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TANNINS OF MEDICINAL PLANTS AND DRUGS[†]

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Abstract —— This review summarizes the research carried out in recent years at the author's laboratory on tannins in several medicinal plants and drugs, most of which have been presumed to contain tannin as the main active principle.

Contents

[†] Dedicated to Professor Tetsuji Kametani on the retirement from the Chair of Organic Chemistry at the Pharmaceutical Institute of Tohoku University.

1. Introduction

A number of medicinal plants and drugs used in oriental countries have been regarded as rich in tannin, and the tannins in many of these plants and drugs have been regarded as the active principles. However, it has been unknown whether the tannins in most of these plants and drugs are the tannin in the rigorous meaning. The description in a literature that a certain plant contains tannin, often just means that this plant is rich in phenolic compounds, since it sometimes happened that natural product chemists put the name "tannin" to some intractable syrup or gum, which were met upon fractionation of the extracts, and were positive to the colour reactions of phenolics.

The type of tannins contained in these medicinal plants, and accordingly the structure of each tannin, have also been mostly unknown. It has been customary in the medical sciences that only the activities of commercially available "tannic acid" is discussed by the name of tannin, while the main constituents of "tannic acid" which is mostly produced from Chinese gall or Turkish gall, chemically belong to gallotannins which compose a subgroup of hydrolysable tannins.

Since the time of E. Fischer who took up tannin in his research, there have been research works by many groups of organic chemists, as represented by the works of O. T. Schmidt, and of E. Haslam and their collaborators. However, the subjects of research at the earlier time were mostly commercially available plant extracts used for leathering. The works of botanists who tried to establish the correlation between plant phylogeny and distribution of tannins were mostly based on the easily detectable small molecules which are produced upon decomposition of tannins in the extracts, or the monomers which may produce tannins upon oligomerisation. There are recent works of tannins, which stand on the realisation of these problems. However, the main interests concerning utilization of tannins were for those in the technological and agricultural fields, and usually attention has not been paid to the tannins playing important roles in medicinal palnts.

There have been some confusions concerning the term "tannin". Several plant constituents such as those named "acertannin" (from *Acer* species[1]) and "hamamelitannin" (from witch hazel, *Hamamelis virginiana*[2]), have been found to have molecules[3,4] which are not large enough to exhibit the tanning activity.

Some works have been published in recent years concerning the other constituents which are regarded as the true tannins of these plants[5,6]. An appropriate comment about this kind of matter concerning tannins in general has been given[7].

Tannins have been defined as follows: They are water soluble phenolic compounds, having molecular weights between 500 and 3,000, and besides giving the usual phenolic reactions they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins[7]. This definition may be regarded too restrictive as it is based on the association of plant polyphenols with one protein —— collagen[7]. Tannins in medicinal plants may be defined in a way somewhat different from that described above. Anyhow, argument in the future concerning the activities of tannins in medicinal plants, should be based accurate knowledge of the structure of tannin in each plant, and the activities of isolated tannins.

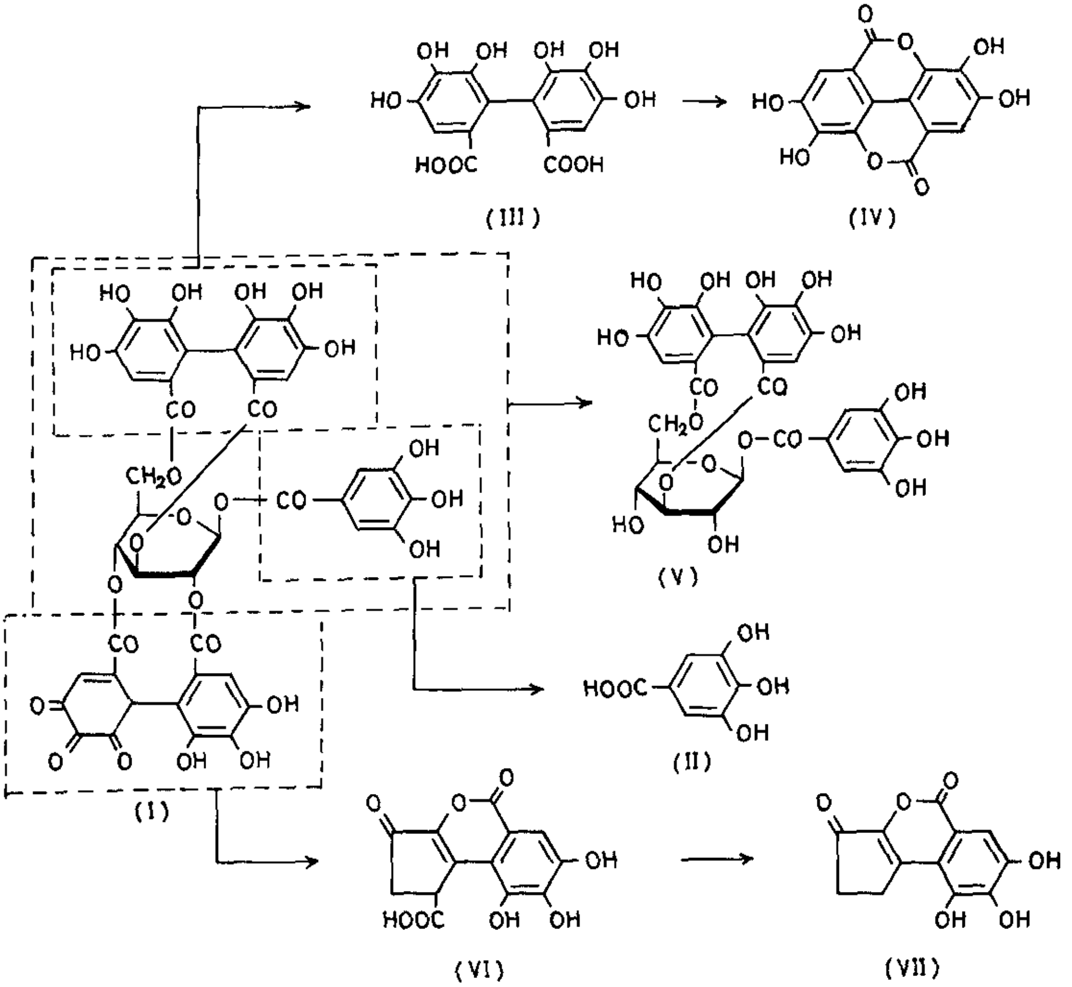
We have been carrying on researches on some tannin-rich medicinal plants and drugs used in Japan and China. In many of them, tannins have been regarded as the active principles, without knowing the characters of these tannins. The tannins isolated by our research up to the present time have been found mostly to be defined as tannins according to the general definition described above. We summarize here the results hitherto obtained.

2. Geraniin from Geranium thunbergii

The overground part of Geranium thunbergii (Japanese name; Gen-no-shoko) is one of the most popular folk medicines, and is an official antidiarrheics in Japan. There have been many research works reported concerning this plant in both chemical and pharmacological field. Although the active constituent has been regarded as tannin, the character of tannin in this plant has been entirely unknown.

