[Chem. Pharm. Bull. 33(4)1424—1433(1985)]

Relationship of the Structures of Tannins to the Binding Activities with Hemoglobin and Methylene Blue[1)]

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(Received July 25, 1984)

The determination of relative astringency (*RA*) and relative affinity to methylene blue (*RMB*), based on those of geraniin (*RAG* and *RMBG*), shows good reproducibility with small amounts of samples for the estimation of the tannin content of plant extracts, and has been applied to the evaluation of the basal activity of 84 tannins and related compounds. The values obtained for polyphenols of lower molecular weight, which are not regarded as tannins, were zero or almost zero. An increase of these two values of up to about 1.3—1.4 times with increase of molecular weight of polyphenols (particularly such increase due to galloylation) was observed for each type of tannin tested. The *RMBG* determination gives values somewhat larger than the *RAG* values for hydrolyzable tannins, and rather smaller than the *RAG* values for condensed tannins.

Keywords——tannin determination; tannin; *RMB*; *RA*; tannin activity; structure–activity correlation; hydrolyzable tannin; condensed tannin; caffeetannin; colorimetry

The ability of tannins to precipitate water-soluble proteins and alkaloids is the main basal activity by which various naturally occurring polyphenols are defined as tannins.[2)] Several methods for quantitative determination of tannins based on these activities have been developed. Among them, determination of relative astringency (*RA*),[3)] and relative affinity to methylene blue (*RMB*)[4)] have been used by the authors for the determination of tannin content in plant extracts, as simple methods with good reproducibility,[3,4)] which can be performed with small amounts of sample in a short time.[3,4)]

While these methods have been applied to plant extracts, which are mixtures containing tannins of various structures, the *RA* and *RMB* values of each component, and also their contribution to the *RA* and *RMB* values of the whole extract are important in order to characterize the tannin of any plant. Since only polyphenols of molecular weights between 500 and 3000 are considered to have tanning ability,[2)] the correlation of the molecular weight of each tannin with these values is one of the main problems to be investigated. It is also desirable to know whether the values obtained by using different reactants (blood and methylene blue) coincide for each tannin. Any marked difference of these values for each tannin should be identified.

Tannic acid JP (Japanese Pharmacopoeia) used as the reference in the determination of *RA* and *RMB* values is a mixture of polygalloylglucoses which cannot be expected to have an identical composition for every lot. In the present investigation, therefore, we have determined the *RAG* and *RMBG* values of various isolated tannins as relative activities to geraniin, which is obtainable in a crystalline form; this approach should give better reproducibility.

The *RAG* and *RMBG* values were obtained by colorimetry of the supernatant liquor after centrifuging the mixture of tannin and hemolyzed blood or methylene blue. The precipitates formed upon admixture of bases with tannins have sometimes been found to be dissolved by addition of larger amount of tannins, depending on the structures of the bases and the

tannins.[5] However, as the concentration of tannins for the *RMB* (or *RMBG*) determination (about 0.001% tannic acid JP with 10^{-5} M methylene blue)[4] is far lower than that at which redissolution of the precipitates occurs (about 1.0% tannic acid JP for 10^{-4} M berberine),[5] redissolution, which may interfere the determination, should not occur in these measurements.

We have now measured the *RMBG* and *RAG* values of various tannins which have been isolated at our laboratory, and compared these values for each tannin. We have also examined how differences in the chemical structures and molecular weights of tannins are reflected in these values.

Experimental

Materials——Tannins and related polyphenols, **5**—**9**, **12**—**29**, **31**—**33**, **35**—**67** and **69**—**84**, were isolated from plants.[2,6] Three compounds, **4**,[7] **30**[7] and **34**,[8] were prepared according to the cited methods. Tannic acid JP was purchased from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. All other chemicals were reagent-grade products obtained commercially.

Determination of *RMBG* Values——Each sample solution (2 ml) was added to a test solution containing 7.0×10^{-5} M aqueous solution of methylene blue (2 ml) and 0.2 M phosphate buffer (pH 7.0, 1 ml), and the mixture was shaken vigorously then left to stand for 30 min. After centrifugation at 3000 rpm for 10 min, the absorbance at 660 nm of the supernatant was determined. The *RMB* value is the ratio of the concentration of tannic acid JP to that of the sample solution required for 50% decrease of the absorbance of the test solution containing additional water (2 ml). The *RMBG* values of tannins were calculated by dividing the results by the *RMB* value of geraniin, which was obtained using the same lot of tannic acid JP.

Determination of *RAG* Values——Fresh human blood was hemolyzed by dilution with water to make a hemoglobin solution which showed an absorbance of *ca.* 2.5 at 578 nm. Each sample solution (2 ml) was added to a test solution containing the hemoglobin solution (2 ml) and 0.2 M phosphate buffer (pH 6.0, 1 ml). The mixture was shaken vigorously and left to stand for 1 h. After centrifugation at 12000 rpm for 10 min, the absorbance at 578 nm of the supernatant was determined. The *RA* and *RAG* values were based on the amounts required for 50% decrease of the absorbance of the test solution containing additional water (2 ml), analogously to the *RMB* and *RMBG* determinations.

Results and Discussion

The results are shown in Tables I and II. The *RMBG* values were practically identical with the *RMB* values obtained in the case of the tannic acid JP used in the present study. In the *RMBG* and *RAG* determinations, the deviation of values for an identical sample is within 5% of the mean values.[3,4]

RMBG Values of Tannins and Related Compounds

The *RMBG* values for polyphenols of low molecular weight, except for ellagic acid (**7**) and valoneic acid dilactone (**9**), which will be discussed later, are generally low, as found for pyrogallol (**1**) (*RMBG* 0) and 11-*O*-galloylbergenin (**17**)[6a] (*RMBG* 0.07). The observation that the polyphenols of molecular weight smaller than 500 show low binding activity is in agreement with the concept[2] that the molecular weight of polyphenols which are regarded as tannins must be larger than 500.

