Communications to the Editor

[Chem. Pharm. Bull. 34(2) 941—943 (1986)]

ELAEOCARPUSIN, A PROTO-TYPE OF GERANIIN FROM GERANIUM THUNBERGII

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The co-occurrence of geraniin (2) and a novel ellagitannin, elaeocarpusin (1) in Geranium thunbergii has been demonstrated. In addition, enzymatic transformation of 1 into 2 has been achieved, leading to the evidence that dehydroascorbic acid (4) participates as a co-enzyme in the oxidative metabolism of the hexahydroxydiphenoyl group to the corresponding dehydro group. Furthermore, flavan-3-ol derivatives and gallotannins containing a glucose and a quinic acid core have been isolated, together with previously reported ellagitannins.

KEYWORDS——Geranium thunbergii; Geraniaceae; elaeocarpusin; geraniin; ellagitannin; dehydrohexahydroxydiphenic acid; hexahydroxydiphenic acid; dehydroascorbic acid; enzymatic transformation; oxidative metabolism

Among various hydrolyzable tannins, those with a dehydrohexahydroxydiphenoyl ester group(s) represent a relatively rare group of compounds which occur rather widely in the plant kingdom, mostly predominating in plant extracts.[1] Recent structural studies on hydrolyzable tannins have shown that tannins of this class are formed biosynthetically by the oxidation of a 4,4',5,5',6,6'-hexahydroxydiphenoyl ester group(s) attached to the polyalcohol (mostly D-glucopyranoside) moiety,[2] although details of the dehydrogenation step are not clear. Previously, we isolated an unstable hydrolyzable tannin, elaeocarpusin (1) from the fresh leaves of Elaeocarpus sylvestris var. ellipticus (Elaeocarpaceae), and elucidated its structure as a novel ellagitannin with a unique acid ester group, probably derived enzymatically by a condensation of a hexahydroxydiphenoyl group and dehydroascorbic acid (4).[3] From its structural feature, elaeocarpusin (1) is quite likely a key intermediate in the biosynthesis of geraniin (2) from 1-O-galloyl-2,4;3,6-bis[(R)-hexahydroxydiphenoyl]-β-D-glucose.[3] Furthermore, dehydroascorbic acid seems to play an important role in the enzymatic oxidation of a hexahydroxydiphenoyl ester group to a dehydrohexahydroxydiphenoyl group. The present

communication deals with the isolation of elaeocarpusin (1), together with flavan-3-ols and gallotannins based on a glucose and a quinic acid core, from Geranium thunbergii (Gennoshoko in Japanese)(Geraniaceae) which is shown to be a rich source of ellagitannins containing the dehydrohexahydroxydiphenoyl ester group.[5] It also describes the enzymatic transformation of 1 into 2.

The 80% aqueous acetone extract of the fresh whole plant of G. thunbergii collected at the flowering stage (October) was chromatographed on Sephadex LH-20. Elution with water containing increasing proportions of methanol separated tannin ingredients according to their molecular weights. A mixture of simple gallic acid esters and flavan-3-ol derivatives obtained from earlier fractions was successfully separated by reverse-phase chromatography on MCI-gel CHP 20P and Bondapak C_{18} Porasil B: these were identified as 3-O-, 4-O-, 5-O- and 3,5-di-O-galloylquinic acids,[6] m-digallate,[7] 1,6-di-O-galloyl-β-D-glucose,[8] (-)-epicatechin and (-)-epigallocatechin[9] by comparing their physical and spectral data with those of authentic samples. The fraction consisting of higher-molecular-weight tannins was chromatographed on Sephadex LH-20 with a solvent system of ethanol-water-acetone.[7] Subsequent purificaiton by a combination of Bondapak C_{18} Porasil B and MCI-gel CHP 20P chromatographies with water-methanol (7:3) afforded compound 1 in a relatively high yield (ca. 0.01% from the fresh material), together with previously reported hydrolyzable tannins[5] including geraniin (2).

The structure of compound 1 was readily apparent from its facile decomposition into geraniin (2) during chromatographic separation[3] and also from ^{1}H- and ^{13}C-NMR examinations. The absence of resonances for an olefinic group conjugated with a carbonyl function and the appearance of additional six carbon signals arising from a dehydroascorbic acid moiety suggested that compound 1 is elaeocarpusin. The identity was finally established by comparison of its ^{1}H- and ^{13}C-NMR spectra with those of a sample obtained previously.[3]

Incubation of elaeocarpusin (1) with crude enzyme preparations[10] obtained from fresh leaves and stems of G. thunbergii, afforded geraniin (2) and ascorbic acid (5) which were identified by HPLC analysis (Fig. 1).

