comparable to that of penta-*O*-galloyl-β-D-glucose (**4**). The differences in the location of acyl groups on the glucopyranose core(s) of hydrolyzable tannins, resulting in differences in the molecular conformation, appear to affect

TABLE I. Inhibitory Effects of Hydrolyzable Tannins and Related Polyphenols on Xanthine Oxidase

Tannin and related polyphenol	Molecular weight	IC_{50} (μM)
Monomeric hydrolyzable tannins		
Galloylglucoses		
1,2,6-Tri-*O*-galloyl-β-D-glucose (**1**)	636.5	39
1,2,3,6-Tetra-*O*-galloyl-β-D-glucose (**2**)	788.6	12
1,3,4,6-Tetra-*O*-galloyl-β-D-glucose (**3**)	788.6	8.1
Penta-*O*-galloyl-β-D-glucose (**4**)	940.7	3.3
Ellagitannins and Related Tannins		
Corilagin (**5**)	634.5	>40
Gemin D (**6**)	634.5	35
Tellimagrandin I (**7**)	786.6	6.9
Pedunculagin (**8**)	784.6	18
Tellimagrandin II (**9**)	938.7	3.1
Casuarictin (**10**)	936.7	11
Casuarinin (**11**)	936.7	36
Rugosin A (**12**)	1106.8	8.0
Oenothein C (**13**)	784.6	11
Isoterchebin (**14**)	954.7	14
Geraniin (**15**)	952.7	>40
Dimeric and trimeric hydrolyzable tannins		
Cornusiin A (**16**)	1571.1	27
Oenothein B (**17**)	1569.1	20
Camptothin B (**18**)	1723.2	12
Cornusiin D (**19**)	1723.2	12
Coriariin A (**20**)	1875.3	9.8
Rugosin D (**21**)	1875.3	3.1
Cornusiin E (**22**)	1875.3	7.4
Cornusiin C (**23**)	2355.7	17
Derivatives of caffeic acid		
3,5-Di-*O*-caffeoylquinic acid (**24**)	516.5	34
Rabdosiin (**25**)	718.6	>40
Low-molecular-weight polyphenols and others		
Gallic acid (**26**)	170.1	24
Methyl gallate (**27**)	176.1	29
Ellagic acid (**28**)	302.2	3.1
Valoneic acid dilactone (**29**)	470.3	0.76
Allopurinol	136.1	0.17

TABLE II. Inhibitory Effects of Condensed Tannins and Related Polyphenols on Xanthine Oxidase

Tannin and related polyphenol	Molecular weight	IC_{50} (μM)
Flavanols having *o*-trihydroxy structure on the B-ring		
(−)-Epigallocatechin (**30**)	306.3	>40
(−)-Epigallocatechin gallate (**31**)	458.4	>40
Flavanols having *o*-dihydroxy structure on the B-ring and their oligomers		
Epicatechin and its oligomers		
(−)-Epicatechin (**32**)	290.3	>40
Procyanidin B-2 (**33**)	578.5	>40
Procyanidin C-1 (**34**)	866.8	>40
Epicatechin gallate and its oligomers		
(−)-Epicatechin gallate (=3-*O*-galloyl-epicatechin) (**35**)	442.4	>40
3-*O*-Galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin (**36**)	882.7	7.2
3-*O*-Galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloyl-epicatechin (**37**)	1323.1	5.9
3-*O*-Galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloyl-epicatechin-(4-β→8)-3-*O*-galloylepi-catechin (**38**)	1763.5	4.4



