**Ellagitannins as Active Constituents of Medicinal Plants**

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**Abstract**

Isolation and structure determination, accompanied by measurement of various biological activities of each isolated tannin, particularly of ellagitannins, have brought about a marked change in the concept of tannins as active constituents of medicinal plants. Their biological activities should now be discussed on the basis of the structural differences among each tannin, in a way similar to that of the other types of natural organic compounds. The antitumor activity exclusively exhibited by several oligomeric ellagitannins, and their anti-HIV activities are examples of such biological activities. The inhibitory activity against lipid peroxidation, which is different in the strength among tannins of various structures, is exhibited in general more strongly by ellagitannins than by the other types of tannins of similar structures. The radical-scavenging activities of tannins as the mechanism of their inhibition, which is regarded to participate in several biological activities of tannins, have been supported by the ESR spectral measurements. Other biological activities, i.e., inhibition of mutagenicity of carcinogens, inhibition of tumor promotion, etc., have been found for tannins including ellagitannins.

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**Introduction**

Ellagitannins belong to the group of hydrolyzable tannins which, along with condensed tannins, comprise one of the two large groups of tannins, and have the hexahydroxydiphenoyl (HHDP) group, or a similar group derivable from the HHDP group, in their molecules. The number of ellagitannins hitherto isolated is by far larger than that of gallo- 

lactannins, which constitute another group belonging to the hydrolyzable tannins, and are often present as complex mixtures. Ellagitannins are generally different from the other types of tannins in that each of them can be isolated as a comparatively stable compound, as exemplified by geraniin which is a crystalline tannin (1), and can be handled as a single compound of defined structure. This property of ellagitannins made it possible to measure the biological activities of each isolated tannin of determined chemical structure, like those of each compound belonging to the alkaloids, terpenoids, and steroids, etc., although it is also true that there are some properties common to most of tannins. Although tannins, in the past, were generally regarded as intractable mixtures having unfavorable biological activities, regardless of the structural differences among each tannin, the recent isolation and structural determination of a number of ellagitannins, including their oligomers among which agrimoninin was the first one (2, 3), aided by the progress in analysis methods (4) and in screening procedures for biological activities of each tannin thus brought about a marked change in the concept of tannins. Ellagic acid, which has recently been a topic of interest because of its anti-carcinogenic activity (5), should be considered as a compound derived from ellagitannins, since ellagic acid is mostly produced by a hydrolysis of ellagitannins taking place during their extraction and the concentration of the extracts from plants, and is rarely found in living plants (6). The discovery of marked anti-tumor (7) and anti-HIV activities (8) specifically exhibited by several of the oligomeric hydrolyzable tannins among a number of tannins screened, further demonstrated the necessity for a change of the old concept of tannins.

**Biogenesis of Ellagitannins**

The HHDP group in ellagitannins has been presumed biogenetically to be produced by the C-C bond formation between two galloyl groups (9). Recent investigation of the seasonal changes in the structures of hydrolyzable tannins in *Liquidambar formosana*, revealed the formation of an HHDP group in several tannins, in place of two galloyl groups with the same locations on the glucopyranose ring in the tannin molecule found in the same plant in earlier seasons (10). Liquidambin, a compound which has a partly hydrated aldehyde group and is regarded as an intermediate product in the biosynthesis of C-glucosidic ellagitannins, has also been isolated (11). An example of a series of biosyntheses of several types of ellagitannins, supported by these findings, can be depicted as shown in Scheme 1.

The distribution of ellagitannins in plant families which have been thus biosynthesized, is therefore fundamentally the same as that of gallotannins (15).

**Tannins Related to Ellagitannins**

There are a number of monomeric ellagitannins, in which the HHDP group (a) has been further metabolized to the other aromatic groups (b)–(f) by structural modifications such as oxidation, reduction, ring cleavage, and C-O(C) oxidative coupling. These tannins are often regarded as belonging to the ellagitannin group on the basis of their pre-
sumable biogenetic relationship with the tannins having the HHDP group, although there are tannins such as geraniin (1) having two or more of these types of groups, and/or a galloyl group in a molecule. Some ellagitannins also have the other bonding in their molecules, as those referred to in groups (i)—(iv). The representative ellagitannins having the groups (a)–(f) are shown in Scheme 2.

