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Studies on the Blue Pigments Produced from Genipin and Methylamine. II.[1] On the Formation Mechanisms of Brownish-Red Intermediates Leading to the Blue Pigment Formation[2]

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The mechanisms of the formation of brownish-red pigments having a 2-methyl-4-carbomethoxy-2-pyrindine nucleus as a basic skeleton by reaction of genipin with methylamine under an atmosphere of inert gas are discussed based on the isolation of 5,6-dihydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine as a precursor and on comparisons of the results obtained from the reactions of genipin congeners and methylamine. The origin of the extra methyl group at C-6 in the structures of some of the brownish-red pigments was clarified to be the carbon atom of the hydroxymethyl group at C-8 of genipin by using deuterium-labelled genipin.

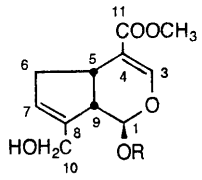
Keywords iridoid; genipin; methylamine; brownish-red pigment; 2-methyl-2-pyrindine formation mechanism; oligomerization mechanism

In basic studies on the blue pigments produced by reaction of genipin (**1**) and primary amines, we recognized the prior formation of brownish-red pigments A—I (**3**—**11**), considered to be precursors, on reaction of genipin (**1**) and methylamine (MA) under an atmosphere of argon and determined their structures, having a unique 2-methyl-4-carbomethoxy-2-pyrindine basic skeleton.[1)] In this paper we discuss the formation mechanism of these brownish-red pigments.

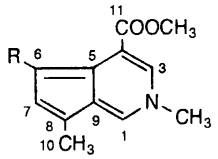
Precursor of Brownish-Red Pigments In the formation of the brownish pigments A—I (**3**—**11**) by reaction of genipin (**1**) and MA under an atmosphere of inert gas, the reaction mixture turned yellow before turning brownish-red. A yellow spot also developed at a higher *Rf* value on silica gel TLC and a yellow substance eluted first when a mixture of the brownish-red pigments was separated by aluminum oxide column chromatography. Based on these observations, the yellow substance was considered to be a precursor of the brownish-red pigments and was subsequently isolated as an amorphous powder. This substance was very unstable and easily turned red during the isolation procedures and even when the NMR spectrum was being recorded. Its structure was elucidated as **12** based on comparisons of the ^{1}H-NMR data (see Experimental) with those of genipin (**1**) and monomeric brownish-red pigments A (**3**), and on the results obtained from internuclear double resonance experiments. When dissolved in 50% aqueous EtOH and stirred for 3 h at 25 °C or 75 °C, **12** gave pigments A—G (**3**—**9**). Although pigments H and I (**10**—**11**) were not obtained in this experiment, **12** might also be a precursor for their formation considering the close similarity of their structures. Thus, the role of **12** as an intermediate in the formation of the brownish-red pigments was established.

Formation Mechanism of Brownish-Red Pigment A (3) To investigate the formation mechanism of **3** *via* **12** from **1** and MA, the reaction of deoxyloganin aglycone (**13**) (7,8-dihydro-10-deoxy derivative of **1**) with MA was examined. Deoxyloganin aglycone (**13**) was allowed to react with MA in a mixture of EtOH and McIlvaine buffer (pH 7.2) at 70 °C under a nitrogen atmosphere to give **14** and **15** as products. Compound (**16**) was obtained when the reaction was effected in 50% aqueous EtOH at 70 °C for 10 min followed by 45 min at the same temperature after the pH of the reaction mixture had been adjusted to pH 9.1 by addition of $NaHCO_3$. The structures of the products were elucidated as **14**—**16** based mainly on analyses of their ^{1}H- and ^{13}C-NMR data (see Experimental). The presumed mechanism for the formation of the compounds (**14**—**16**) is shown in Chart 1. Namely, a presumed intermediate (I) might be formed through the dehydration of **16**, which is formed by a nucleophilic attack by MA on the olefinic carbon atom at C-3 of deoxyloganin aglycone (**13**), followed by opening of the dihydropyran ring and attack by the secondary amino group on the resulting aldehyde group. Under neutral conditions, the intermediate (I) further gave **14** and **15**. The formation of **14** and **15** *via* bimolecular oxidation–reduction of I′ is also a possibility. Based on these considerations, we presumed the formation mechanism for pigment A (**3**) to be as shown in Chart 2. That is, a yellow-colored intermediate (**12**) which corresponds to I (see Chart 1) is formed first, and then the successive removal of a hydride, a proton and a hydroxyl group gives an intermediate (II), to which a hydride adds, affording pigment A (**3**). The formation of II is also possible *via* elimination of the hydroxyl group at C-10 of **12** followed by migration of the double bond, deprotonation and removal of a hydride from C-5 by way of an intermediate (III). Another possible route to **3** would be protonation at C-10 of III followed by migration of the double bond and deprotonation.