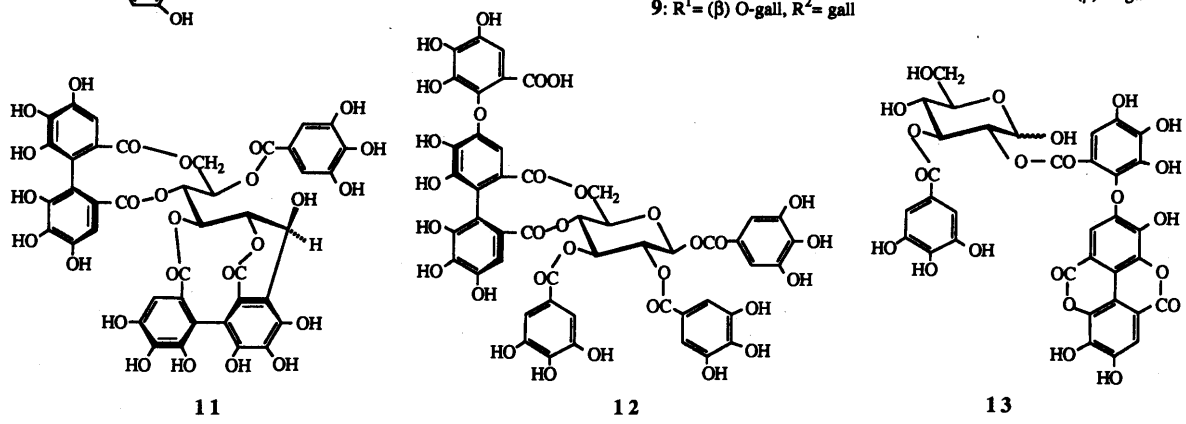


Chart 1

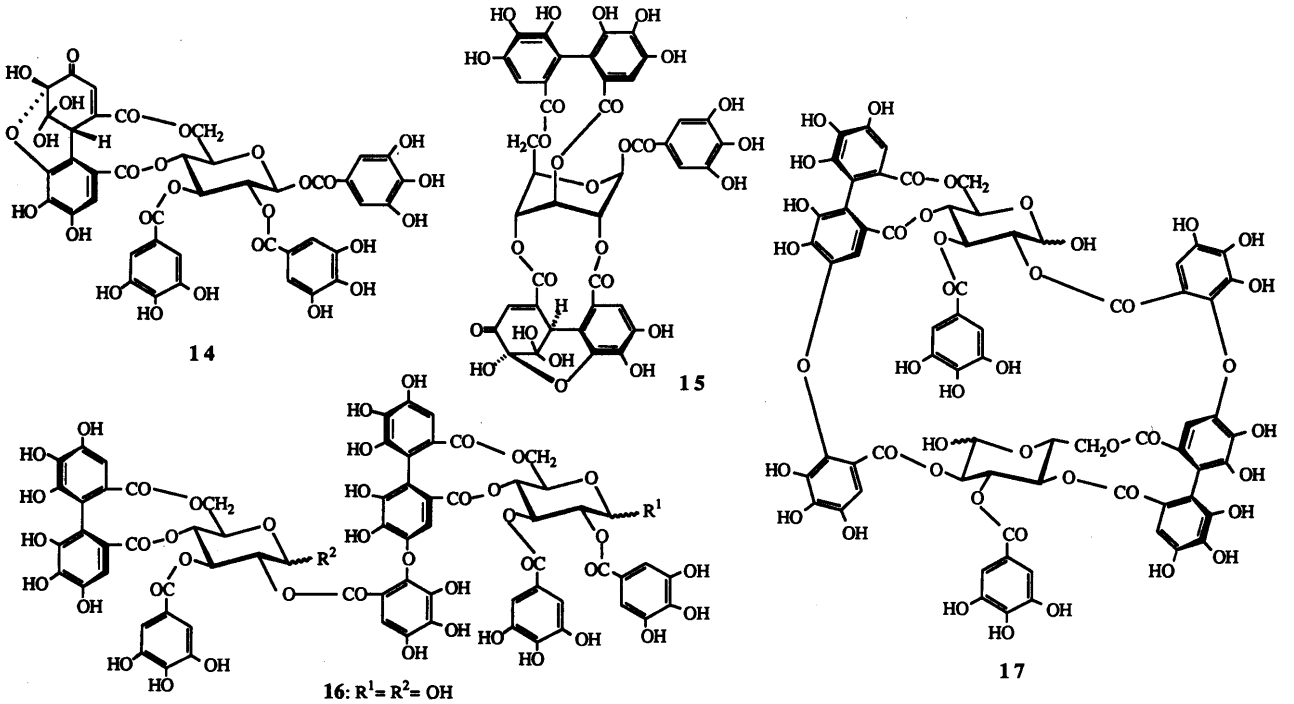


Chart 2

the inhibitory activity.

Although penta-*O*-galloyl-*β*-D-glucose (**4**) was reported to inhibit the enzyme with the same order of IC_{50} as that of allopurinol,[10] the present experiment showed that the inhibitory activity of penta-*O*-galloyl-*β*-D-glucose was far weaker than that of allopurinol.