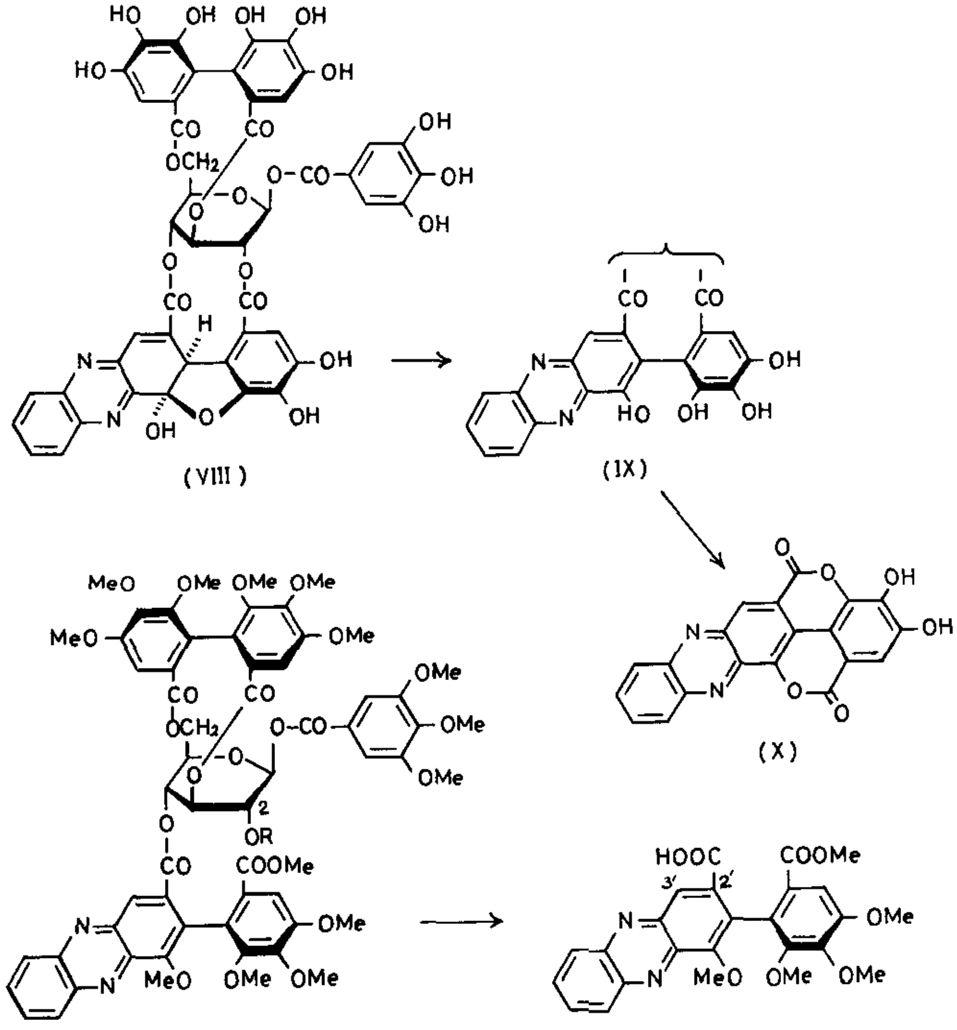
We found that the compounds isolated by the conventional extraction method at the beginning of our research were the decomposition products of the original tannin[8]. Brevifolin (VII) and corilagin (V) were included in the mixture of decomposition products[8,9]. We then carried out isolation of the original tannin, upon which droplet counter-current chromatography (DCC) of the fraction showing the highest value of Relative Astringency (RA)[10,11] was employed.

This tannin was named geraniin (I)[9]. It formed yellow crystals, $C_{41}H_{28}O_{27}\cdot xH_2O$, $[\alpha]_D^{15}$ -141° (c 0.5, MeOH, x=5)[12,13], and showed mutarotation in acetone-water (9:1) ($[\alpha]_D^{19}$ -148° → -132°, 5h, x=7). Its activities as the tannin, determined by the RA and RMB[14] methods, were similar to those of tannic acid of Japanese pharmacopoeia (tannic acid JP). However, this tannin showed almost no astringent taste on the tongue, in marked contrast to the unpleasant irritating astringent taste of tannic acid JP. Although a doubt was aroused that the lack of unpleasant taste may be due to low solubility of isolated geraniin in water, comparison of the tastes of geraniin and tannic acid JP in solutions also showed marked difference of the taste between these two tannins. Such a mild activity



of geraniin to the mucous membrane should be important for the utilization of this plant which has been used orally, very frequently for long years.

The structure of geraniin was found to be shown fundamentally by (I) as the result of following experiments[12,13,15]. Upon hydrolysis in boiling water, geraniin yielded gallic acid (II), hexahydroxydiphenic acid (III), ellagic acid (IV) and corilagin (V). Brevifolincarboxylic acid (VI) which was decarboxylated to give brevifolin (VII), was also detected in the hydrolysates mixture. Geraniin condensed with o-phenylenediamine to give a product named "phenazine A" (VIII)



(VIII)

(IX)

(X)

(XI) R = H

(XIII) R = Ac

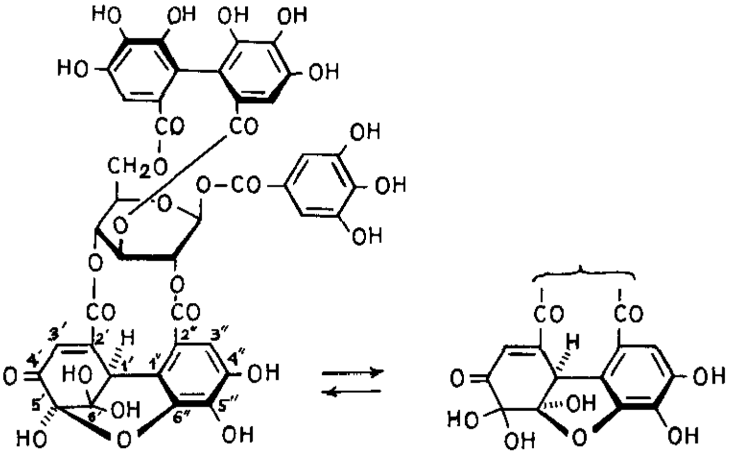
(XII)

which was slowly converted to "phenazine B" (IX) in the solutions. Upon the hydrolysis of "phenazine A and "phenazine B" in acidic solutions, "phenazine C" (X) was precipitated out, and corilagin was isolated from the mother liquor.

The orientation of dehydrohexahydroxydiphenoyl (DHHDP) group in geraniin was determined as follows[15]. Methylation of (IX) with moist diazomethane yielded a tetradecamethyl derivative (XI) which was hydrolysed in 10% hydrochloric acid to give nona-*O*-methylcorilagin and a monocarboxylic acid (XII). Comparison of the downfield shift of H-3', which was induced on addition of pyridine-d_5 to a solution of (XII) in deuteriochloroform, with the downfield shifts in the analogues, indicated that the free carboxyl group in (XII) is at C-2'. The location of free hydroxyl group in (XI) was determined to be at C-2 of the glucose moiety, by the PMR spectra of (XI) and its monoacetate (XIII).

