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STRUCTURE AND ANTIHERPETIC ACTIVITY AMONG THE TANNINS

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Key Word Index—Tannins; antiherpetic activity; structure–activity relationships; galloyl group; cytotoxicity; tannin–protein interaction.

Abstract—In order to investigate the relationship between the antiherpetic activity and the structure of tannins, the activities of 38 such compounds were examined. The results indicate that the activities of hydrolysable tannins were dependent on the number of galloyl or hexahydroxydiphenoyl groups and those of condensed ones on the degree of condensation. On the other hand, the more active tannins were the more cytotoxic.

INTRODUCTION

We have previously purified the antiherpetic (antiviral) substances from the bud of *Syzygium aromatica* and the root of *Paeonia* species and these substances were identified as eugeniin [1] and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose [2], respectively. From these results, it appears that tannins can show antiherpetic activity. In previous reports on the virus-inactivating actions of tannins, some authors [3–6] tested hydrolysable tannins and others [7–10] condensed tannins; but the chemical structures of tannins used were not clearly described.

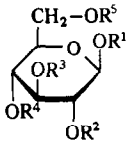
In this work, we have attempted to elucidate the relationship between activity and the structure of the tannin.

RESULTS AND DISCUSSION

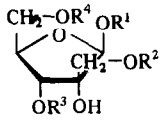
As shown in Table 1, hydrolysable tannins which have the greater number of galloyl or hexahydroxydiphenoyl groups in the molecule show the greater activity. The nature of the constitutive alcohol, however, was not related to activity.

As shown in Table 2, tannins which are more highly condensed have the greater activity, but the galloyl group contributes more to the activity, than the degree of condensation. The activities of hydrolysable tannins or galloylated condensed tannins were considerably diminished by tannase treatment.

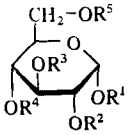
It has been reported that galloyl or *o*-dihydroxyphenoyl groups are related to polyphenol–protein complex forma-



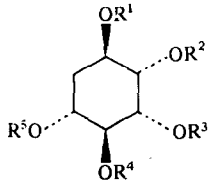
1 $R^1 = R^2 = R^3 = R^4 = H$, R^5 = Galloyl
2 $R^3 = R^4 = H$, $R^1 = R^2 = R^5$ = Galloyl
3 $R^4 = H$, $R^1 = R^2 = R^3 = R^5$ = Galloyl
4 $R^1 = R^2 = R^3 = R^4 = R^5$ = Galloyl
5 $R^1 = R^2 = R^3$ = Galloyl, $R^{4,5}$ = (s) –Hexahydroxydiphenoyl



8 $R^1 = R^2 = R^3 = H$, R^4 = Galloyl
9 $R^1 = R^3 = H$, $R^2 = R^4$ = Galloyl
10 $R^1 = H$, $R^2 = R^3 = R^4$ = Galloyl



6 R^1 = Galloyl, $R^{2,3} = R^{4,5}$ = (s) – Hexahydroxydiphenoyl
7 $R^4 = H$, $R^1 = R^5$ = Galloyl, $R^{2,3}$ = (s) –Hexahydroxydiphenoyl

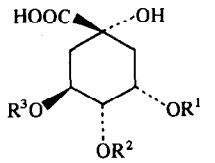


11 $R^1 = R^2 = R^3 = H$, $R^4 = R^5$ = Galloyl
12 $R^1 = R^2 = H$, $R^3 = R^4 = R^5$ = Galloyl
13 $R^2 = H$, $R^1 = R^3 = R^4 = R^5$ = Galloyl

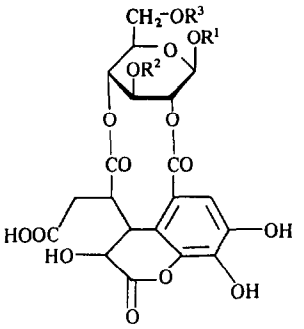
Table 1. Antiviral activities and cytotoxicities of hydrolysable tannins

