

BIOMIMETIC SYNTHESIS OF ELAEOCARPUSIN

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Abstract — Elaeocarpusin, a tannin-ascorbic acid condensation product, isolated from Geranium thunbergii as one of the water-soluble forms of tannin, has been biomimetically synthesized from geraniin and ascorbic acid, and shown to occur widely in the geraniin-containing species of plants.

Geraniin (1), the crystalline tannin originally found as the main component of Geranium thunbergii Sieb. et Zucc.,¹ and then found in many other species of plants,² is almost insoluble in water once it is isolated, nevertheless geraniin often forms aqueous solutions of high concentration upon the extraction from plants. Similar phenomena are frequently observed for natural organic compounds including tannins. Although participation of some coexisting substances in the solubilization of these compounds is presumed, no proof for such solubilization of tannins has yet been reported.

Upon investigation of water-soluble polyphenolic compounds in the extract of G. thunbergii, we have isolated a compound (2) which is composed of equimolar geraniin and ascorbic acid, and may be regarded as one of the water-soluble forms of geraniin in plant tissues (more than 1 g dissolves in 1 ml of water at 15°C). The mother liquor, from which geraniin and accompanied tannins were separated out, was submitted to the centrifugal partition chromatography (CPC)³ and then to the column chromatography on Sephadex LH-20 and Toyopearl HW-40F, to give 2, $C_{47}H_{34}O_{32} \cdot 5H_2O$, $[\alpha]_D +31^\circ$ (c=1, MeOH), UV λ_{max}^{MeOH} nm (log ϵ), 223 (4.89) and 282 (4.50), FAB-MS m/z 1133 $[M+Na]^+$, 1149 $[M+K]^+$, as a pale brown amorphous powder which showed the positive color reaction of ellagitannins with $NaNO_2$ -AcOH. The presence of geraniin structure as a part of 2 was confirmed by production

of 1 upon keeping 2 in 0.2M $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer (pH 7.0) at 37°C for 6 h. These properties and the ^1H - and ^{13}C -NMR spectral data indicated that 2 is identical with elaeocarpusin which has been presented very recently.^{4,5,6} The analogy to ascorbigen (3),⁷ suggests that elaeocarpusin is biogenetically the product of condensation between ascorbic acid and geraniin. We have carried out the biomimetic synthesis of 2 as follows: When a solution of 1 and L-ascorbic acid was kept in McIlvaine's buffer (pH 4.0) at 37°C for 5 h, production of 2 was recognized by HPLC of normal-phase development.⁸ Then synthesis of 2 in a preparative scale was achieved by keeping a solution of 1 and L-ascorbic acid in the buffer solution as described above, or a mixture of methanol and water, at 37°C for 15 h, and the product (over 60% yield)⁹ was identified with 2 in all respects. This facile biomimetic synthesis, and the consideration of the presence of a large amount of 1 in this plant, support the biogenesis of 2 occurring by either enzymatic or non-enzymatic condensation of geraniin and ascorbic acid in a way shown in Chart 1, in spite of the proposition⁵ that the possible precursors of 2 are dehydroascorbic acid and 1-O-galloyl-2,4;3,6-di-O-hexahydroxydiphenyl- β -D-glucose which has not yet been isolated from natural sources.

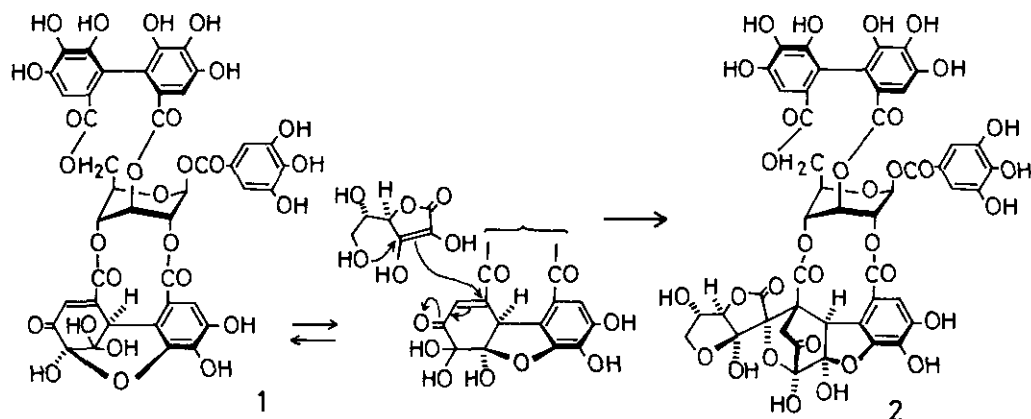
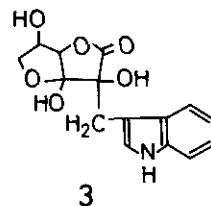


Chart 1

We have also examined the distribution of 2 in other species of Geraniaceae by the HPLC analysis, and found the presence of 2 in the extracts of fresh leaves of *Erodium stephanianum* Willd., *Geranium carolinianum* L., *G. robertianum* L. and *G. yoshinoi* Makino. Therefore elaeocarpusin can be regarded as



a constituent generally present in the geraniin-containing species of plants. This assumption has been further verified by co-occurrence of 1 and 2, as revealed upon HPLC analysis of the extracts of several species of other families: Acer nikoense Maxim., A. buergerianum Mig., A. saccharum Marsh. and A. palmatum Thunb. (Aceraceae); Rhus trichocarpa Mig., R. succedanea L. (Anacardiaceae); Cercidiphyllum japonicum Sieb. et Zucc. (Cercidiphyllaceae).

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9. Yield was calculated after subtracting recovered geraniin.

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