The hydrolyzable tannins with molecular weights larger than 500, having common substituents at the same locations on the glucose core, show increased *RMBG* values as their molecular weight increase (see the chart for a comparison of structures): gemin D (**25**)[6c] (m.w. 634.5, *RMBG* 0.50) < tellimagrandin I (**27**)[6c] (786.6, 1.00) < tellimagrandin II (**31**)[6c] (938.7, 1.20); casuariin (**35**)[6f] (784.6, 0.57) < casuarinin (**36**)[6f] (936.7, 1.18); furosin (**44**)[6k] (650.5, 0.45) < geraniin (**47**)[6l] (952.7, 1.00); 1,2,3-tri-*O*-galloyl-*β*-D-glucopyranose (**21**)[6d] (636.5, 0.90) < 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucopyranose (**26**)[6g] (788.6, 1.08) < penta-*O*-galloyl-*β*-D-glucopyranose (**30**)[7] (940.7, 1.21). The correlation coefficient between the *RMBG* values and the molecular weights of the monomeric hydrolyzable tannins, *i.e.*, **18**—**59**, is 0.80 (Fig. 1).

TABLE I. *RMBG* and *RAG* Values of Hydrolyzable Tannins and Related Compounds

Tannin or related compound	Molecular weight	*RMBG*	*RAG*
Low molecular weight compounds			
Pyrogallol (**1**)	126.1	0	0.08
Protocatechuic acid (**2**)	154.1	0	0.11
Gallic acid (**3**)	170.1	0.09	0.11
Digallic acid (**4**)	322.2	0.27	0.26
Hexahydroxydiphenic acid (**5**)	338.2	0.20	0.23
Luteic acid (**6**)	320.2	0.65	0.39
Ellagic acid (**7**)	302.2	1.01	0.14
3,4,3′-Tri-*O*-methylellagic acid (**8**)	344.3	0	0.17
Valoneic acid dilactone (**9**)	470.3	1.19	0.71
Quinic acid (**10**)	192.2	0	0
Caffeic acid (**11**)	180.2	0.005	0.23
Chlorogenic acid (**12**)	354.3	0.004	0.06
Bergenin (**13**)	328.3	0	0
11-*O*-Galloylbergenin (**14**)	480.4	0.07	0.10
Hamamelitannin (**15**)	484.4	0.07	—
Acertannin (**16**)	468.4	0.08	—
2,3-*O*-Hexahydroxydiphenoyl-D-glucose (**17**)	482.4	0.18	—
Monomeric hydrolyzable tannins (molecular weight > 500)			
3,5-Di-*O*-caffeoylquinic acid (**18**)[a]	516.5	0.25	0.20
3,4-Di-*O*-caffeoylquinic acid (**19**)[a]	516.5	0.25	0.10
4,5-Di-*O*-caffeoylquinic acid (**20**)[a]	516.5	0.15	0.12
1,2,3-Tri-*O*-galloyl-*β*-D-glucopyranose (**21**)[b]	636.5	0.90	0.64
1,2,6-Tri-*O*-galloyl-*β*-D-glucopyranose (**22**)[b]	636.5	1.03	0.58
Corilagin (**23**)[c]	634.5	0.22	0.17
Strictinin (**24**)[c]	634.5	0.37	0.17
Gemin D (**25**)[c]	634.5	0.50	0.49
1,2,3,6-Tetra-*O*-galloyl-*β*-D-glucopyranose (**26**)[b]	788.6	1.08	1.11
Tellimagrandin I (**27**)[c]	786.6	1.00	0.82
Praecoxin B (**28**)[c]	786.6	1.08	—
Pedunculagin (**29**)[c]	784.6	0.98	0.24
Penta-*O*-galloyl-*β*-D-glucopyranose (**30**)[b]	940.7	1.21	1.29
Tellimagrandin II (**31**)[c]	938.7	1.20	0.93
Potentillin (**32**)[c]	936.7	1.04	0.74
Casuarictin (**33**)[c]	936.7	1.20	0.59
1-*O*-Galloyl-2,4 : 3,6-bis-*O*-hexahydroxydiphenoyl-*β*-D-glucopyranose (**34**)[c]	936.7	1.13	0.67
Casuariin (**35**)[c]	784.6	0.57	—
Casuarinin (**36**)[c]	936.7	1.18	0.54
Stachyurin (**37**)[c]	936.7	1.17	0.78
Castalagin (**38**)[c]	934.6	1.07	—
Alnusiin (**39**)[c]	934.6	1.17	1.21
Oenothein C (**40**)[c]	784.6	1.23	—
Cornusiin B (**41**)[c]	1086.7	1.43	—
Punicalin (**42**)[c]	782.5	0.87	0.40
Punicalagin (**43**)[c]	1084.7	1.07	0.87
Furosin (**44**)[c]	650.5	0.45	—
Furosinin (**45**)[c]	816.6	0.45	0.23
Dehydrogeraniin (**46**)[c]	968.7	0.87	0.60
Geraniin (**47**)[c]	952.7	1.00	1.00
Granatin A (**48**)[c]	800.6	0.39	0.27
Granatin B (**49**)[c]	952.7	0.96	0.53

TABLE I. (continued)