Several derivatives of caffeic acid, and their mixtures have been called caffeetannin[4m] or labiataetannin.[11] An example of caffeetannin is 3,5-di-*O*-caffeoylquinic acid (**24**), which has been isolated from *Artemisia* species as a con-

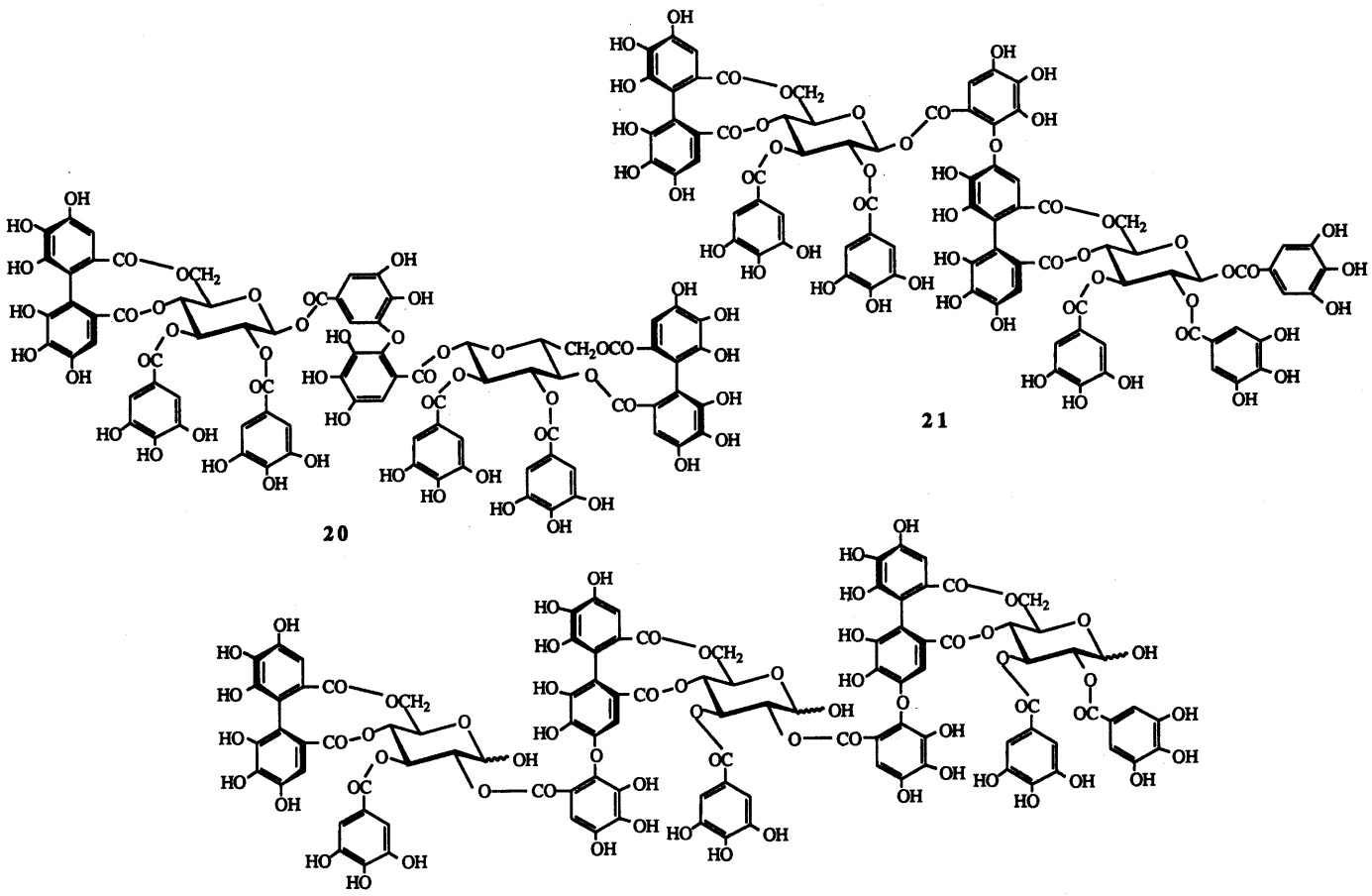


Chart 3

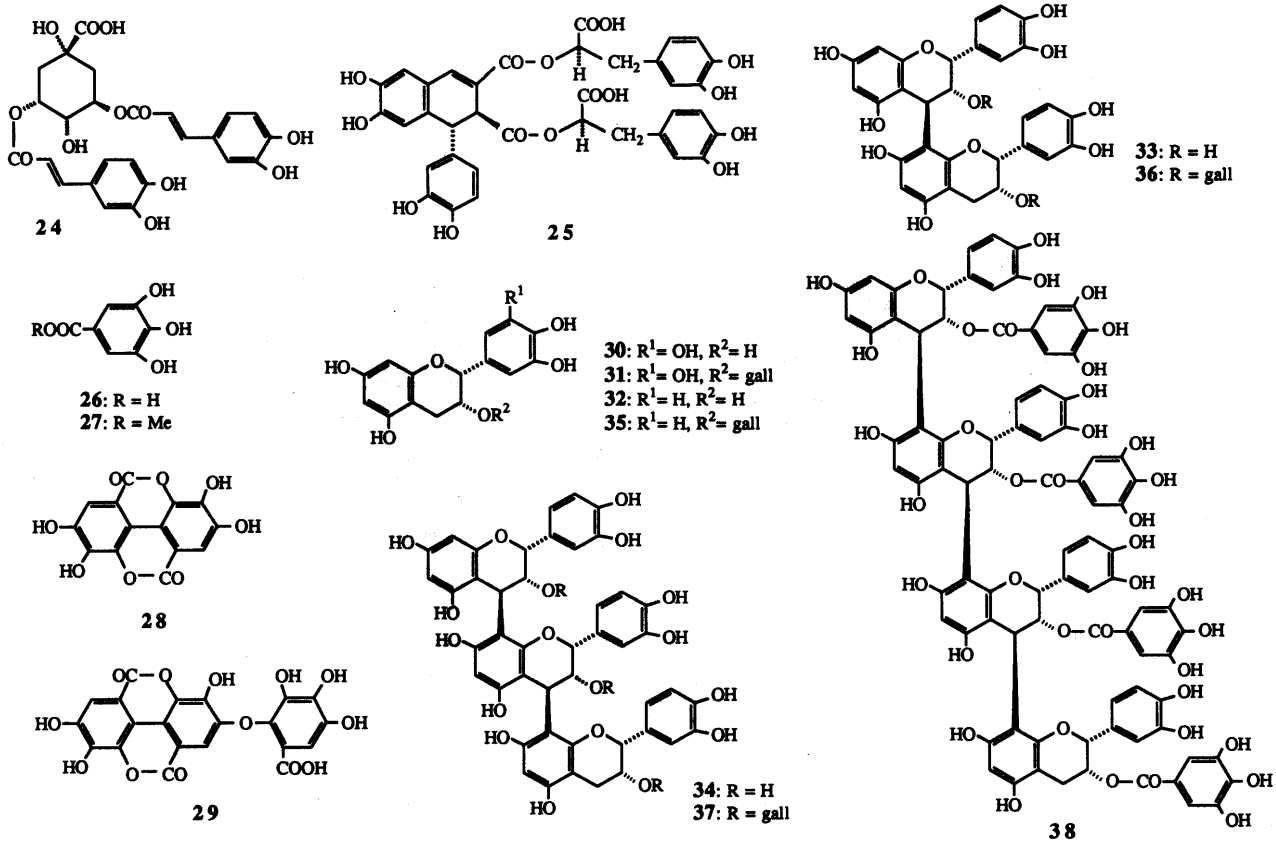


Chart 4

stituent responsible for the astringency of the extracts from these plants.[4n] The caffeetannin originally found in coffee beans is mainly composed of a monocaffeoylquinic acid (chlorogenic acid) which showed only negligible activity of tannin as far as the binding to hemoglobin is concerned. However, di-*O*-caffeoylquinic acids which are also contained in small amounts in coffee beans showed RA (relative astringency; relative binding activity to hemoglobin) values comparable to those of tannins.[4n] In the present experiment, 3,5-di-*O*-caffeoylquinic acid inhibited XOD with an IC_{50} value of 3.4×10^{-5} M. However, rabdosiin (**25**), a caffeic acid tetramer which was isolated from *Rabdosia japonica*,[4o] showed only very weak inhibition of XOD (IC_{50} 1.5×10^{-4} M).

The oligomeric proanthocyanidins having galloyl groups showed appreciable inhibition, depending on the degree of polymerization [dimer (**36**) < trimer (**37**) < tetramer (**38**)] (Chart 4). However, monomeric epicatechin gallate (**35**) and epigallocatechin gallate (**31**) showed practically no inhibition of the activity of XOD. Ordinary proanthocyanidins lacking an acyl group (**33** and **34**), and their constituent monomers (**30** and **32**), also showed practically no inhibition.