The fundamental structure of geraniin which was determined to be (I) in this way, was then extended to the hydrated structures (Ia $\rightleftarrows$ Ib) as follows[16]. The CMR spectrum of geraniin measured in acetone-d_6 showed the peaks of the corilagin moiety[17]. However, among the ketone carbonyls in the DHHDP moiety, only one was shown in the region of conjugated ketone carbon (191.8 ppm) when the CMR spectrum was obtained in a short time after dissolution of crystalline geraniin (a-form). The other two ketone carbons were not observed in the region of carbonyl carbon, and two peaks at 92.5 ppm and 96.3 ppm indicated that the two carbonyl groups are hydrated, or forming hemiacetals.

The mutarotation of geraniin was exhibited by the PMR and CMR spectra to be



(Ia)

(Ib)

due to the structural transformation at the DHHDP moiety, but not due to the epimerisation at C-1', as H-1' was not substituted by deuterium upon the mutarotation of dried geraniin in the presence of D_2O. The equilibrium mixture (a-form *and b-form) of geraniin showed four singlets in the region of hydrated ketone or* hemiacetal in the off-resonance spectrum. These peaks which are due to a mixture of the a-form and the b-form, were shown as doublets or triplets by the gated-decoupling technique [92.3,t(b); 92.5,d(a); 96.3,t(a) and 108.9,d(b) ppm] as indicated in Fig. 1. As these couplings *are attributable to those of* ^{13}C-C-H *and* ^{13}C-C-C-H on the cyclohexene ring, the doublets were assigned to C-6', and the triplets were to C-5'. The downfield shift of C-6' peak from 92.5 ppm to 108.9 ppm upon the mutarotation was attributable to the formation of an ether linkage, or a transformation similar to that from pyranose to furanose[18]. The formation of a five-membered hemiacetal ring in the b-form was supported by the analogy of the chemical shift of C-6' in the CMR spectra, and also of the allyl coupling in the PMR spectra, between the b-form of geraniin and "phenazine A" in which only five-membered ring can be formed upon the hemiacetal formation.

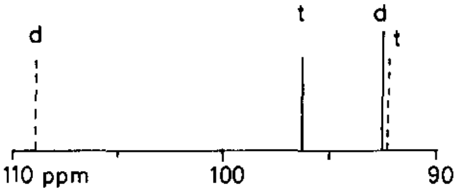
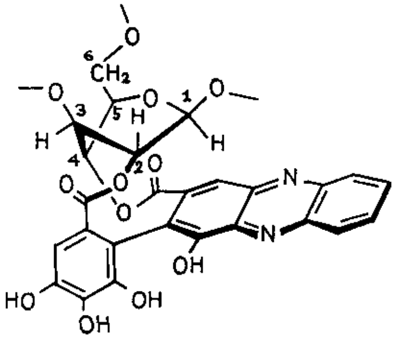


Fig.1

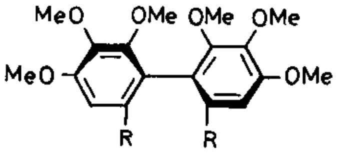
Between the two possible structures for the a-form, i.e., six-membered hemiacetal-ring structure, or the one which has gem-diol groups, the former was supported by the deuterium-induced differential isotope shift (DIS) measurement. Although this technique was originally developed for the analysis of carbohydrates[19], we confirmed in the preliminary test that this method can be applied to phenolic compounds, using phenol derivatives of lower molecular weights. The aromatic carbons which carry free hydroxyl group were clearly differentiated from those carrying ether oxygen, by a dual peak (difference of shifts, 0.1 - 0.25 ppm) of the former, and a single peak of the latter. The DIS measurement of geraniin showed that among the phenolic carbons, only C-6" is exhibited as a single peak in the spectra of both of the a-form and the b-form. All of the other phenolic carbons were shown as dual peaks. This result indicated formation of a hemiacetal ring in each of the two forms of geraniin, and hence that a six-membered hemiacetal ring is formed in the a-form. This structure was also supported by the sharp singlets of H-1' and H-3' in the PMR spectrum, which show the absence of allyl coupling, as bondings (H-1')~(C-1') and (H-3')~(C-3') in this structure are almost in the same plane. The upfield shift of C-5' peak in the CMR spectra upon the mutarotation (96.3→92.3 ppm) was also in accord with this structural correlation of the a-form and the b-form.

The atropisomerism R at the phenylphenazine moiety in "phenazine B" was assigned on the basis of the large upfield shift of H-1 of the glucose moiety (6.62→6.14 ppm) occured upon the transformation of "phenazine A" to "phenazine B", as this shift is attributable to the conformation of "phenazine B" shown by (IX-c). The configuration of H-1' in geraniin was then assigned α, as the atropisomerism R in "phenazine B" is regarded as the result induced when H-1 in "phenazine A", and accordingly in geraniin is α-oriented.

The atropisomerism at the hexahydroxydiphenoyl (HHDP) group in corilagin was determined to be identical as that of the polymethoxydiphenyl group in schizandrin[20], as the optical activities of dimethyl hexamethoxydiphenoate (XIV)



(IX-c)



(XIV) R = COOMe

(XV) R = CH_2OH

($[\alpha]_D^{25}$ +21°, $CHCl_3$) and dihydroxymethylhexamethoxydiphenyl (XV) ($[\alpha]_{240}^{25}$ +2800°, MeOH) obtained from nona-O-methylcorilagin were identical with those of the same compounds derived from schizandrin. Although S-configuration had been once proposed for the HHDP group in corilagin as the result of extended application of the amide rule of glyconic acids to biphenyls[21], our result unambiguously determined this atropisomerism to be R as the atropisomerism of schizandrin had been determined to be R[20]. These results assign structure (Ia) to the a-form of geraniin, and (Ia)⇄(Ib) to the equilibrium mixture[16]. The PMR spectrum indicates the conformation of the glucose moiety in geraniin to be 1B.

The structures (Ia)⇄(Ib) are presumed to be the genuine forms in the living plant of Geranium thunbergii, as the high-performance liquid chromatography (HPLC) performed with the extract prepared by homogenizing the fresh plant frozen immediately upon collection, showed identical peak as that of isolated geraniin[22]. The geraniin content in the plant determined by the peak area in the chromatogram (12% in the dried leaf, and 1.8% in the fresh overground part) were also close to those of isolated geraniin (1.5% from fresh overground part). These data show significance of geraniin as the tannin of Geranium thunbergii, since the total tannin in the overground part of the plant, which was determined by the RA method, was about 2%.

Geraniin in Geranium thunbergii is the first example of vegetable tannin which is composed mainly by a single compound in a species of plant.

2. Hydrolysis of geraniin

As tannins are generally regarded as labile substances, it has been often hard to say whether a tannin isolated from a plant is the original tannin in the living plant or not. A more complex problem occurs for the commercially available extracts from some "tannin drugs", concerning whether the extract contains the original tannin. Although the latter problem is important for qualification of many drugs and their preparations, there has been practically no method developed for the analysis of tannins on this basis.