Tannin or related compound	Molecular weight	*RMBG*	*RAG*
Terchebin (**50**)[c]	954.7	1.12	1.04
Isoterchebin (**51**)[c]	954.7	1.00	0.91
Mallotusinic acid (**52**)[c]	1120.8	1.21	0.87
Rugosin A (**53**)[c]	1106.8	1.33	1.08
Rugosin B (**54**)[c]	954.7	1.29	—
Rugosin C (**55**)[c]	1104.8	1.25	1.07
Chebulagic acid (**56**)[c]	954.7	1.20	0.74
Chebulinic acid (**57**)[c]	956.7	1.28	1.05
Praecoxin C (**58**)[c]	1086.8	1.05	—
Praecoxin D (**59**)[c]	934.6	1.29	—
Dimeric or trimeric ellagitannins			
Agrimoniin (**60**)	1871.3	1.17	1.12
Gemin A (**61**)	1873.3	1.00	1.15
Coriariin A (**62**)	1875.3	1.16	0.95
Cornusiin A (**63**)	1571.1	1.10	1.19
Rugosin D (**64**)	1875.3	1.19	1.02
Rugosin E (**65**)	1723.2	1.15	1.04
Rugosin F (**66**)	1873.3	1.13	0.84
Rugosin G (**67**)	2812.0	1.08	—

a) Caffeetannin. *b*) Galloylglucose. *c*) Ellagitannin or related tannin.

TABLE II. *RMBG* and *RAG* Values of Condensed Tannins and Related Polyphenols

Tannin or polyphenol	Ratio of galloylation (%)	Molecular weight[a]	*RMBG*	*RAG*
(+)-Catechin (**68**)	0	290.3	0.01	0.10
(−)-Epicatechin (**69**)	0	290.3	0.01	0.08
(−)-Epigallocatechin (**70**)	0	306.3	0	0.07
(−)-Epicatechin gallate (**71**)	100	442.4	0.60	0.81
(−)-Epigallocatechin gallate (**72**)	100	458.4	0.95	0.84
Procyanidin-B2 (**73**)	0	578.5	0.05	0.10
Procyanidin-B2-3′-gallate (**74**)	50	730.6	0.64	0.46
Procyanidin-B2-3,3′-digallate (**75**)	100	882.7	0.98	1.01
Ss-tannin 1 (**76**)	96	2300	1.02	1.32
RSF-tannin H (**77**)	81	3100	0.84	1.24
RG-tannin A (**78**)	43	710	0.58	0.67
RG-tannin B (**79**)	61	1100	0.83	0.79
RG-tannin C (**80**)	79	1100	0.91	1.03
RG-tannin D (**81**)	74	1200	0.85	1.08
RG-tannin E (**82**)	84	2300	0.91	1.08
RG-tannin F (**83**)	82	2300	0.84	0.93
RG-tannin G (**84**)	85	2600	0.87	0.80

a) Number-average molecular weight for **76**—**84**.

The *RMBG* values of the ellagitannin dimers, as found for agrimoniin (**60**)[6c] (m.w. 1871.3, *RMBG* 1.17) and rugosin D (**64**)[6q] (1875.3, 1.19), and those of the trimer, as shown by rugosin G (**67**)[6q] (2812.0, 1.08), are larger than those of most of the monomeric hydrolyzable