Among the polyphenols of low molecular weight, ellagic acid (**28**) and valoneic acid dilactone (**29**), both of which have two lactone groups, showed inhibitory effects stronger than those of gallic acid (**26**) and methyl gallate (**27**). The inhibitory effect of valoneic acid dilactone on XOD was the most prominent among those of the compounds tested in the present experiment; the IC_{50} value was of the same order as that of allopurinol. Kinetic studies using Lineweaver–Burk plots showed that valoneic acid dilactone inhibited XOD non-competitively at the concentration of 0.8 μM (Fig. 1). Since the inhibition of the mutagenicity of benzo[*a*]pyrene-7,8-diol-9,10-epoxide by ellagic acid (**28**) was suggested to be due to the formation of a complex in which the mutagen and ellagic acid were arranged in parallel,[12] the flat dilactone structure in ellagic acid and valoneic acid dilactone may also contribute to the inhibition of XOD.

On the other hand, oenothein C (**13**), in which the dilactone (**29**) is linked to the glucose core, showed relatively weak inhibition.

Correlation of the Inhibitory Effects on XOD and Binding Activity to Hemoglobin In order to establish whether the inhibition of XOD is due to non-specific binding or not, the inhibitory activity was compared with the binding activity to hemoglobin.[13,14] Inhibitory activity of each tannin was expressed as RIA_{PGG} value, relative inhibitory activity based on the activity of penta-*O*-galloyl-β-D-glucose (PGG). This value was calculated according to the following equation:

$$RIA_{PGG}=\frac{IC_{50}\text{ of PGG}}{IC_{50}\text{ of tested tannin}}$$

Binding activity of each tannin was expressed as RBA_{PGG} value, relative binding activity to hemoglobin based on the activity of PGG. This value was recalculated according to the following equation using the *RAG* (relative astringency based on geraniin) value of each tannin,[14b] since the *RAG* value has been estimated based on the weight of each tannin required to give a 50% decrease of the absorbance of

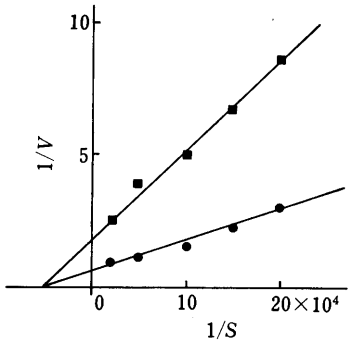


Fig. 1. Inhibitory Effects of Valoneic Acid Dilactone (**29**) on XOD

Lineweaver–Burk plots in the absence (●—●) and in the presence (■—■, 0.8 μM) of **29**. *V*, μmol substrate metabolized/mg enzyme/min; *S*, molar concentration of substrate.

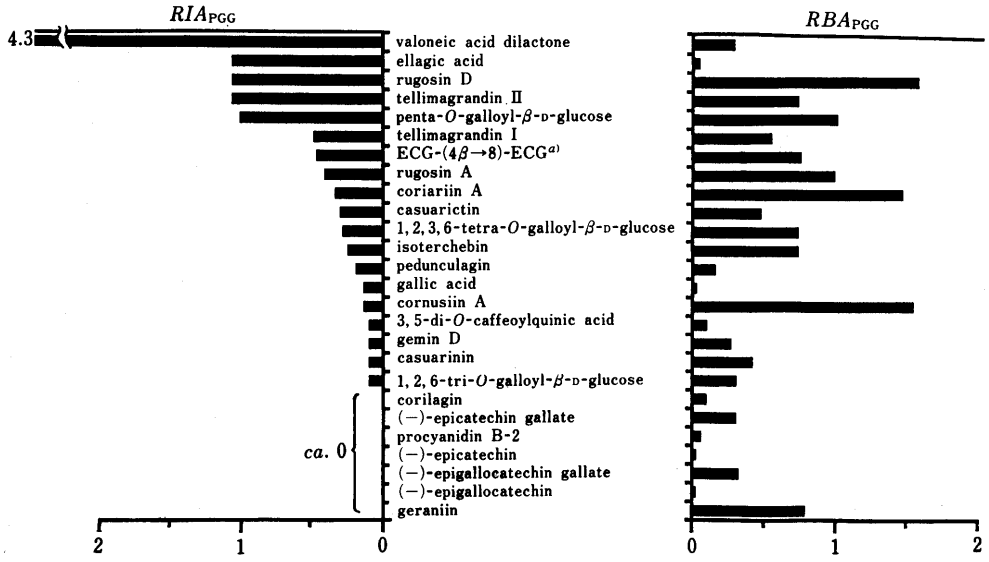


Fig. 2. Comparison of Inhibitory Activity of Tannins and Related Polyphenols on XOD, with Binding Activity to Hemoglobin