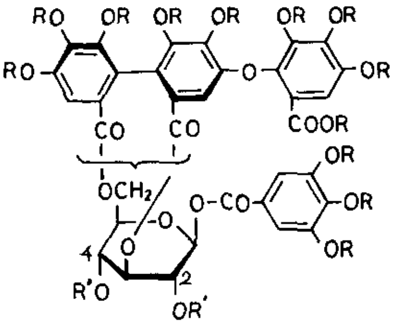
Geraniin is a very suitable compound in several aspects to be used for the experiments to answer this problem. It is obtained as crystals, it composes the major part of the tannin in Geranium thunbergii, and this plant is a very useful drug. As has been mentioned in several papers, it is obvious that the tannin is the main active principle of Geranium thunbergii, or at least the starting material of the active principles which may be produced upon the transformation of geraniin. The transformation of geraniin occuring in the solution is mainly hydrolysis, and several compounds produced upon the hydrolysis of geraniin are now analysable.

As the reproducibility upon the chromatography of tannins by TLC and PPC are often poor, the analysis of geraniin and its hydrolysates by way of HPLC was investigated. The HPLC analysis of geraniin and the extracts from Geranium thunbergii, performed by the reversed-phase development, gave good reproducibility of the retention time and the peak area[22]. The extents of hydrolysis of geraniin in the solutions and the extracts were clearly exhibited by this method[23]. The results of this analysis should give a basis of evaluating several drugs for which chemical analysis was practically impossible before the discovery of geraniin.

4. Mallotusinic acid and mallotinic acid from Mallotus japonicus

Bark and leaf of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) have been used in Japan as remedies of gastric ulcer. We isolated two main tannins of the leaf, using DCC and also by extraction under regulation of acidity of the extract. One of the tannins was identified as geraniin. The other one was named mallotusinic acid[24].

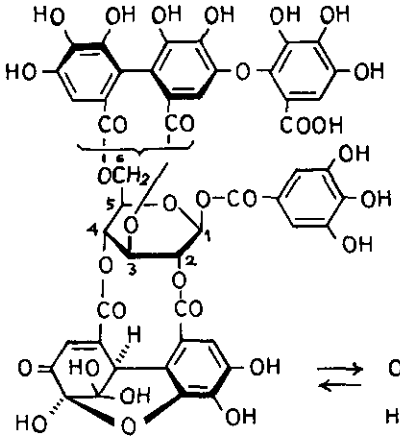
Mallotusinic acid (XVI) formed yellow amorphous powder, $C_{48}H_{32}O_{32}\cdot xH_2O$, $[\alpha]_D^{15}$ -65.4° (c 0.5, MeOH, x=10). The PMR and CMR spectra showed that this tannin is present as an equilibrium mixture which is analogous to that of geraniin and that the molecule of mallotusinic acid is composed of geraniin and a gallic acid residue. Upon the reaction with o-phenylenediamine, mallotusinic acid yielded a condensation product (XVII) which is analogous to "phenazine B" obtained from geraniin. This product was hydrolysed in boiling water to give a precipitate which was identified with "phenazine C" obtained from geraniin. An amorphous degradation product, $C_{34}H_{26}O_{23}$, which showed positive colour reaction of ellagitannin, was isolated from the mother liquor, and was named mallotinic acid (XVIII). Methylation of mallotinic acid with diazomethane followed by methanolysis yielded methyl tri-O-methylgallate, D-glucose, and another product (XIX), $[\alpha]_D^{20}$ +17° (c 1.0, acetone), m/e 660 (M^+), whose PMR spectrum was identical with that of (-)-trimethyl octa-O-methylvaloneate which is obtained from valonia tannin[25]. The optical rotations indicated that (XIX) is the atropisomer of (-)-trimethyl octa-O-methylvaloneate. The analogy of mallotinic acid with corilagin, and their difference which is due to the difference between the HHDP group in corilagin and the valoneoyl group in mallotinic acid, were also shown in the PMR spectra, and in the mass spectra of the permethylated derivatives. The presence of free hydroxyl group at C-2 and C-4 in mallotinic acid was proved by production of 2,4-di-O-methylglucose upon methanolysis of methyl trideca-O-methylmallotinate (XXI) which was produced by permethylation of undeca-O-methylmallotinate (XX). The selective hydrolysis of mallotinic acid at O-1 of glucose gave gallic acid and the residue which was positive to the colour reactions of reducing sugar and



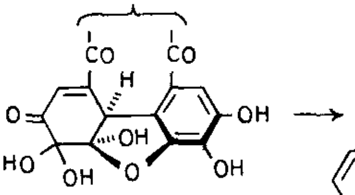
(XVIII) R = R' = H

(XX) R = Me, R' = H

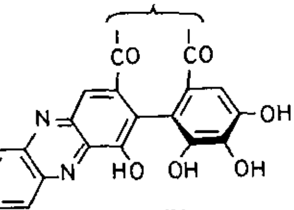
(XXI) R = R' = Me



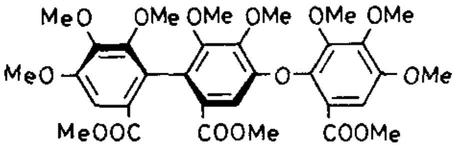
(XVI-a)



(XVI-b)



(XVII)



(XIX)

also of ellagitannin. The structure of mallotinic acid was therefore represented by (XVIII). The atropisomerism R was based on the comparison of the optical activity of (XIX) with that of (XIV).

Mallotinic acid was detected by HPLC, in the extract of leaf, which was prepared with cautions for preventing hydrolysis.

The orientation of the DHHDP group at O-2 and O-4 of the glucose moiety, and the β-configuration of the galloyl group at O-1 of the glucose moiety in mallotusinic acid, were presumed to be identical with those in geraniin, based on the analogy of the upfield shifts of H-1 of glucose upon the formation of "phenazine B" from geraniin, and of the analogous phenazine derivative (XVII) from mallotusinic acid.

The equilibration at the DHHDP moiety in mallotusinic acid was shown by the CMR and PMR spectra to be identical with that in equilibrated geraniin. The structure of mallotusinic acid is therefore represented by (XVIa ⇄ XVIb)^{16}.

The amounts of geraniin and mallotusinic acid in the dry leaf of Mallotus japonicus, determined by HPLC, were 8.4% and 7.1%, respectively.

5. Distribution of geraniin and mallotusinic acid in plants

The isolations and the structure determinations of these tannins which are present in large amounts in plants, offered some questions concerning their distribution; whether occurrence of these tannins is limited to the species from which these tannins were isolated, or their occurrence is limited to a certain group of plants, or they distribute widely in plants regardless of their correlation to the plant taxonomy. Besides the chemotaxonomical significance of the answer to this question, there is also a significance of the answer for the medicinal use of several plants. For instance, several species of Geranium have been used by the name of Gen-no-shoko (G. thunbergii), or as substitutes of Gen-no-shoko. Nevertheless there was no evidence available upon estimating whether the main constituents of these species are identical with those of Gen-no-shoko.

We inspected about 70 species of plants belonging to Geraniales, and also to other Orders, with HPLC[26] and also isolating geraniin from several species[27], and found that large amounts of geraniin is contained in all of the inspected 14 species (including two varieties) of Geranium grown in Japan. The amount of geraniin in the dry leaf of Geranium species was 9.4% on average. Geraniin was also found in 28 species among the inspected 40 species of Euphorbiaceae, and also in some species of the other families of Geraniales. Chemotaxonomically, the obtained results were rather consistent with the contemporary taxonomy of Euphorbiaceae, and were inconsistent with the old taxonomy[26]. The occurrence of mallotusinic acid was limited to 13 species of Euphorbiaceae.