(i) C-Glucosides: Ellagitannins of this type might be biogenetically produced by a phenol-aldehyde coupling as illustrated in Scheme 1. They include tannins such as castalagin (14) having a flavogalloyl group, which is regarded as a condensate through the C-C coupling between an HHDP and a galloyl group.

(ii) Condensates with ascorbic acid: Ascorgeraniin was found in several species of Geranium, Acer, Elaeocarpus, Rhus, and Cercidiphyllum genera (24) (Scheme 3). This type of tannin is easily formed in a high yield when a pentagalloylglucose (12) tannin having the dehydrohexahydroxydiphenoyl (DHHDP) group is mixed with ascorbic acid in solution at room temperature (25).

(iii) Condensates with catechins: Most of this type of ellagitannins are composed of C-glucosidic ellagitannins and catechins, and the C-C bond is formed between C-1 of the former and C-6 or C-8 of the latter (26).

(iv) Oligomers: Oligomeric ellagitannins exemplified by agrimonin (2, 3), oenothein B (dimer) (27), rugosin G (trimer) (28), and nobotanin K (tetramer) (29), etc., are regarded as products of biosyntheses in which a valoneoyl group, or a dehydrodigalloyl group is formed between two molecules of monomeric or oligomeric hydrolyzable tannins (Scheme 4).
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Scheme 2

Scheme 3

Biological Activities of Ellagitannins

Biological activities of any isolable compound should be examined for each compound, however, there are some properties commonly found for ellagitannins. The property most easily noticed for many ellagitannins is their comparatively mild property, which is weakly astringent on the tongue, and is weakly irritating on the mucous membrane. The extracts of medicinal plants containing ellagitannins, therefore have been generally administered orally without marked unfavorable responses.

1. Inhibitory effect on peroxidation of lipids and other co-existing substances, and radical-scavenging effect

Inhibitory effects of tannins on the lipid peroxidation induced by ADP and ascorbic acid in rat liver mitochondria, and on that induced by ADP and NADPH in rat microsomes, were strongly exhibited by some ellagitannins, such as pedunculagin and isoterchebin (30). This effect in condensed tannins was somewhat lower, and that in the polyphenols of small molecules, except for (-)-epigallocatechin gallate (EGCG) which is the main component of so-called green tea tannin, was markedly lower. No positive correlation of the intensity of effect was observed between the inhibition of peroxidation in the experiment with ADP and ascorbic acid and the binding activity, as expressed by RA and RMB values of tannins (31). The experiments on the inhibition of peroxidation by the radical-chain reaction of methyl linoleate, initiated by ultraviolet irradiation of AIBN in solution, also showed similar results (32). These re-
Scheme 4

agrimonin

enothein B

nobotanin K
sults indicate that the inhibitory effects of ellagitannins against lipid peroxidation, which are stronger than those of the other types of tannins, should be due to stronger radical-scavenging effect of the HHDP group (33), although the effect of the galloyl group cannot be underestimated. The ESR spectra of the mixture in the latter experiment exhibited formation of stable free radicals on the polyphenolic group in the tannins of fairly large molecules, thus confirming the mechanism of the inhibition of lipid peroxidation by tannins, which was induced by donation of a hydrogen radical to the peroxide radical of the lipids which were undergoing peroxidation (33). The ESR spectrum of geraniin, which has an HHDP, a DHDP, as well as a galloyl group in its molecule, showed preferential formation of a free radical on the HHDP group (33). Inhibition by several tannins and related polyphenols of the oxidative damage to eye lenses of mice, which was induced by the increase of lipid peroxide with the xanthine-xanthine oxidase system, was observed (34). The oral administration of geraniin and the geraniin-containing extract from *Geranium thunbergii* to rats was found to reduce the lipid peroxide concentrations in serum and liver, which was raised by feeding the animals with peroxidized corn oil. The levels of serum cholesterol, GOT, and GPT were also lowered (35).

### 2. Effects on arachidonate metabolism

Inhibition of 5-lipoxygenase in the arachidonate metabolism in rat peritoneal polymorphonuclear leukocytes was observed with comparatively low concentrations of ellagitannins, e.g., geraniin and corilagin (36). The mechanism of this inhibition may have a correlation with that of the inhibition of lipid peroxidation described above.

### 3. Inhibition of autoxidation of ascorbic acid

Autoxidation of ascorbic acid was strongly inhibited by tannins (geraniin and tannic acid), while the inhibitory effects by polyphenols of small molecules were almost negligible (37). The inhibitory effect of geraniin was stronger than that of tannic acid. The superior effect by this ellagitannin may be attributable to the formation of more stable radicals (32).