Hydrolysable tannin	Number of galloyl groups	Number of hexahydroxy-diphenoyl groups	PRD_{50} (μM)	CSD_{50} (μM)	References
1	1	0	*	†	
8	1	0	*	†	
9	2	0	*	†	
11	2	0	*	†	
14	2	0	*	†	
2	3	0	55	†	[13]
10	3	0	53	†	
12	3	0	58	†	
15	3	0	56	†	
3	4	0	22	195	[14]
13	4	0	23	198	
4	5	0	12	117	[15]
16	1	1	26	182	[18]
17	1	1	27	184	[19]
7	2	1	20	168	[17]
5	3	1	15	122	[16]
6	1	2	19	165	
18	2	3	29	61	[17]
19	2	4	27	52	[20]

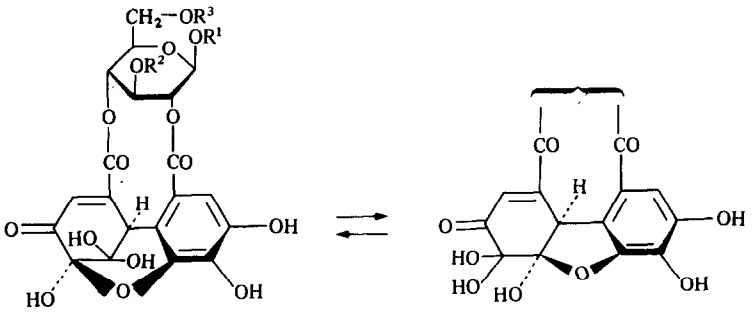
*PRD_{50} = > 100 μM.
†CSD_{50} = > 200 μM.



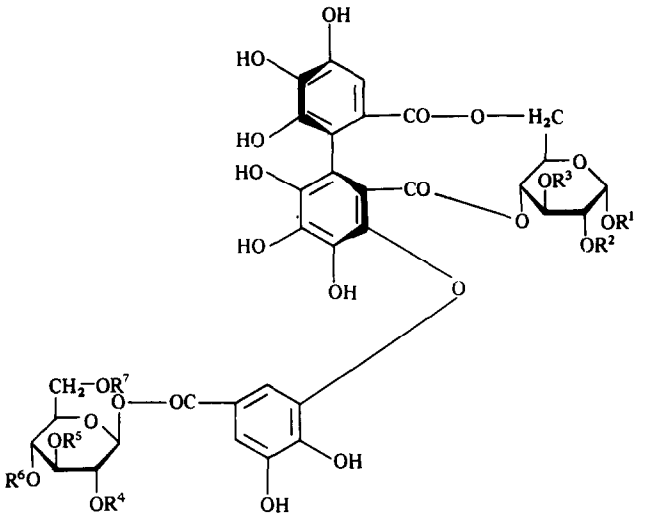
14 R^3 = H, R^1 = R^2 = Galloyl
15 R^1 = R^2 = R^3 = Galloyl



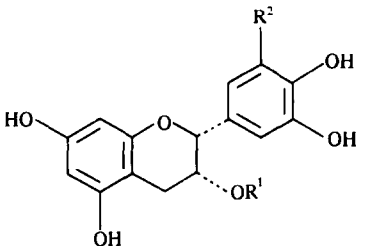
16 R^1 = Galloyl, $R^{2,3}$ = (s) –Hexahydroxydiphenoyl



17 R^1 = Galloyl, $R^{2,3}$ = (s) – Hexahydroxydiphenoyl



18 $R^6 = R^7 = H$, R^1 = Galloyl, $R^{2,3} = R^{4,5}$ = (s) –Hexahydroxydiphenoyl
19 R^1 = Galloyl, $R^{2,3} = R^{4,5} = R^{6,7}$ = (s) – Hexahydroxydiphenoyl