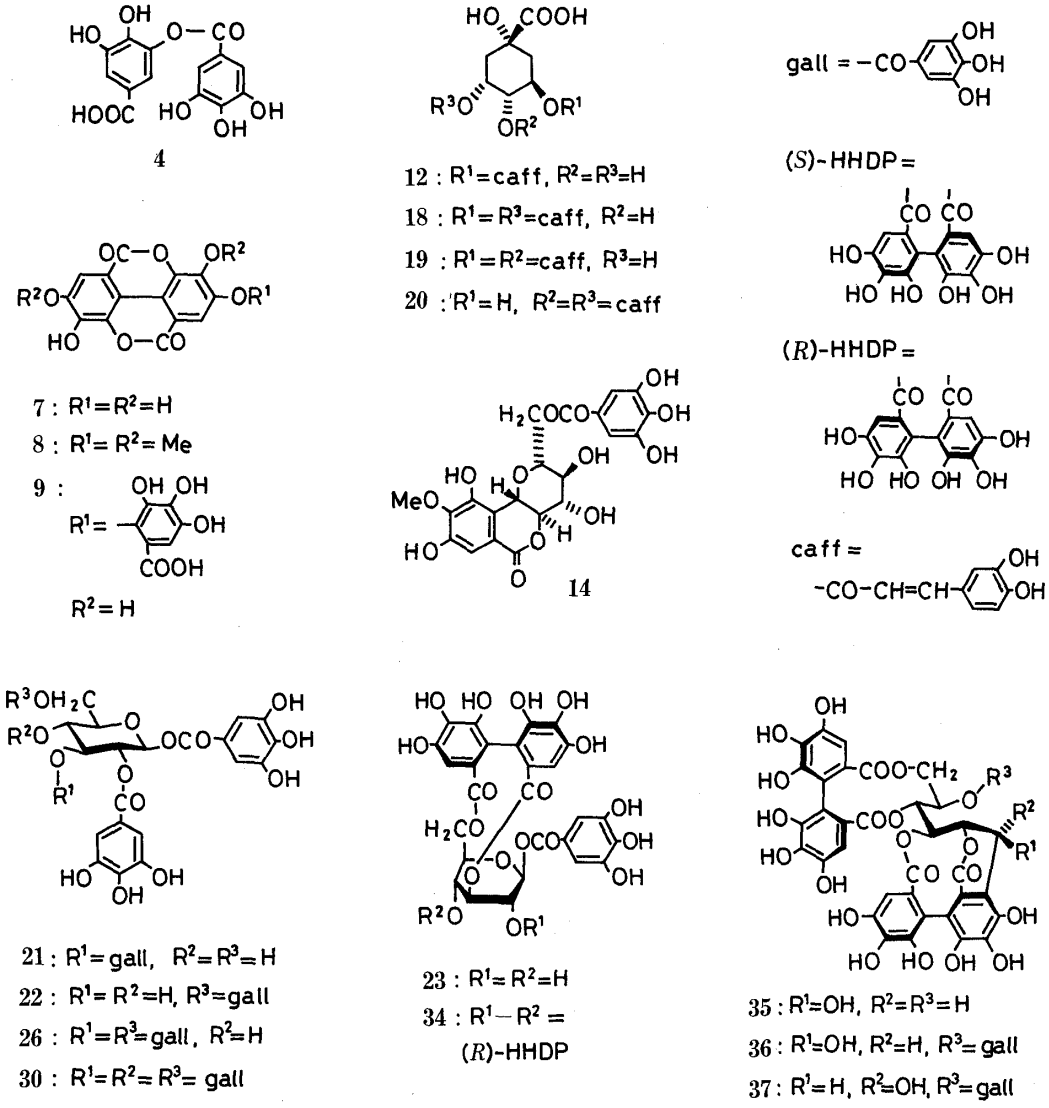

Chart 1

tannins. However, the increase of the molecular weight is not directly reflected in these values for the dimeric and trimeric tannins. The upper limit of the *RMBG* values of each tannin, observed for the tannins in the tables, is about 1.4.

It is noticeable that ellagic acid (**7**) and valoneic acid dilactone (**9**), which have small molecular weights, exhibit unexpectedly large *RMBG* values, 1.01 and 1.19. These large values might be due to some specific interaction between each of these dilactones and methylene blue, as their *RAG* values are comparable to those of the other polyphenols of low molecular weight. The large *RMBG* values of oenothein C (**40**)[6j] (*RMBG* 1.23) and cornusiin B (**41**)[6g] (1.43) are accordingly attributable to the presence of a valoneic acid dilactone moiety in the molecule.

Ellagic acid (**7**) is known to be an effective inhibitor of the mutagenicity of benzo[*a*]pyrene-7,8-diol-9,10-epoxide.[9] The large *RMBG* value of ellagic acid and the dilactones may be accounted for by complex formation through hydrogen bondings between methylene blue and the dilactone, which are presumed to be oriented in parallel with each other in a way similar to the hypothetical complex formation between the epoxide and ellagic acid.[9] The presence of free phenolic hydroxyl groups in these dilactones, as in other polyphenols, may be essential for the interaction with the methylene blue molecule, since

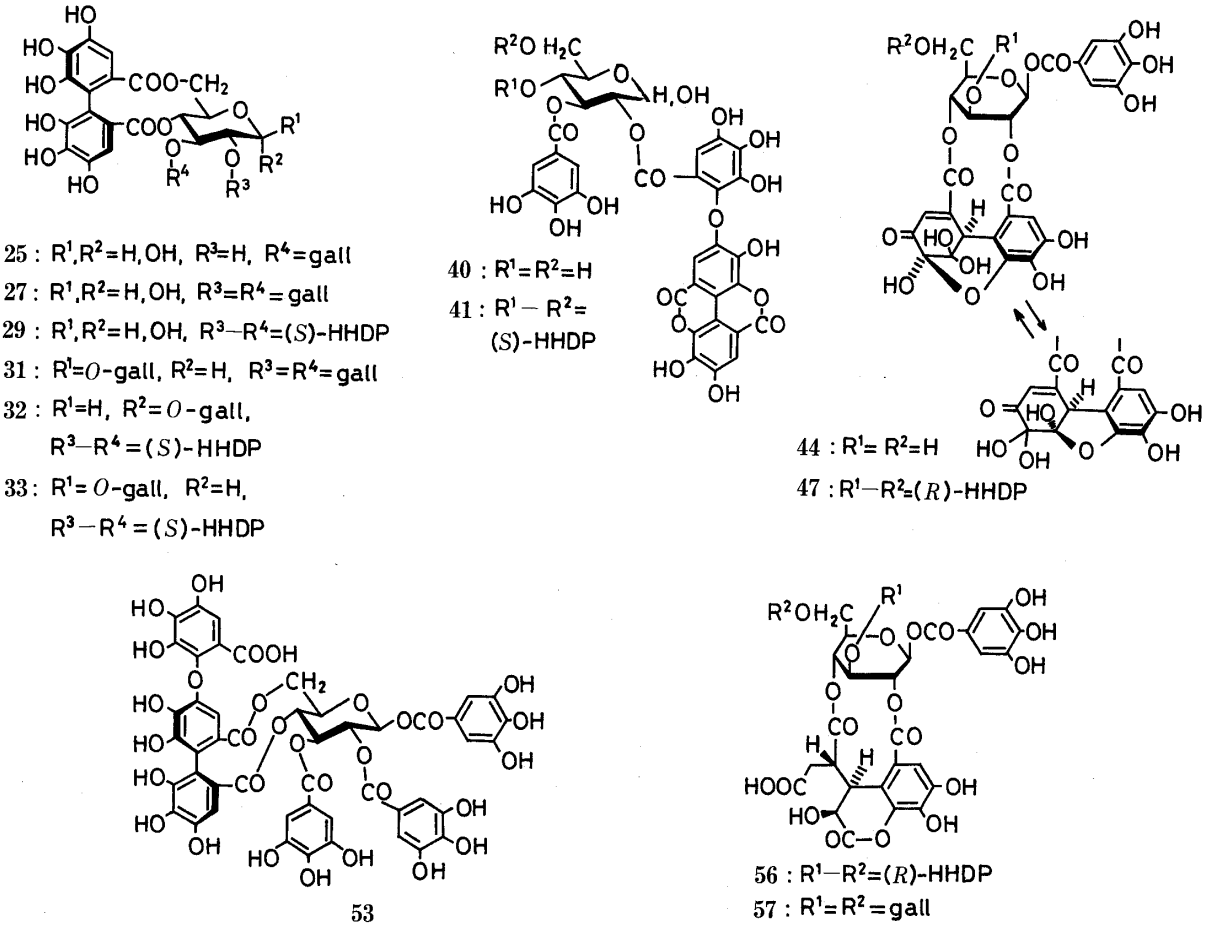

Chart 2

3,4,3′-tri-*O*-methylellagic acid (**8**)[6a] shows no affinity for methylene blue (*RMBG* 0).