RIA_{PGG}, relative inhibitory activity on XOD based on the activity of penta-*O*-galloyl-β-D-glucose (**4**); RBA_{PGG}, relative binding activity to hemoglobin based on the activity of **4**. *a*) 3-*O*-Galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin.

hemoglobin in diluted blood, and not on the molar concentration.

$$RBA_{PGG}=\frac{(RAG \text{ of tested tannin})\times(MW \text{ of tested tannin})}{(RAG \text{ of PGG})\times(MW \text{ of PGG})}$$

MW means molecular weight

As shown in Fig. 2, the inhibitory effects of tannins and related compound on XOD are not due to the non-specific binding of tannins to the protein, since there is no correlation between the RIA_{PGG} values and RBA_{PGG} values. Some tannins such as coriariin A (**20**), cornusiin A (**16**) (dimeric hydrolyzable tannin) and geraniin (**15**) (monomeric hydrolyzable tannin), which showed moderate or strong binding activity to hemoglobin, merely showed weak inhibitory effects on XOD. Epigallocatechin gallate (**31**) and epicatechin gallate (**35**), which showed relatively strong binding activity among the polyphenols of low molecular weight,[14b] showed practically no inhibition of XOD. On the other hand, ellagic acid (**28**), with a weak binding activity to hemoglobin, showed a strong inhibitory effect on XOD.

Correlation of the Inhibition of XOD and the Inhibition of Superoxide-Anion Radical Generation If the inhibitory effects of tannins on the generation of O_2^- show a good correlation with the inhibitory effects on XOD, then the inhibition of the O_2^- generation may be regarded as the result of the inhibition of the enzyme. However, the present experiment showed that the order of the strength of inhibition of XOD among the tannins was considerably different from that of the inhibitory effects on the O_2^- generation from the hypoxanthine–XOD system[1]: Compounds which showed strong inhibition of generation of O_2^- [such as geraniin (**15**), cornusiin A (**16**), coriariin A (**20**), epigallocatechin (**30**) and epigallocatechin gallate (**31**)], showed weak inhibition of XOD, while ellagic acid (**28**), which showed weak inhibition of O_2^- generation, strongly inhibited XOD. Thus, it may be concluded that the inhibition of the O_2^- generation by tannins is due to their radical-scavenging activity, as proposed previously,[1] and not due to their inhibitory activity upon the enzyme.