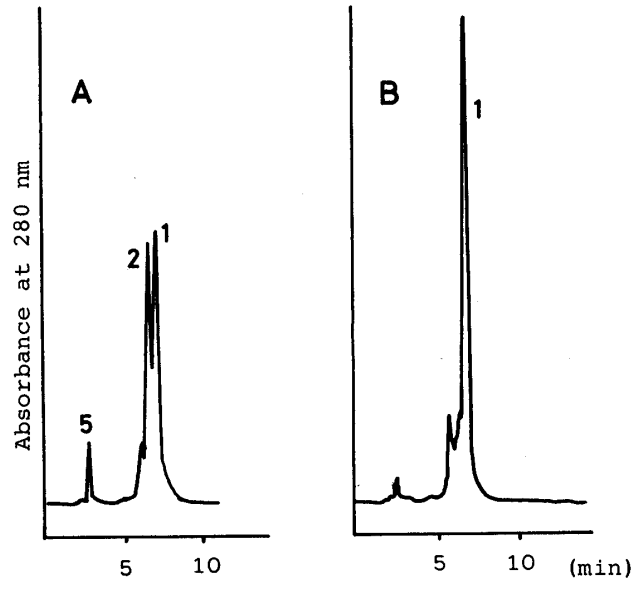

Fig. 1. HPLC of Enzymatic Reaction Products (after 3 h)

A: incubation with crude enzyme solution.

B: incubation with heat-treated crude enzyme solution.

Column: Nucleosil $5C_{18}$.

Solvent: CH_3CN-H_2O (3:17).

Flow rate: 0.75 ml/min.

1: Elaeocarpusin, 2: Geraniin, 5: ascorbic acid.

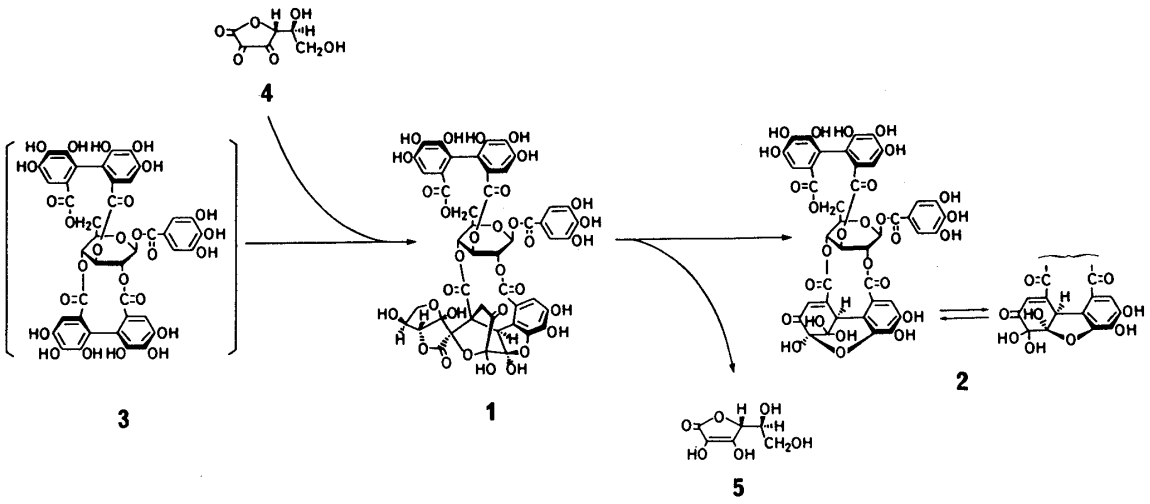

Fig. 2. Possible Biogenesis

The occurrence of elaeocarpusin (1), accompanied by geraniin (2), in G. thunbergii, as well as the enzymatic transformation of 1 into 2 with release of ascorbic acid, supports our earlier prediction[3] that dehydroascorbic acid (4) may be involved in the oxidative metabolism of a hexahydroxydiphenoyl group to a dehydrohexahydroxydiphenoyl group. We have also isolated structurally related ellagitannins in which a similar acyl group is located at different positions in the glucopyranose moiety. Details of their structures will be reported soon.

ACKNOWLEDGEMENTS We thank Mr. Y.Tanaka and Miss K.Soeda for ^{13}C- and ^{1}H-NMR measurements. This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

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(Received December 4, 1985)