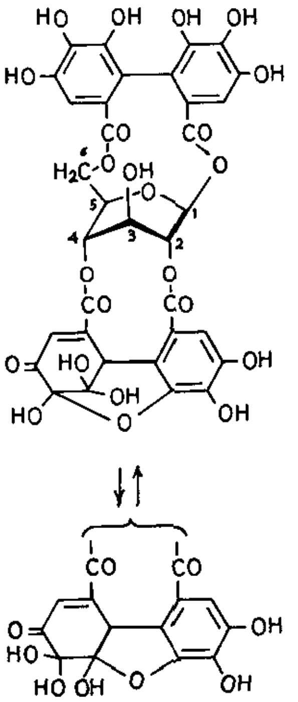
6. Granatin A and granatin B from Punica granatum

Although bark of pomegranate, Punica granatum L., has been known as an anthelmintics, the main way of medicinal use of this plant in China has been the utilization of the fruit peel as an astringent drug. We carried out isolation of the tannins contained in the fruit peel, and obtained four hydrolysable tannins which were named granatin A, B, C and D[28]. Among them, granatin C and granatin D were found identical as punicalagin and punicalin, which were reported recently[29].

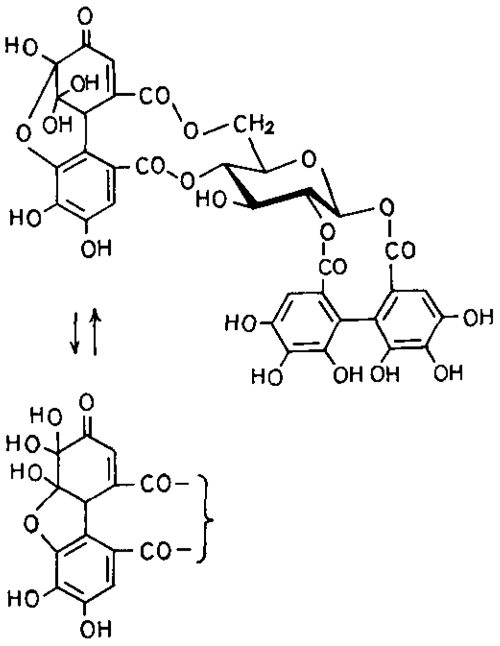
Granatin A (XXII) and granatin B (XXIII) were isolated by DCC of the ethyl acetate-soluble fraction.

Granatin A forms yellow amorphous powder, $C_{34}H_{26}O_{24}\cdot xH_2O$, $[\alpha]_D^{15}$ +65.2° (c 0.5, EtOH, x=7), and gives positive colour reaction of ellagitannin. The PMR and CMR spectra showed that granatin A composes an equilibrium mixture which is similar to that of geraniin. Granatin A condensed with o-phenylenediamine to give "phenazine 1" which was shown by the PMR and CMR spectra to be analogous to "phenazine A". Upon hydrolysis of "phenazine 1" in boiling water, the precipitated product was identified as "phenazine C". The molecular residue in the mother liquor, which was positive to the colour reaction of ellagitannin, was inspected by combination of densitometry and colour reactions on PPC and TLC of the methyl derivative, and was found that O-1 of the glucose moiety is acylated. The decoupling experiment of the PMR spectrum of granatin A showed that O-1 and O-2 of the glucose moiety is acylated, while O-3 is not acylated.

These results indicate that the structure of granatin A should be either 1,6-HHDP-2,4-DHHDP-glucose (XXII) in which the glucose moiety will take most



(XXII)*



(XXIV)*

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*The orientation of the DHHDP group is not determined.

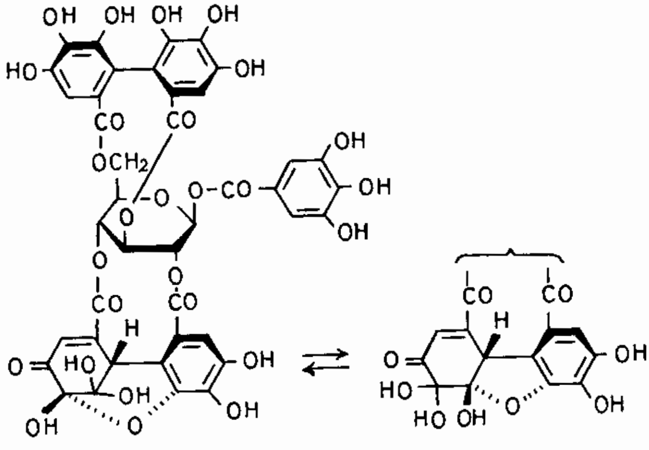
probably the B3 conformation, or 1,2-HHDP-4,6-DHHDP-glucose (XXIV) in which the glucose moiety will take the C1 conformation. As H-1 of the glucose moiety in granatin A in the PMR spectrum does not show separation of coupled peak, structure (XXIV) is excludable. Structure (XXII) was then assigned to granatin A.

Granatin B (XXIII) forms yellow crystals, $C_{41}H_{28}O_{27}\cdot xH_2O$, which shows mutarotation in the reversed direction of that of geraniin, ($[\alpha]_D^{28}$ -109° → -123°, 4h, acetone-water, 9:1, x=8). The PMR and CMR spectra of granatin B before and after the mutarotation showed practically all of the peaks corresponding to those of geraniin, with small differences of the chemical shifts.

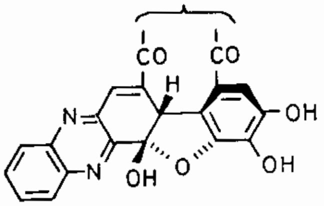
Condensation of granatin B with o-phenylenediamine gave a product (XXV) which was converted to another product (XXVI) in acidic solution. These products were shown by the PMR and CMR spectra to be isomeric to "phenazine A" and "phenazine B", respectively. Unlike "phenazine B", the upfield shift of H-1 of the glucose moiety in the PMR spectrum was not observed in (XXVI). Upon hydrolysis, both (XXV) and (XXVI) gave "phenazine C" along with corilagin.

Although methylation of (XXVI) with dry solution of diazomethane yielded tridecamethyl derivative, the methylation with diazomethane in a mixture of wet ether and methanol gave a tetradecamethyl derivative (XXVII), which gave monoacetate (XXVIII) upon acetylation. The H-4 peak of the glucose moiety, which presumably shifted upfield in (XXVII) as the result of cleavage of the ester linkage at C-4 of glucose, was hidden by other protons. This proton showed downfield shift to δ5.64 in (XXVIII). The difference of the chemical shift of H-2 in (XXVII) and (XXVIII) was only 0.02 ppm. These data indicated that the ester linkage at C-4 of glucose was methanolysed upon the methylation of (XXVI). Hydrolysis of (XXVIII) in 10% hydrochloric acid, followed by extraction with chloroform and fractionation by preparative TLC, gave an acid (XXIX). The free carboxyl group in (XXIX) was found to be at C-2", by the comparison of the downfield shift of H-3" (δ7.36 → 7.44) in the PMR spectrum, which occurred upon addition of pyridine-d_5 into the deuteriochloroform solution, with the shifts in the analogues. These data indicated structure (XXVII) for the product of methylation of (XXVI), and showed that the cyclohexenetrione moiety in the DHHDP group in granatin B links to O-4 of the glucose moiety in analogous way as in geraniin.