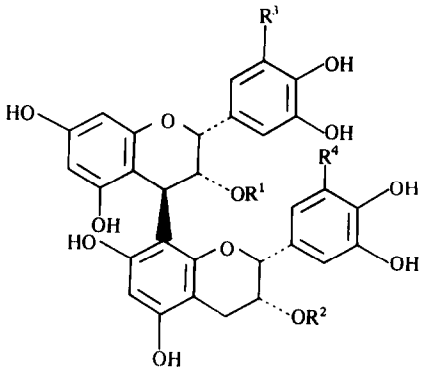


20 $R^1 = R^2 = H$
21 R^1 = Galloyl, $R^2 = H$
22 $R^1 = H$, $R^2 = OH$
23 R^1 = Galloyl, $R^2 = OH$

tion [11]. Therefore, it seems likely that the active groups interact with the proteins of virus particles and host cell surfaces, resulting in a reduction or loss of viral infectivity. Probably the process of the interaction is dependent on pH or the nature of the protein [12]; therefore, the activity will vary according to the types of viruses or host cells used. On the other hand, the cytotoxicities of tannins parallel their antiviral activities and may be based on the interaction with the proteins of FL cell surfaces.

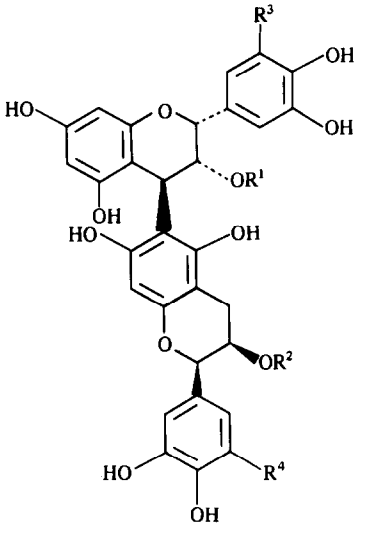
EXPERIMENTAL

Tannase (E.C. 3.1.1.20) prepared from *Aspergillus niger* was kindly provided by Kyowa Fermentation Industry Co., Ltd., Japan and the specific activity was 920 U/g. Tannins were purified from the following crude drugs and their structures had been determined [13–26]. 6-*O*-Galloyl-β-D-glucose (**1**), 1,2,6-tri-*O*-galloyl-β-D-glucose (**2**), 3-*O*-galloyl-(−)-epicatechin (**21**) and 3,3′-di-*O*-galloylprocyanidin B-2 (**26**) were from commercial rhubarb. 1,2,3,6-Tetra-*O*-galloyl-β-D-glucose (**3**) was from the galls of *Quercus infectoria*. 1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose (**4**) was from the galls of *Rhus javanica*. Eugeniin (**5**) was from the buds of *Syzygium aromatica*. 1-(α)-*O*-Galloylpedunculagin (**6**), sanguiin H-1 (**7**), sanguiin H-3 (**18**) and sanguiin H-6 (**19**) were from the underground parts of *Sanguisorba officinalis*. 5-*O*-Galloyl-D-hamamelose (**8**), 2′,5-di-*O*-galloyl-D-hamamelose (**9**) and 2′,3,5-tri-*O*-galloyl-D-hamamelose (**10**) were from the barks of *Castanea crenata*. 4,5-di-*O*-Galloylprotoquercitol (**11**), 3,4,5-tri-*O*-galloylprotoquercitol (**12**), 1,3,4,5-tetra-*O*-galloylprotoquercitol (**13**), 3,4-di-*O*-galloylquinic acid (**14**) and 3,4,5-tri-*O*-galloylquinic acid (**15**) were from the bark of *Quercus stenophylla*. Chebulagic acid (**16**) was from the fruits of *Terminalia chebula*. Geraniin (**17**) was from the aerial parts of *Geranium thunbergii*. (−)-Epicatechin (**20**) and (−)-epigallocatechin (**22**) were from the stems of *Ephedra sinica*. 3-*O*-Galloyl-(−)-epigallocatechin (**23**),3′-*O*-galloylprodelphinidin B-2 (**27**), 3,3′-di-*O*-galloylprodelphinidin B-2 (**28**), prodelphinidin B-5 (**30**) and 3,3′-di-*O*-galloylprodelphinidin B-5 (**31**) were from the bark of *Myrica rubra*. Procyanidin B-2 (**24**) was from the roots of *Polygonum multiflorum*. 3′-*O*-Galloylprocyanidin B-2 (**25**) was