References

1) For Part VI in the series entitled "Effects of Interaction of Tannins with Co-existing Substances," see T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, T. Yasuhara, T. Yoshida and T. Okuda, *Chem. Pharm. Bull.*, **37**, 2016 (1989).
2) *a*) Y. Fujita, K. Komagoe, Y. Niwa, I. Uehara, R. Hara, H. Mori, T. Okuda and T. Yoshida, *Yakugaku Zasshi*, **108**, 528 (1988); *b*) Y. Fujita, I. Uehara, Y. Morimoto, M. Nakajima, T. Hatano and T. Okuda, *ibid.*, **108**, 129 (1988); *c*) Y. Fujita, K. Komagoe, H. Mori, Y. Niwa and T. Okuda, in "Medical, Biomedical and Chemical Aspects of Free Radicals," ed. by O. Hayaishi, E. Niki, M. Kondo and T. Yoshikawa, Elsevier Science Publishers, Amsterdam, 1989, pp. 853—856.
3) *a*) T. Noro, Y. Oda, T. Miyase, A. Ueno and S. Fukushima, *Chem. Pharm. Bull.*, **31**, 3984 (1983); *b*) T. Hatano, T. Yasuhara, T. Fukuda, T. Noro and T. Okuda, *ibid.*, **37**, 3005 (1989), and literatures cited therein.
4) *a*) T. Okuda, T. Hatano, K. Yazaki and N. Ogawa, *Chem. Pharm. Bull.*, **30**, 4230 (1982); *b*) T. Okuda, T. Hatano and N. Ogawa, *ibid.*, **30**, 4234 (1982); *c*) T. Okuda, T. Hatano and T. Yasui, *Heterocycles*, **16**, 1321 (1981); *d*) T. Hatano, N. Ogawa, R. Kira, T. Yasuhara and T. Okuda, *Chem. Pharm. Bull.*, **37**, 2083 (1989); *e*) T. Hatano, T. Yasuhara and T. Okuda, *ibid.*, **37**, 2665 (1989); *f*) T. Yoshida, L. Chen, T. Shingu and T. Okuda, *ibid.*, **36**, 2940 (1988); *g*) T. Hatano, S. Hattori and T. Okuda, *ibid.*, **34**, 4092 (1986); *h*) *Idem, ibid.*, **34**, 4533 (1986); *i*) T. Okuda, T. Yoshida, M. Ashida and K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1765; *j*) T. Okuda, T. Yoshida and H. Nayeshiro, *Chem. Pharm. Bull.*, **25**, 1862 (1977); *k*) T. Okuda, T. Yoshida and T. Hatano, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 9; *l*) T. Hatano, T. Yasuhara, M. Matsuda, K. Yazaki, T. Yoshida and T. Okuda, *Chem. Pharm. Bull.*, **37**, 2269 (1989); *m*) T. Hatano, Y. Ikegami, T. Shingu and T. Okuda, *ibid.*, **36**, 2017 (1988); *n*) T. Okuda, T. Hatano, I. Agata, S. Nishibe and K. Kimura, *Yakugaku Zasshi*, **106**, 894 (1986); *o*) I. Agata, T. Hatano, S. Nishibe and T. Okuda, *Phytochemistry*, **28**, 2447 (1989); *p*) T. Okuda, K. Mori and H. Hayatsu, Abstracts of Papers, 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982, p. 588; *q*) T. Hatano, K. Urita and T. Okuda, Abstracts of Papers, 24th Annual Meeting of the Chugoku–Shikoku Branch of the Pharmaceutical Society of Japan, Tokushima, October 1985, p. 45; *r*) T. Hatano, K. Urita and T. Okuda, *J. Med. Pharm. Soc., Wakan-Yaku*, **3**, 434 (1986).
5) P. Crabtree, E. Haslam, R. Haworth, S. Mills and J. Stangroom, *J. Chem. Soc.*, **1965**, 6888.
6) O. Th. Schmidt and E. Komarek, *Justus Liebigs Ann. Chem.*, **591**, 156 (1955).
7) K. Miyamoto, N. Kishi, R. Koshiura, T. Yoshida, T. Hatano and T. Okuda, *Chem. Pharm. Bull.*, **35**, 814 (1987).
8) M. Asanaka, T. Kurimura, R. Koshiura, T. Okuda, M. Mori and H. Yokoi, Abstracts of Papers, 4th International Conference on Immunopharmacology, May 1988, Osaka, p. 47.
9) N. Kakiuchi, M. Hattori, T. Namba, M. Nishizawa, T. Yamagishi and T. Okuda, *J. Nat. Prod.*, **48**, 614 (1985).
10) T. Hayashi, K. Nagayama, M. Arisawa, M. Shimizu, S. Suzuki, M. Yoshizaki, N. Morita, E. Ferro, I. Basualdo and L. H. Berganza, *J. Nat. Prod.*, **52**, 210 (1989).
11) T. Okuda, T. Hatano, I. Agata and S. Nishibe, *Yakugaku Zasshi*, **106**, 1108 (1986).
12) A. W. Wood, M.-T. Huang, R. L. Chang, H. L. Newmark, R. E. Lehr, H. Yagi, J. M. Sayer, D. M. Jerina and A. H. Conney, *Proc. Natl. Sci. U.S.A.*, **79**, 5513 (1982).
13) E. C. Bate-Smith, *Phytochemistry*, **12**, 907 (1973).
14) *a*) T. Okuda, K. Mori and K. Aoi, *Yakugaku Zasshi*, **97**, 1267 (1977); *b*) T. Okuda, K. Mori and T. Hatano, *Chem. Pharm. Bull.*, **33**, 1424 (1985); *c*) T. Hatano, H. Kagawa, T. Yasuhara and T. Okuda, *ibid.*, **36**, 2090 (1988).