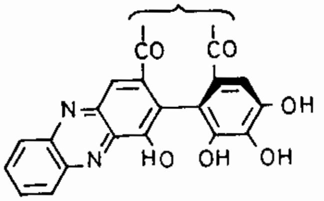
Methylation of acid (XXIX) with diazomethane yielded dimethyl ester (XXX),



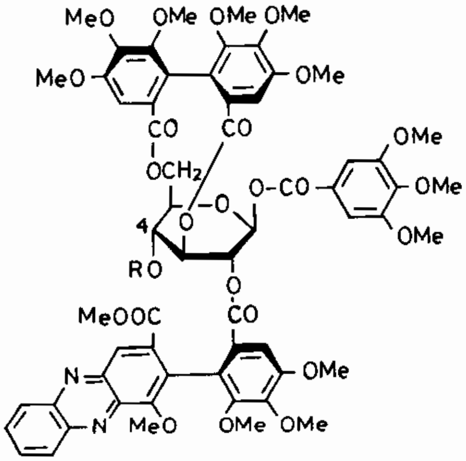
(XXIII)



(XXV)

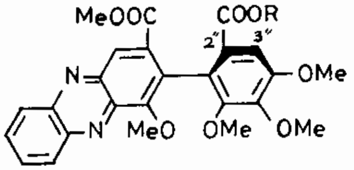


(XXVI)



(XXVII) R = H

(XXVIII) R = Ac



(XXIX) R = H

(XXX) R = Me

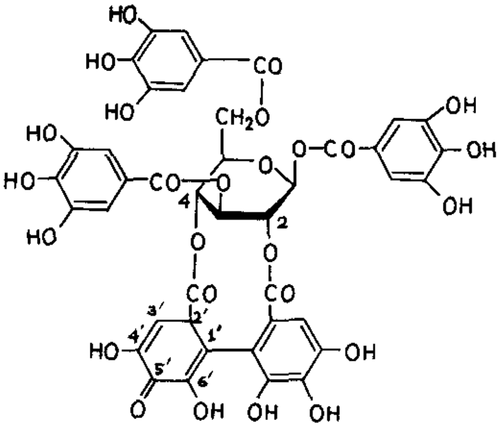
$[\alpha]_D^{23}$ -39°, c 1.0, EtOH), which was found to be the atropisomer of dimethyl ester prepared by methylation of (XII) which was derived from geraniin. The stereostructure and the absolute configurations of granatin B were therefore shown by (XXIII)[28,30]. This is a stereoisomer of geraniin, in which the DHHDP moiety is enantiomeric to that in geraniin.

7. Tannins of myrobalans —— revised structures of terchebin, chebulinic acid and chebulagic acid, and isolation of punicalagin

Fruits of Terminalia chebula Retz. (Combretaceae), called myrobalans, and used in Europe for leathering, have been used as an astringent drug in China and Japan.

The investigations of the tannins in this fruit have been done by several groups of workers, i.e., those of E. Fischer[31], K. Freudenberg[32], O. T. Schmidt[33-35], R. D. Haworth[36,37], and E. Haslam[38]. Among them, Schmidt gave most remarkable results, presenting structures of chebulinic acid, chebulagic acid, and terchebin, along with those of the other tannins. We found that the structures of these main tannins should be reinvestigated.

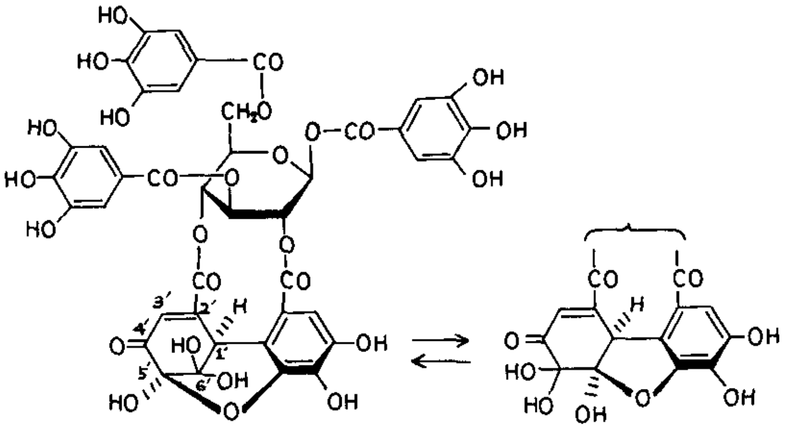
Structure of terchebin was reported to be (XXXI), in which the cyclohexanetrione moiety forms dihydroxycyclohexadienone. This partial structure which does not tautomerise to pyrogallol group in (XXXI) should require a further evidence. The location of this group at O-4 of glucose, not at O-2, also lacks proof.



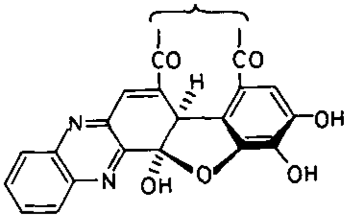
(XXXI)

We isolated terchebin practically in the same way as reported by Schmidt et al[33], with partial improvement at the final stage by applying DCC[39].

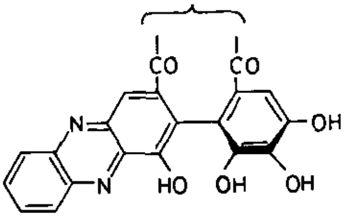
One of the bases by which cyclohexanetrione instead of cyclohexenetrione, as in (XXXI), was presumed is that chloroellagic acid was not isolated upon hydrolysis of terchebin in concentrated hydrochloric acid. However, we found production of chloroellagic acid by the mass spectrum measured after methylation of the precipitate occurred upon the hydrolysis. It showed the ion peaks of tetra-O-methylchloroellagic acid (M^+, m/e 392; M+2, m/e 394) along with the peaks due to tetra-O-methylellagic acid (M^+, m/e 358) in analogous way as in the experiments



(XXXII)



(XXXIII)

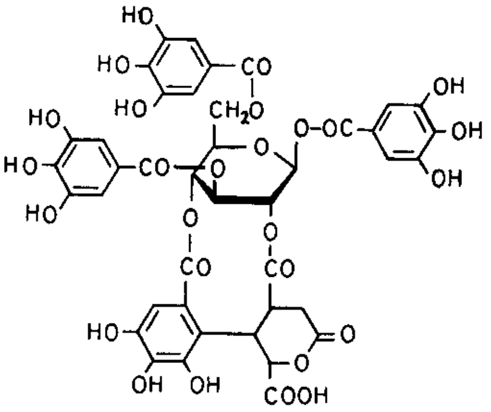


(XXXIV)

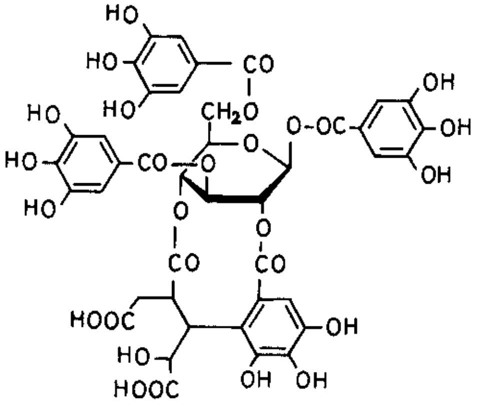
with geraniin. Another basis of presuming cyclohexanetrione as the partial structure in (XXXI) was that hydrogenized terchebin decolorized Tillman's reagent. However, we found that this property is consistent with the presence of cyclohexenetrione, as hydrogenized geraniin also decolourized Tillman's reagent.