24 $R^1 = R^2 = R^3 = R^4 = H$
25 $R^1 = R^3 = R^4 = H$, R^2 = Galloyl
26 $R^1 = R^2$ = Galloyl, $R^3 = R^4 = H$

27 $R^1 = H$, R^2 = Galloyl, $R^3 = R^4 = OH$
28 $R^1 = R^2$ = Galloyl, $R^3 = R^4 = OH$

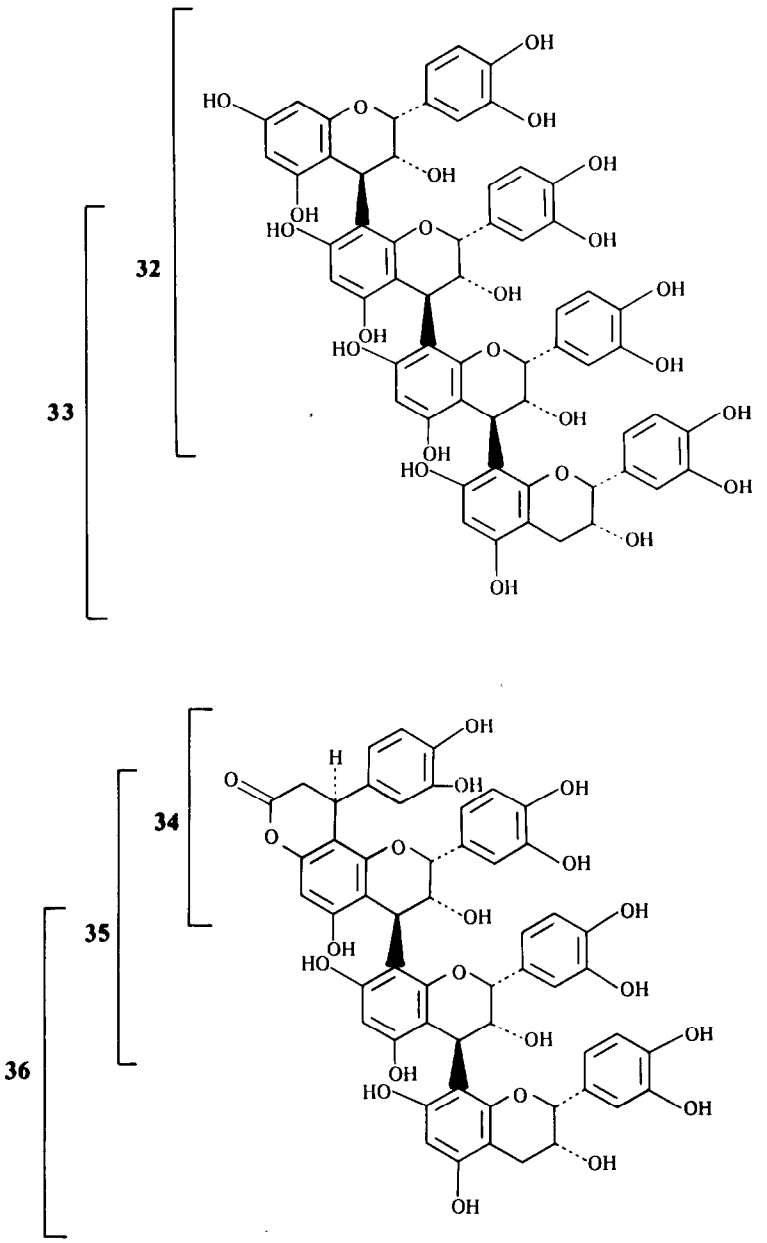


29 $R^1 = R^2 = R^3 = R^4 = H$
30 $R^1 = R^2 = H$, $R^3 = R^4 = OH$
31 $R^1 = R^2$ = Galloyl, $R^3 = R^4 = OH$

