# Isolation and structure elucidation of tannins

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Abstract- Chemical examinations show that a new class of tannins designated as 'complex tannin' occurs widely in the plants containing both condensed and hydrolyzable tannins. The complex tannins are shown to contain in the molecules a hydrolyzable tannin moiety connected through a carbon-carbon linkage to flavan-3-ol (flavano-ellagitannin), procyanidin (procyanidino-ellagitannin) and flavonoid glucoside (flavono-ellagitannin) moieties. Some of the significant findings obtained in the fields of hydrolyzable and condensed tannins are also briefly described herein.

### INTRODUCTION

Difficulties in isolating vegetable tannins from an extremely complex mixture have been overcome in recent years by development of new separation procedures based mainly on reverse-phase chromatography, and thereby enormous progress has been made in our understanding of the chemistry of vegetable tannins. In the classical work, tannins were in principle divided on the basis of their chemical properties into two groups; the hydrolyzable and the non-hydrolyzable of condensed. However, recent accumulated data of their structural studies have shown that they may be classified into three major groups based mainly on the structural features rather than on the chemical properties. The third group is now designated here as 'complex tannin', and its isolation and structure are the major subjects of this paper. In addition, some of the significant findings obtained in the fields of condensed and hydrolyzable tannins are briefly discussed herein.

### **COMPLEX TANNIN**

The complex tannin is a series of compounds first isolated from a Fagaceous plant (ref. 1) and now found to occur widely in the plants containing both hydrolyzable and condensed tannins (ref. 2-6). The isolation of these tannins is almost invariably achieved by a combination of Sephadex LH-20 dextran gel (with a mixture of water and methanol) and reverse-phase (such as high-porosity polystyrene, ODS, etc. with a water and methanol mixture) chromatographies. The former chromatography allows separation from more mobile lower-molecular-weight polyphenolics, while the latter is effective for the separation of each structural isomer. The structure elucidation is based on chemical means (hydrolysis, degradation, synthesis, etc.) in conjunction with high-resolution <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy.

More than thirty tannins of this class have so far been isolated and structurally elucidated, and from their structural features, they are further divided into three groups. The most commonly occurring is 'flavano-ellagitannin', which possesses in the molecule a flavan-3-ol, the component unit of condensed tannins, connected to a hydrolyzable tannin moiety through a carbon-carbon linkage. As typical examples, the structures of stenophyllanin A (1)(ref. 1), acutissimin B (2)(ref. 3), mongolicain A (3)(ref. 4) and stenophynin A (4)(ref. 2) are shown in the text.

358 G. NONAKA

The second is 'procyanidino-ellagitannin'. Although only one compound, mongolicanin (5), has so far been known for this type tannin (ref. 6), it is considered to exist rather widely in the plants containing both proanthocyanidins and ellagitannins. The structure of 5 principally follows from thiolytic degradation and subsequent desulfurization to yield acutissimin A (6) and also from the preparation of 5 by condensation of procyanidin B-3 (7) and vescalagin (8).

The 'flavono-ellagitannins', mongolicins A (9) and B (10), isolated from the plant of the genus Quercus (Fagaceae)(ref. 5) have novel structures in which a flavonoid glucoside is linked to an ellagitannin moiety through a carbon-carbon bond. The structures have been established on the basis of analysis of the  $^{1}\text{H}-^{13}\text{C}$  long-range COSY spectra and preparation by condensation of the corresponding flavonoid and ellagitannin. The co-occurrence with (+)-taxifolin 3-0-glucoside and 8 (and castalagin, the C-1 epimer of 8) in the same plant suggests that 9 and 10 are biosynthetically derived by condensation of these compounds.

## HYDROLYZABLE TANNIN

During the past decade, more than two-hundred hydrolyzable tannins and related compounds have been isolated from about fifty plant materials in our laboratory. From their structural work, it is shown that gallic acid is metabolized, in the form of the ester attached to the polyalcohol (mostly D-glucopyranose) moiety, by i) oxidative coupling leading to oligomerization and ii) oxidation of the aromatic ring as shown in Chart 1.

Chart 1. Metabolism of gallic acid

360 G NONAKA

Among various hydrolyzable tannins, those with a dehydrohexahydroxydiphenoyl ester group represent a relatively rare group of compounds which occur rather widely in the plant kingdom, mostly predominating in the plant extracts. The isolation of the novel tannin, elaeocarpusin (11), which possesses an ascorbic acid moiety connected to the dehydrohexahydroxydiphenoyl group, suggests that dehydroascorbic acid is involved in the oxidative metabolism of the hexahydroxydiphenoyl group to the dehydrohexahydroxydiphenoyl group (ref. 7, 8).

### **CONDENSED TANNIN**

Application of the above-mentioned chromatographic procedures has led to the isolation of several oligomeric procyanidins including trimers, tetramers, pentamers and hexamers in their pure forms. As typical examples, the procyanidins isolated from Ceylonese cassia bark (ref. 9) and Areca seed (ref. 10) are shown here. It should be noted that the trimeric proanthocyanidins, cinnamtannins B-1 (12) and D-1 (ref. 11) possess a strongly sweet taste, whereas other compounds taste astringent, suggesting that tannins combine with proteins specifically.

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