### 4. Reduction of co-existing substance

Reduction of metallic ions, Cu²⁺, Fe³⁺, and Cr⁶⁺ to Cu⁺, Fe²⁺, and Cr³⁺, occurred in the presence of tannins at room temperature (38). This reducing property of tannins will be attributable to the property of tannins by which they are easily oxidized to presumable quinoids.

### 5. Host-mediated anti-tumor activity of oligomeric ellagitannins

Several oligomeric, hydrolyzable tannins, among the more than fifty of those hitherto isolated, showed strong anti-tumor activity when they were injected intraperitoneally into mice at 4 days before inoculation of Sarcoma 180 cells, while the about 80 tannins of the other types, including polyphenols of small molecules, screened were inactive (7). Among the oligomeric, hydrolyzable tannins, coriariin A (39), rugosin E (28), and oenothein B (27), etc., which are dimeric ellagitannins, showed the strongest anti-tumor activity. Similar effects were observed for other types of tumors including solid type tumors, during experiments with oenothein B. These anti-tumor effects may be due to the enhancement of the immune response of the host animals through the actions on tumor cells and some immunocytes, as shown by several experiments (40). Orally administered oenothein B also inhibited the Ehrlich ascites tumor (40). It is noticeable that this kind of anti-tumor effect was exclusively exhibited by a limited number of oligomeric ellagitannins, including the tetramer.

### 6. Anti-HIV activity

These dimeric ellagitannins also inhibited replication of human immunodeficiency viruses (HIV) (8). Oenothein B, among these ellagitannins, most strongly inhibited the virus growth at the concentrations 1 μg/ml and 10 μg/ml. The investigation of the mechanism of this inhibitory effect indicated that the antiviral activity of these ellagitannins may be ascribable to the inhibition of adsorption of HIV on the cells, and also to other effects such as the inhibition of reverse transcriptase activity (8). Mouse sera taken after the oral administration of oenothein B also inhibited replication of HIV and herpes virus, thus indicating that this dimeric ellagitannin may be effectively used by oral administration (8).

### 7. Inhibition of mutagenicity of carcinogens

Strong inhibitions of the mutagenicity of the carcinogens Trp-P-1, Trp-P-2, MNNG, and also of direct acting mutagens, N-OH-Trp-P-2 and benzopyrene diol epoxide, were exhibited in the Ames test by geraniin and other ellagitannins (41). Positive correlations between the inhibitions of Trp-P-1 and Trp-P-2, and the binding activities of each tannin were observed, while this correlation was reversed in the inhibition of MNNG. Although the inhibition of benzopyrene diol epoxide by ellagic acid was stronger than that by tannins, several tannins, including ellagitannins, showed markedly stronger inhibition for the mutagenicity of N-OH-Trp-P-2 in the absence of enzyme, than that by polyphenols of small molecules in a way analogous to the inhibition for Trp-P-2, indicating that the inhibition of this type of mutagens by tannins is due to a direct action of the tannins (41).

### 8. Inhibition of tumor promotion

In a two-stage carcinogenesis experiment in mouse skin treated with DMBA plus teleocidin, EGCG and penta-O-galloyl-β-d-glucose showed marked inhibitions of tumor growth at the stage of tumor-promotion (42, 43). Inhibition of the activation of protein kinase C induced by teleocidin was observed. EGCG was also found to inhibit duodenum cancer which was produced in a similar way (44). Ellagitannins also inhibited the binding of [³²P]-TPA to a particulate fraction of mouse skin in a preliminary screening of the inhibition of the tumor-promotion (43).

### 9. Other biological activities

Besides the inhibition of reverse transcriptase (8, 45), inhibition and also promotion, by diluted solutions of each tannin, of the activities of the other enzymes to various extents depending on the structure of each tannin, have been observed (46–48). Antiproteolytic activities assayed utilizing CCl₄- and GaIn-induced cytotoxicity in primary cultured rat hepatocytes was revealed (49). Inhibitions of adrenalin- and ACTH-induced lipolysis and also insulin-induced lipogenesis from glucose in fat cells of rat were also found (50–52).
The change in the concept of tannins, initiated by that of ellagitannins, still requires further experimental support including that of absorption and metabolism, and also of the unfavorable effects, for each tannin, now based on pure tannins of determined structures.

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References