The *RMBG* value of valoneic acid dilactone, which is larger than that of ellagic acid, may be partially due to the presence of a free carboxyl group; other tannins having a free carboxyl group also show large *RMBG* values, as exemplified by chebulinic acid (**57**)[6p] (*RMBG* 1.28) and rugosin A (**53**)[6d] (1.33). However, since the *RMBG* value of the polyphenols of low molecular weight having a carboxyl group are small, as observed for protocatechuic acid (**2**) and gallic acid (**3**), a polyphenolic structure of fairly large molecular weight, in addition to the presence of a carboxyl group in the molecule, may be requisite for large *RMBG* values.

An analogous correlation of the *RMBG* values with molecular weight is observed for monomeric and oligomeric catechin analogs (Table II). A similar correlation is also shown by "caffeetannins,"[6b,10] among which di-*O*-caffeoylquinic acids (**18**—**20**)[6b] show some activity, while chlorogenic acid (**12**)[6b] and caffeic acid (**11**) show negligible activity.

Among the structural changes which contribute to the increase of molecular weight, galloylation contributes significantly to the enhancement of the *RMBG* values for the compounds described above. It is also noticeable that the catechin analogs having a galloyl group at O-3 give fairly large *RMBG* values in spite of their low molecular weight, as shown by (−)-epigallocatechin gallate (**72**) (*RMBG* 0.95) and (−)-epicatechin gallate (**71**) (*RMBG* 0.60), which are the main active components of the "tannin" in tea leaf and green tea, while non-galloylated (−)-epigallocatechin (**70**) (*RMBG* 0) and (−)-epicatechin (**69**) (0.01) show practically no activity. A similar correlation is observed among procyanidin-B2-3,3′-digallate (**75**)[6r] (0.98), procyanidin-B2-3′-gallate (**74**)[6r] (0.64) and procyanidin-B2 (**73**)[6r] (0.05). The enhancement of *RMBG* values of catechin analogs including condensed tannins of rhubarbs

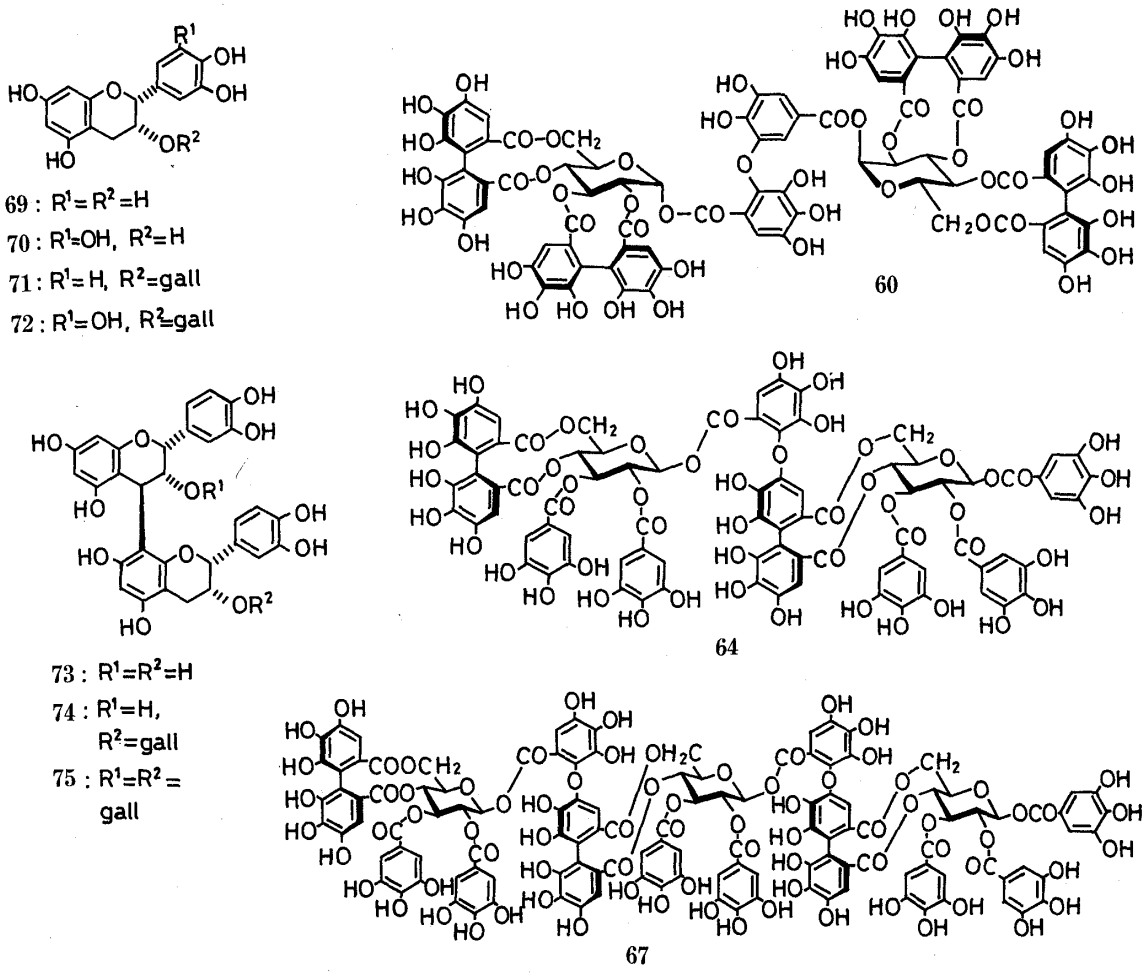

Chart 3

and *Saxifraga stolonifera* MEERB. (**66**—**82**),[6r] due to the increase of galloylation (correlation coefficient, 0.94) is shown in Fig. 3.