Table 2. Antiviral activities and cytotoxicities of condensed tannins

Condensed tannin	Degree of condensation	Number of galloyl groups	PRD_{50} (μM)	CSD_{50} (μM)	References
20	1	0	*	†	
22	1	0	*	†	
34	1	0	*	†	[24]
21	1	1	50	†	[13]
23	1	1	45	†	[20]
24	2	0	62	†	[22]
29	2	0	55	†	
30	2	0	57	†	[21]
35	2	0	60	†	[25]
37	2	0	64	†	[26]
38	2	0	67	†	[26]
25	2	1	21	129	
27	2	1	19	123	[21]
26	2	2	15	93	[13]
28	2	2	14	81	[21]
31	2	2	15	84	[21]
32	3	0	35	176	[23]
36	3	0	37	182	
33	4	0	19	75	

*PRD_{50} = > 100 μM. †CSD_{50} = > 200 μM.

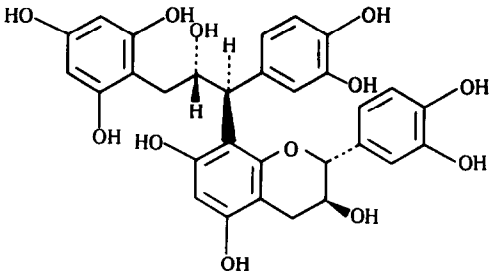


from the leaves of *Eucalyptus globulus*. Procyanidin B-5 (**29**) and cinnamtannin A_1 (**33**) were from the barks of *Cinnamomum cassia*. Procyanidin C-1 (**32**) was from the seeds of *Areca catechu*. Cinchonain Ib (**34**), IIb (**35**) and IIIb (**36**) were from the bark of *Cinchona succirubra*. Gambiriin A_1 (**37**) and B_3 (**38**) were from the dried aqueous extract of the leaves and young twigs of *Uncaria gambir*.

Tannase treatment. 2 mg of each galloylated tannin were dissolved in 2 ml 0.1 M acetate buffer (pH 5.5) and the solution was incubated at 37° with 0.2 mg tannase for 15 hr. After the complete release of gallic acid was confirmed by checking with ferric chloride reagent on a cellulose TLC plate in MeOH–H_2O (9:1), each digest was filtered under sterile conditions and subjected to the antiviral assay.

Antiviral assay. FL cell cultures were incubated in maintenance medium comprising Eagle's minimal essential medium and 2% calf serum. Assays were carried out in confluent FL cell monolayers in Falcon multiwell plates with 24 flat-bottom wells of 1.6 cm diameter. FL cell monolayers were infected with 50 PFU of Herpes simplex virus type I and, after an incubation at 37° for 60 min, exposed to varying concentrations of the compounds in maintenance medium containing 1% methylcellulose. Three days later, the overlay medium was washed off with saline and the cells were stained with 0.2% crystal violet solution for 20 min. After washing the cells with saline, plaques were counted and the number was averaged from three cultures. Antiviral potency was expressed as PRD_{50}, that is, the concentration of compound required to reduce the number of plaques to 50% of that in the control cell cultures.

Cytotoxic assay. Cytotoxicity of the compounds against FL cells was examined by the trypan blue exclusion test. Confluent FL cell cultures were exposed to various concentrations of each compound in maintenance medium for 24 hr at 37°. After removal of the solution, the cells were dispersed by treating with



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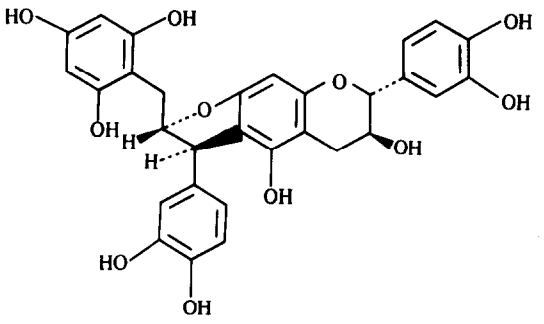

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0.2% trypsin solution at 37° for 5 min and stained with 0.1% trypan blue solution. Experiments of each series were repeated three times. Cytotoxicity was expressed as 50% cell staining dose (CSD_{50}), that is, the concentration of compound required to stain 50% of whole cell numbers in the culture.

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