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Separation of Oligomeric Proanthocyanidins from Fresh Tea Leaves

N. Savitri Kumar

Institute of Fundamental Studies, Kandy, Sri Lanka
E-mail: nskumar3@gmail.com

A range of polyphenolic compounds, including, catechins, flavanol glycosides and proanthocyanidins have been isolated from tea leaves. Oligomeric and polymeric proanthocyanidins (PAs), also known as condensed tannins and leucocyanidins, are an ubiquitous group of plant phenols and have attracted a great deal of attention in the fields of nutrition, health and medicine because of their potent antioxidant properties. These compounds are also important as plant defense compounds that have a general toxicity towards fungi, yeast and bacteria, and provide protection against insects and large herbivores. PAs are flavan-3-ols in which these units are linked by C-C bonds and have the typical C6-C3-C6 skeleton. In B-type PAs inter-flavanol links are C-C bonds between C4 of one flavanol unit (upper unit) and the C8 or C6 of another (lower unit). A type PAs have two linkage (C-O and C-C) between two of the flavanol units and have been found less frequently in nature. B-type PAs have been isolated from many different plant species while A-type PAs have been reported from fewer natural sources.

The wide distribution of PAs in the plant kingdom, their occurrence as active compounds in many plants and plant-derived extracts used in traditional medicines, and their ability to interact with biological systems have led to the isolation and study of PAs from different plants and plant parts. Interactions of PAs with biological sysmtems may be attributed to the polyphenolic nature of the proanthocyanidins, their anti-oxidant and radical scavenging activities, and their ability to form complexes with metal ions and other biological compounds such as proteins, carbohydrates and alkaloids (Ferreira and Slade 2002).

During our studies PAs were isolated from 70% aqueous extracts of fresh tea leaves, and passed though Sephadex LH-20 and then fractionated further using high-speed counter-current chromatography (HSCCC) using the solvent system Hex-EtOAc-MeOH-water (1/5/1/5). The aqueous phase was used as the mobile phase. Five dimeric proanthocyanidins were isolated from tea by us in one study (Kumar et al., 2009). Four of these were prodelphinidins while one was a procyanidin. PAs with a higher degree of polymerization were not isolated.

In a more recent study, fractions containing tetrameric and trimeric PAs were separated after Sephadex LH-20 chromatography and HSCCC. The separation of higher oligomers was made possible by limiting the amount of aqueous 50% methanol that was used for elution of the Sephadex LH-20 column which resulted in the separation of additional oligomeric PA rich fractions, which were subjected to HSCCC under the same conditions. Electro Spray Ionization Mass Spectrometry (ESI-MS) indicated that the oligomeric proanthocyanidin fractions PA_1 and PA_2 isolated, are tetrameric in nature, while PA_3 was trimeric in nature. ESI-MS evidence suggests the presence of A-type PAs in the PA_1 fraction isolated during the study.

Twenty one PAs including an A-type PA, prodelphinidin A-2,3'-O-galllate, were isolated from an aqueous 80% acetone extract of Oolong tea (Hashimoto et al., 1989). To our knowledge this has been the only previous report of an A-type proanthocyanidin from tea.

References

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