RAG Values of Tannins and Related Compounds

Like the *RMBG* values, the *RAG* values are small for polyphenols of low molecular weight, as found for gallic acid (**3**) (*RAG* 0.11) and 11-*O*-galloylbergenin (**17**) (0.10). Ellagic acid (**7**) (0.14), in spite of its large *RMBG* value, gave a small *RAG* value.

There is a significant correlation of the *RAG* values with the molecular weights. This correlation for monomeric hydrolyzable tannins with molecular weights larger than 500 is shown in Fig. 2 (correlation coefficient, 0.72); examples of tannins having common substituents at the same location on the glucose core are as follows (see Charts 1 and 2): gemin D (**25**) (m.w. 634.5, *RAG* 0.49) < tellimagrandin I (**27**) (786.6, 0.82) < tellimagrandin II (**31**) (938.7, 0.93); corilagin (**23**)[6e] (634.5, 0.17) < geraniin (**47**) (952.7, 1.00); 1,2,6,-tri-*O*-galloyl-*β*-D-glucopyranose (**22**)[6d] (636.5, 0.58) < 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucopyranose (**26**) (786.6, 1.11) < penta-*O*-galloyl-*β*-D-glucopyranose (**30**) (940.7, 1.28).

The *RAG* values of dimeric hydrolyzable tannins, exemplified by agrimoniin (**60**) (m.w. 1871.3, *RAG* 1.12) and rugosin D (**64**) (1875.3, 1.02), are larger than those of most tannins of other types, but these values are not proportional to the molecular weights, and this phenomenon is analogous to that observed in the *RMBG* determination. The largest value among the tannins in the present study was about 1.3, which is similar to that found in the *RMBG* determination.

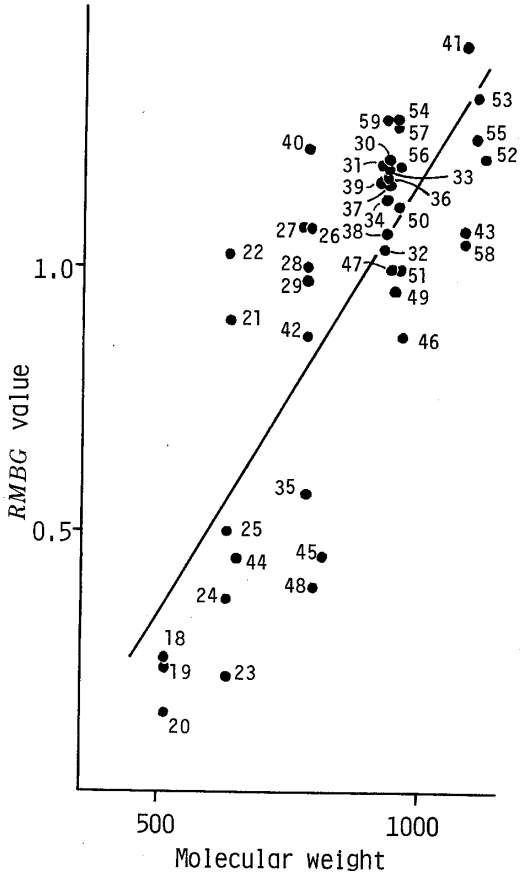

Fig. 1. Correlation of *RMBG* Values with Molecular Weights for Monomeric Hydrolyzable Tannins

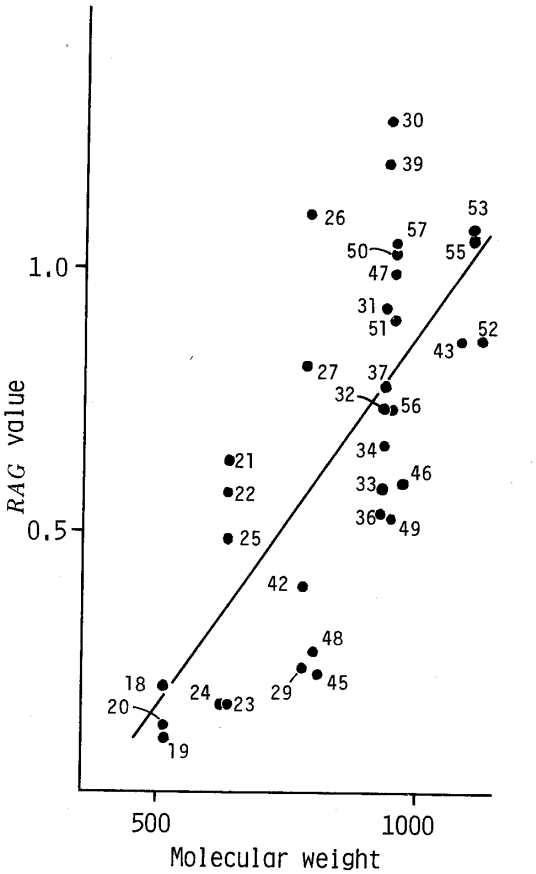

Fig. 2. Correlation of *RAG* Values with Molecular Weights for Monomeric Hydrolyzable Tannins