The PMR spectrum of terchebin showed protons at δ4.66 (1/2H, d, J 1.3Hz), 4.96 (1/2H, s), 6.24 (1/2H, d, J 1.3 Hz) and 6.48 (1/2H, s), which were regarded by Schmidt et al. as due to H-2' and H-3' in the epimers concerning C-2' in structure (XXXI). However, we found that these protons are assignable to H-1' and H-3' in structure (XXXII) based on the analogy of the peak pattern to that of geraniin.

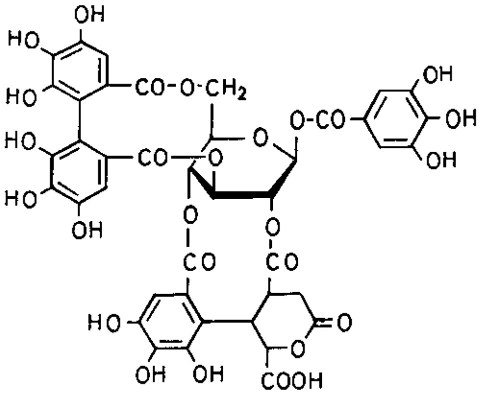
The peaks in the CMR spectrum, which were assigned to the carbons of hydrated cyclohexenetrione moiety in the equilibrated structure (XXXII), are as follows: (acetone-d_6, δ) C-1' (46.5 ⇄ 52.4), C-2' (155.2 ⇄ 150.0), C-3' (130.0 ⇄ 126.5), C-4' (193.0 ⇄ 195.6), C-5' (96.8 ⇄ 93.1) and C-6' (93.1 ⇄ 109.7). These peaks which were analogous to those of geraniin and mallotusinic acid, excluded



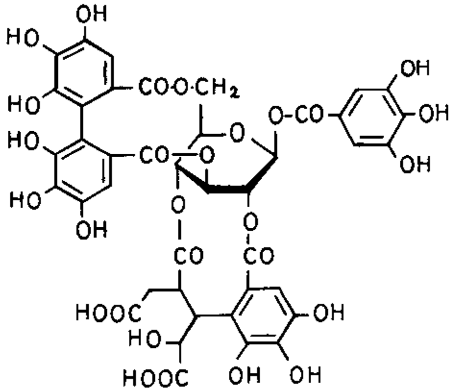
(XXXV)



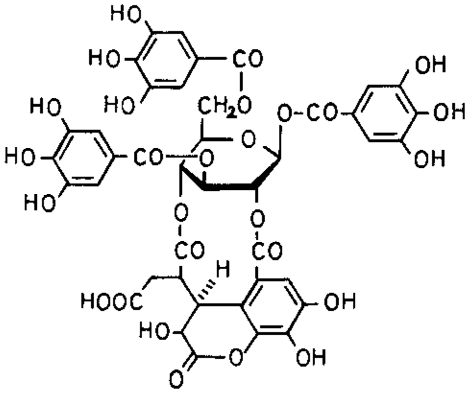
(XXXVI)



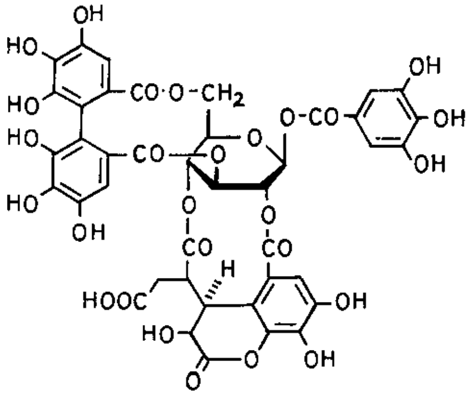
(XXXVII)



(XXXVIII)



(XXXIX)



(XL)

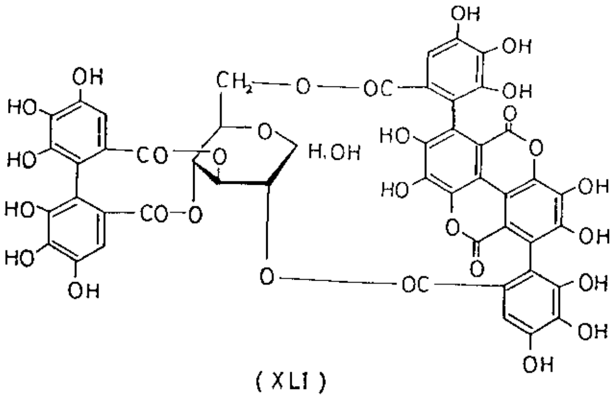
structure (XXXI).

Upon condensation with o-phenylenediamine, terchebin yielded a product (XXXIII) which was converted to another product (XXXIV) in an acidic solution. The PMR and CMR spectra showed that these products are analogous of "phenazine A" and "phenazine B". Hydrolysis of these products yielded a precipitate which was found to be identical as "phenazine C". The supernatant liquor gave an amorphous powder which was identified as 1,3,6-trigalloyl-β-D-glucopyranose[40].

These results indicated that the structure of terchebin should be revised to (XXXII)[30,39]. The orientation of DHHDP group and the configuration at C-1' in this structure were based on the upfield shift of H-1 (δ6.65 → 6.26) upon the transformation of (XXXIII) to (XXXIV), as this shift showed analogy of the relative spatial locations of H-1 of glucopyranose and cyclohexenetrione in terchebin to those in geraniin.

Chebulinic acid is contained in the largest amount among the tannins in myrobalans. Structure (XXXV) given to this tannin by Schmidt et al.[33] was later revised to (XXXVI)[35] by the work of Haslam et al.[38]. Structure of chebulagic acid was also revised from (XXXVII) to (XXXVIII) at the same time[35]. We applied the DIS measurement to this tannin, and reinvestigated the methylation products, and found that the structures of chebulinic acid and chebulagic acid should be (XXXIX) and (XL), respectively[39].

An additional tannin was isolated and was identified as punicalagin (XLI)[29].

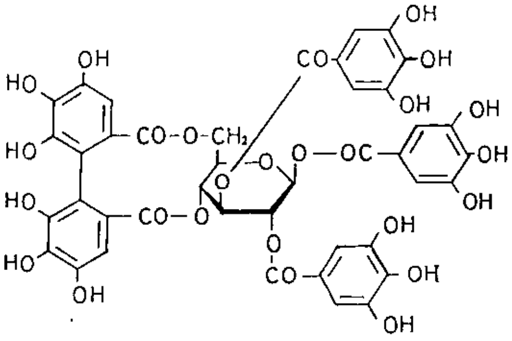


(XLI)

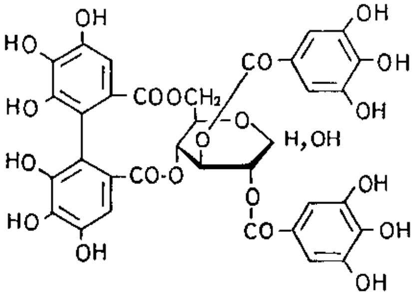
8. Hydrolysable tannins in other medicinal plants

Cloves, the dry flower-buds of *Eugenia caryophyllata* Thunberg (Myrtaceae), is known to contain a large amount of essential oil. However, this bud is also known to be rich in tannin.

