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Structure of Genipocyanin G_1, A Spontaneous Reaction Product Between Genipin and Glycine

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A spontaneous reaction of genipin and glycine gave a blue dimeric 1:1 adduct, whose structure was determined as **1** based on instrumental analyses, in particular, nOe experiments in ^{1}H-NMR spectroscopy.

Genipin,[1,2] a major iridoid constituent of *Genipa americana* fruits, is known to form a dark blue pigment upon spontaneous reaction with amino acids or peptides. This particular blue pigment formed from genipin and a mixture of peptides or amino acids has become one of the most important natural blue food dyestuffs. On a commercial base, the blue pigment is produced from an aqueous extract of gardenia fruits (*Gardenia jasminoides*)[3] which contains geniposide, a β-D-glucopyranoside of genipin, by a spontaneous reaction *in situ* with amino acids and peptides (or proteins) after treatment with glucosidase.[4] However the chemical nature of the constituents in the blue dyestuff mixture is not known.[5]

In order to determine the structure of the adduct formed between genipin and amino acids, the simplest amino acid, glycine, was chosen. A stoichiometric study[6] of this spontaneous reaction revealed that equimolar amounts of genipin and the amino acid is required for the reaction at pH 7.0 (80°, 4 hrs); suggesting that the reaction should occur between genipin and amino acid in 1:1 manner. The reaction mixture was purified successively on Diaion HP-20 and ODS columns with aqueous methanol system to give four blue components. Among them, a reasonable amount of the major component, which was named genipocyanin G_1,[7] was obtained in pure form: mp. >280°; λ_{max}(EtOH) 600(log ε 4.79) and 556nm(sh, 4.36), λ_{max}(H_2O) 595(4.64) and 555nm(sh, 4.48); IR(film), 1685, 1600($\nu_{C=C}$, $\nu_{C=N}$) and 1720 cm^{-1}($\nu_{C=O}$). SIMS data, *m/z* 505(M+H), 527(M+Na), 549(M+2Na-H), suggested the molecular formular to be $C_{27}H_{24}O_8N_2$, and therefore, this component should be a dimer[5] of the 1:1 adduct between genipin and glycine. This was confirmed by ^{1}H-NMR studies: Pairs of ester methyl and arylic methyl groups were present as well as a pair of methylene groups belonging to glycine residues. ^{1}H-NMR studies (in CD_3OD and/or D_2O) finally allowed us to conclude the total structure of the pigment as **1** (Fig. 1): in the COSY spectrum, the presence of 2 sets of cross peaks between two broad singlets (in CD_3OD), 2.52 and 7.97, and 2.47 and 7.64 ppm disclosed the long-range couplings

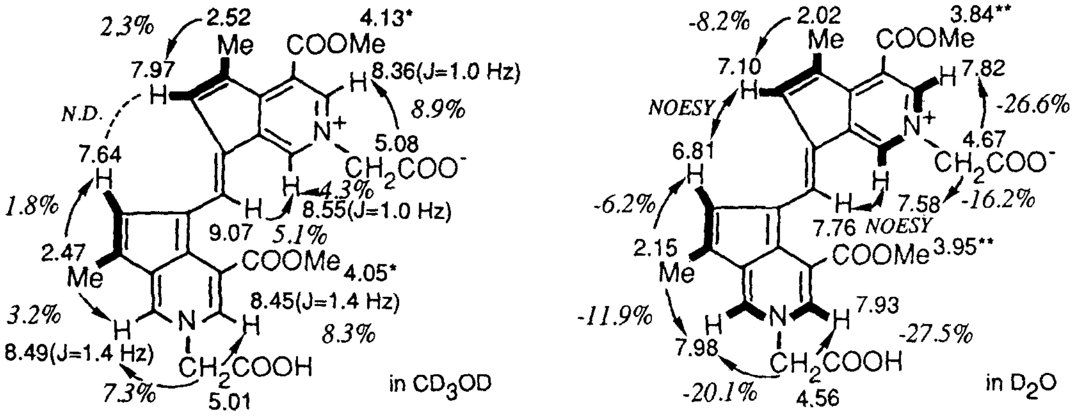

Fig. 1. Structure of Genipocyanin G_1(1) and its ^{1}H-NMR Data.
Another tautomeric form is omitted. Heavy lines denote couplings obtained by COSY experiments.
*,** interchangeable; *N.D.* not determined; *NOESY* obtained by NOESY experiment.

between the aryl methyl and the aromatic proton (mentioned in heavy lines). These four singlets as well as the glycine-methylene groups and flanking aromatic methynes, which could be assigned upon nOe experiments, were good starting points to obtain further connectivities of protons. It is noteworthy that in CD_3OD, the nOe was positive, whereas in D_2O it was negative when measured on a 360 MHz ^{1}H-NMR spectrometer.[8]

From the structure of the pigment, it is obvious that genipin reacted spontaneously as azaphilone does with an amino group to form a nitrogen-iridoid, which undergoes dehydration to form an aromatic monomer. Dimerization occurred at a second stage, perhaps by radical reaction,[5] with or without migration of a methyl group. Further studies on other components are in progress.[9]

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5. Inouye *et al.*, obtained the structure of several non-blue adducts formed between genipin and methylamine, and concluded that the blue pigments formed should have average molecular weight of *ca.* 9000. They have also examined that the polymerization occurred by a radical reaction. H. Inouye, Y. Takeda, K. Inoue, I. Kawamura, M. Yatuzuka, *"26th Symposium on the Chemistry of Natural Product"*, Kyoto(1983), Abstr. pp 577-584.
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7. The suffix, which stands for the originating amino acid, should be followed by the numbers of the compounds.
8. We have expected in the term of association molecular weight; the higher possibility of association in water than in methanol. However, measurement of the association molecular weight on a micro-osmometer did not give a positive result on the molecular weight. So far we have not reached any conclusive result. We are grateful to Shimadzu Corporataion, Kyoto, Japan, for measurement of molecular weight on their OSM-1 instrument.
9. We have obtained another component G_2, which has one aryl methyl group less. However, because of the insolubility of this component in methanol, we could not confirm our tentative structure for this compound.

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