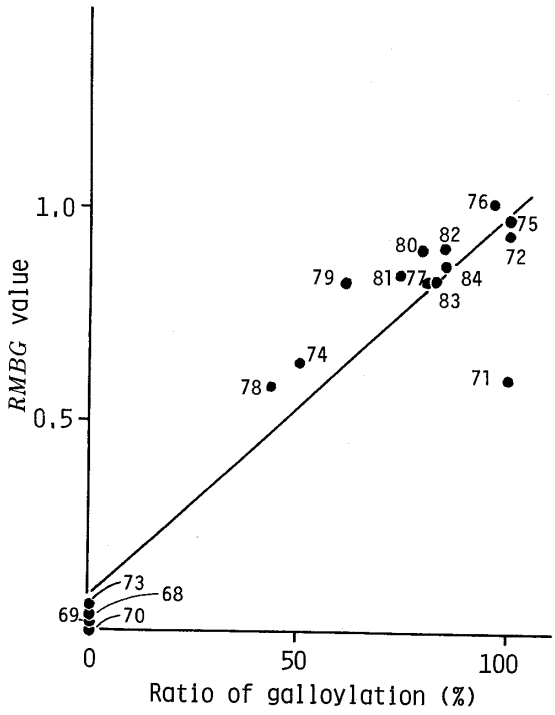

Fig. 3. Correlation of *RMBG* Values with Ratio of Galloylation for Catechin Analogs

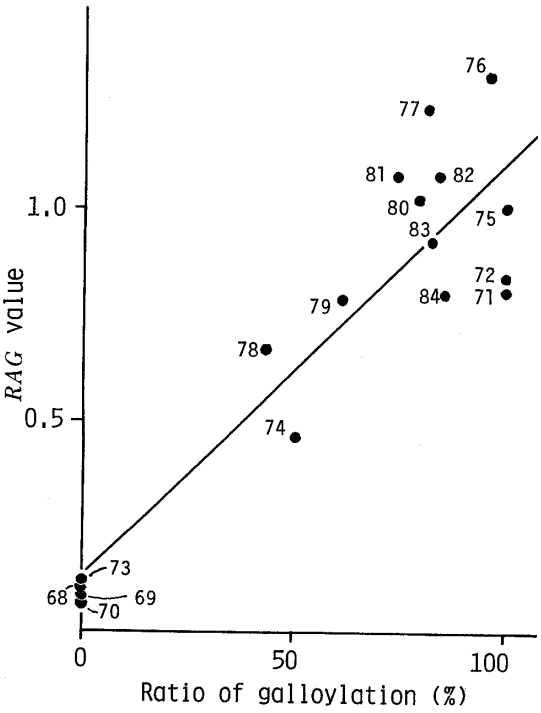

Fig. 4. Correlation of *RAG* Values with Ratio of Galloylation for Catechin Analogs

A significant difference in the contribution to the *RAG* values of the molecule is observed between the galloyl and hexahydroxydiphenoyl (HHDP) groups. Although pedunculagin (**29**)[6f] (m.w. 784.6) has a molecular weight large enough to satisfy the definition of a tannin,[2] and its structure resembles that of tellimagrandin I (**27**) (m.w. 786.6), which shows a relatively large *RAG* value (0.82), the *RAG* value of pendunculagin (0.24) is barely equal to that of digallic acid (**4**)[7] (0.26). This result shows that the contribution of two galloyl groups to the *RAG* value is larger than that of an HHDP group at the same location on the glucose core. Similar correlations were also observed upon comparison of tannins which possess galloyl and/or HHDP group at identical locations, and differ from each other concerning whether they have two galloyl groups or an HHDP group: casuarictin (**33**)[6f] (m.w. 936.7, *RAG* 0.59, 2 HHDP and 1 galloyl) < tellimagrandin II (**31**) (938.7, 0.93, 1 HHDP and 3 galloyl) < penta-*O*-galloyl-β-D-glucopyranose (**30**) (940.7, 1.29, 5 galloyl); chebulagic acid (**56**)[6p] (954.7, 0.74) < chebulinic acid (**57**) (956.7, 1.05) (see Charts 1 and 2).

The enhancement of the *RAG* value upon galloylation is also apparent upon comparison of galloylated and non-galloylated catechin analogs (Fig. 4). For example, the *RAG* values of procyanidin-B2-3,3′-digallate (**75**) (1.01) and procyanidin-B2-3′-gallate (**74**) (0.46) are markedly larger than that of procyanidin-B2 (**73**) (0.10). The correlation coefficient between the *RAG* values and the ratio of galloylation of catechin analogs is 0.91.

Comparison of *RMBG* and *RAG* Determinations

Close similarity between the *RMBG* and *RAG* values is shown by several tannins, *i.e.* gemin D (**25**), penta-*O*-galloyl-β-D-glucopyranose (**30**) and agrimoniin (**60**). However, most ellagitannins and related tannins give *RMBG* values larger than the *RAG* values. The ratio of *RMBG* and *RAG* values for the ellagitannins having two HHDP groups, **29**, **32**,[6c] **33**, **34**,[8] **36**[6f] and **37**[6f] are in the range of 1.41—4.08, and the average ratio of the *RMBG* value to the *RAG* value for ellagitannins and related tannins determined in this study, *i.e.* **23**—**25**, **27**—**29** and **31**—**66**, is 1.47. Conversely, the *RAG* values are larger than *RMBG* values for most condensed tannins and related polyphenols, with an average ratio of *RAG* to *RMBG* of 1.11 (except for **68**—**70** and **73**). These results show that the binding activity of ellagitannins and related tannins is generally stronger when methylene blue, which is a polycyclic aromatic compound, is the reactant, while that of condensed tannins is stronger to hemoglobin.