Applying DCC, we isolated an amorphous ellagitannin from the fraction which showed the highest activity upon the RMB determination. This ellagitannin was identified as 1,2,3-trigalloyl-4,6-hexahydroxydiphenoyl-β-D-glucopyranose (XLII)[41] which was previously isolated from *Tellima grandiflora*[42].



(XLII)



(XLIII)

Fruits of *Cornus officinalis* Sieb. et Zucc. have been a kind of tonic, and used in several prescriptions of old Chinese medicine, and has been regarded as rich in tannin.

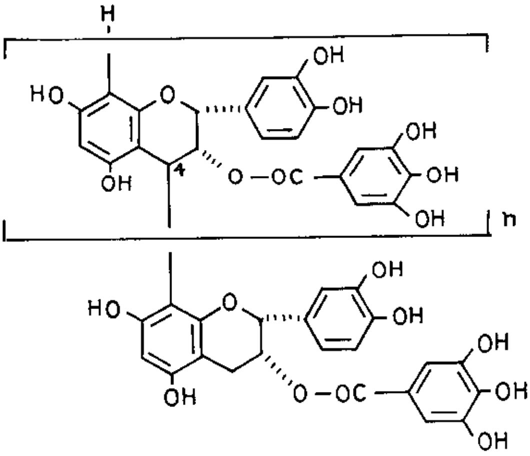
We isolated a tannin which was identified as (XLII), and another tannin which was identified as 2,3-digalloyl-4,6-hexahydroxydiphenoyl-β-D-glucopyranose (XLIII)[43]. The same tannin as the latter also had been isolated from *Tellima grandiflora*[42].

9. Condensed tannins of medicinal plants

There are a number of medicinal plants in which condensed tannins are the main constituents. Rhubarb is an example of the crude drug of this kind taken up in our research. The quantitative determination by the RMB method of the tannin in rhubarb of several varieties, showed that the tannin content in rhubarb belongs to the group of the highest content among that of several crude drugs which have been regarded as rich in tannin. This result may be an unexpected one since rhubarb has been used as a purgative, while the activity of tannin is usually supposed to be of reversed direction.

There had been a report which described presence of a "tannin" named tetrarine in rhubarb[44]. However, researches by several groups of workers in recent years have not detected this tannin in rhubarb. Isolation of polyphenol glucosides which are structurally related to hydrolysable tannins was reported[45]. On the other hand, the tannin in rhubarb was presumed to be that of condensed type[46], and a mixture of condensed tannins, having galloyl-(-)-epicatechin as the chain extension unit, was obtained from a variety of rhubarb[47].

We made determination of the contents of hydrolysable tannin and condensed tannin, and then fractionated the tannins of rhubarb by monitoring with RMB determination of each fraction. The ratio of condensed tannin in the total tannin in a variety of rhubarb (Shinshu-daiwo, rhizome of a hybrid of Rheum coreanum Nakai and R. palmatum L.), estimated by a method which is composed of the salt formation of tannins with cinchonine, and condensation of catechins with formaldehyde, was about 75%. The fractions of strongest activity obtained by gel-permeation chromatography (Sephdex LH-20, EtOH → $MeOH-H_2O$), from this variety of rhubarb, and from Gawo which is a variety of rhubarb imported from China, showed RMB value about 1.0.



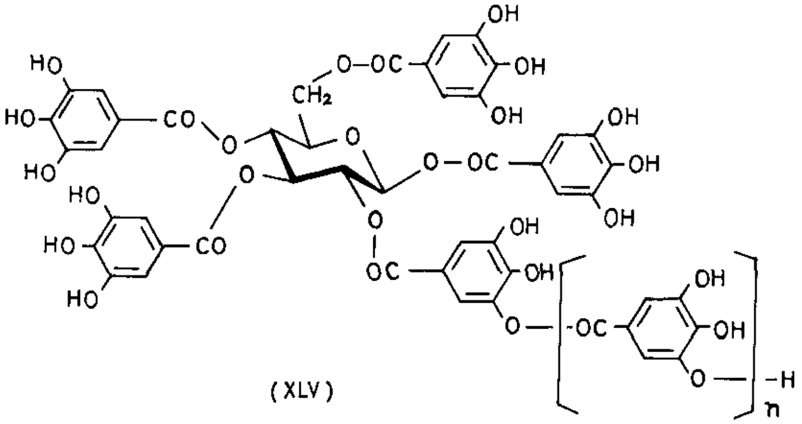
(XLIV)

Degradation of this tannin fraction from Gawo with toluene-α-thiol, followed by the fractionation on a column of Sephadex LH-20, yielded (-)-epicatechin gallate and its benzylthio derivative at C-4 as the main products. A small amount of (+)-catechin was also detected. This tannin is therefore mainly composed of condensates of (-)-epicatechin gallate. The PMR and CMR spectra show that galloyl ester is formed at C-3 of (-)-epicatechin in the oligomeric chain (XLIV). However, the ratio of galloyl group to (-)-epicatechin differed between the two varieties of rhubarb. It was 0.5-0.9/1.0 in Shinshu-daiwo, and about 1/1 in gawo[41], which were used in the experiments.

10. Correlations of the structures of isolated tannins with the activities and the biogenesis

The activities of tannins are usually regarded as due to the phenolic hydroxyl groups in their molecules. The nature of association of polyphenols which forms stable cross-linked structures with proteins, is thought to be multiple hydrogen bondings, without determination of the precise detail[7]. On the other hand, there was a view that quinoids are intermediates in the tanning process[48]. The dehydrohexahydroxydiphenoyl group which is present in several tannins found in our research, forms the quinone-like structures, and presumed to be participating in the activities of these tannins.

This partial structure can be regarded as an oxidised form of the biphenyl type condensate produced by two galloyl groups. However, our experiments concerning the activities of tannins showed that the reducing activities of geraniin to nitro group and heavy metal ions are comparable to that of tannic acid JP which is produced from the gall of Rhus javanica L., and sometimes stronger than the latter[49] which is mainly composed of polygalloyl glucose (XLV)[50]. Some differences of activities of the tannins having the DHHDP group with those of



(XLV)

the tannins of other types have been found in several activity tests[49]. Further investigation should be carried out concerning the differences of activities of the tannins in medicinal plants, due to their structural differences.

Biogenetically this patial structure may be one of the products of oxidation of polyhydroxydiphenoyl group which is formed by oxidative coupling of galloyl ester residues in a gallotannin[51], or conversely an intermediate product between the biphenyl group and its precursor formed by carbohydrate-type condensation[52]. It is hoped that the discovery of the occurrence of enantiomeric partial structures, (Ia $\rightleftarrows$ Ib) and (XXIII), helps determination of biosynthetic route.

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