However, there are good correlations on the whole between the *RMBG* and *RAG* values of the tannins and related compounds determined in this study (correlation coefficient for **1**—**84**: 0.84). The values of each tannin reflect its molecular structure; the polyphenols of low molecular weight, which are not regarded as tannins according to the generally accepted definition of tannin,[2] which is mainly based on the leathering ability, gave a negative result or negligible values in these determinations, and the values increased to about 1.3—1.4 with increase of molecular weight, particularly upon galloylation. Both the *RMBG* and *RAG* determinations, therefore, are useful for the determination of tannins.

Chlorogenic acid and polycaffeoylquinic acids, which are found in coffee, have sometimes been called caffeetannins, though they are often not regarded as tannins. The present data show that dicaffeoylquinic acids are active as tannins, although their values are rather small, while chlorogenic acid is inactive. As the polyphenol most abundantly present in coffee is chlorogenic acid, and the amounts of polycaffeoylquinic acids are small,[6b] coffee can hardly be regarded as a tannin-rich material. However, the extracts of *Artemisia* species, which are rich in dicaffeoylquinic acids,[6b] and give significant values in determinations of *RMBG* and *RAG*, should be regarded as tannin-containing extracts. The name "caffeetannin" could be applied to the polycaffeoyl derivatives in these *Artemisia* species.

Among various ways of determining tannin content in plant extract, the hide-powder method[11] which requires a fairly large amount of sample and a long time for the experiment,

has been regarded as the standard method for determination of the tannins used for leathering. As the tannin values obtained by different methods are often different to some extent, a suitable method should be employed for each particular purpose.

Both the *RMBG* and *RAG* determinations are convenient methods because the procedure is simple, requiring only a short time and a small quantity of sample. The former often has the advantage that it can be performed with only a few milligrams of sample, while the latter, which is based on the binding activity of tannins with protein, might be more suitable in particular cases, depending on the purpose of the determinations.

References

1) Part IV of Effects of the Interaction of Tannins with Coexisting Substances. Part III: ref. 5.
2) T. Okuda, T. Yoshida, K. Mori and T. Hatano, *Heterocycles*, **15**, 1323 (1981).
3) T. Okuda, K. Mori and K. Aoi, *Yakugaku Zasshi*, **97**, 1267 (1977).
4) T. Okuda, K. Mori and R. Murakami, *Yakugaku Zasshi*, **97**, 1273 (1977).
5) T. Okuda, K. Mori and M. Shiota, *Yakugaku Zasshi*, **102**, 854 (1982).
6) *a*) T. Yoshida, K. Seno, Y. Takama and T. Okuda, *Phytochemistry*, **21**, 1180 (1982); *b*) T. Okuda, T. Hatano, S. Tatsumi, I. Agata, S. Nishibe and K. Kimura, Abstract Papers, 104th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, March 1984, p. 192; *c*) T. Yoshida, T. Hatano, T. Okuda, M. U. Memon, T. Shingu and K. Inoue, *Chem. Pharm. Bull.*, **32**, 1790 (1984); *d*) T. Okuda, T. Hatano, K. Yazaki and N. Ogawa, *ibid.*, **30**, 4230 (1982); *e*) T. Okuda, T. Yoshida and K. Mori, *Phytochemistry*, **14**, 1877 (1975); *f*) T. Okuda, T. Yoshida, M. Ashida and K. Yazaki, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 1765; *g*) T. Okuda, T. Hatano, N. Ogawa and K. Kira, Abstract Papers, 30th Annual Meeting of the Japanese Society of Pharmacognosy, Tokushima, October 1983, p. 58; *h*) T. Okuda, T. Hatano and K. Yazaki, *Chem. Pharm. Bull.*, **31**, 333 (1983); *i*) T. Yoshida, M. U. Memon and T. Okuda, *Heterocycles*, **16**, 1085 (1981); *j*) T. Okuda, T. Hatano, K. Yazaki and M. Matsuda, Abstract Papers, 30th Annual Meeting of the Japanese Society of Pharmacognosy, Tokushima, October 1983, p. 57; *k*) T. Okuda, T. Hatano and K. Yazaki, *Chem. Pharm. Bull.*, **30**, 1113 (1981); *l*) T. Okuda, T. Yoshida and T. Hatano, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 9; *m*) T. Okuda, T. Yoshida, T. Hatano and H. Nitta, Symposium Papers, 22nd Symposium on the Chemistry of Natural Products, Fukuoka, October 1979, p. 323; *n*) T. Okuda, T. Hatano, H. Nitta and R. Fujii, *Tetrahedron Lett.*, **21**, 4361 (1980); *o*) T. Okuda and K. Seno, *Nippon Kagaku Kaishi*, **1981**, 671; *p*) T. Yoshida, T. Okuda, T. Koga and N. Toh, *Chem. Pharm. Bull.*, **30**, 2655 (1982); *q*) T. Okuda, T. Hatano and N. Ogawa, *ibid.*, **30**, 4234 (1982); *r*) T. Okuda, T. Hatano, M. Kuwahara, Y. Higashiyama and Y. Maruyama, Abstract Papers, 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, April 1981, pp. 511—512.
7) P. Crabtree, E. Haslam, R. Haworth, S. Mills and J. Stangroom, *J. Chem. Soc.*, **1965**, 6888.
8) E. A. Haddock, R. K. Gupta and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2535.
9) 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. Acad. Sci. U.S.A.*, **79**, 5513 (1982).
10) W. A. Court, *J. Chromatogr.*, **130**, 287 (1977).
11) JIS K6504-1964.