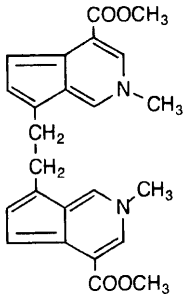
Formation Mechanism of Brownish-Red Pigments B (4)



1 : R=H
2 : R=Glc



3 : R=H
4 : R=CH_3



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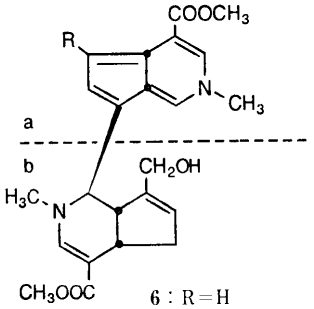
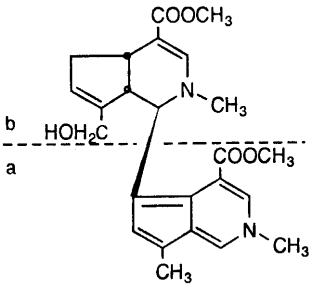


6 : R=H
7 : R=CH_3



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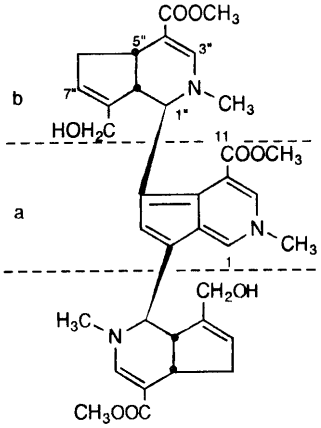

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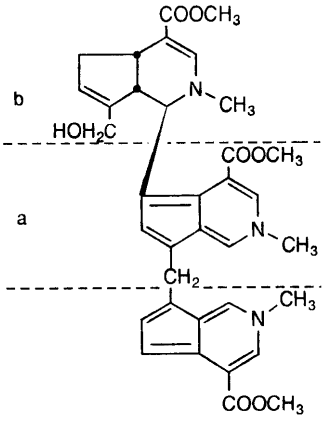

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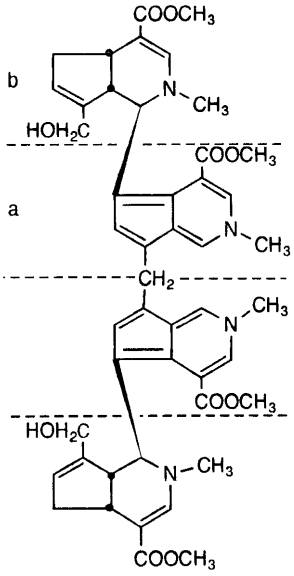

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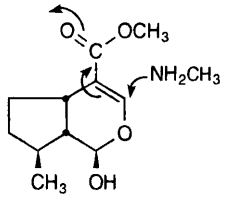

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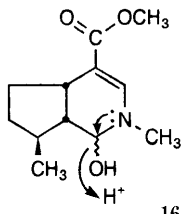

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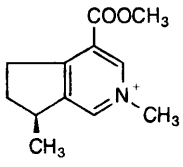

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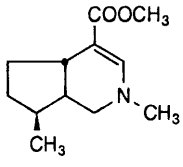

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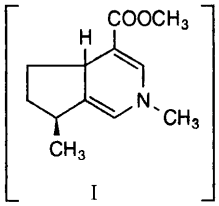
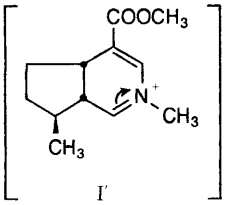


I



I'

Chart 1. Presumed Mechanism for the Formation of Compounds **14**—**16** from Deoxyloganin Aglycone (**13**) and Methylamine

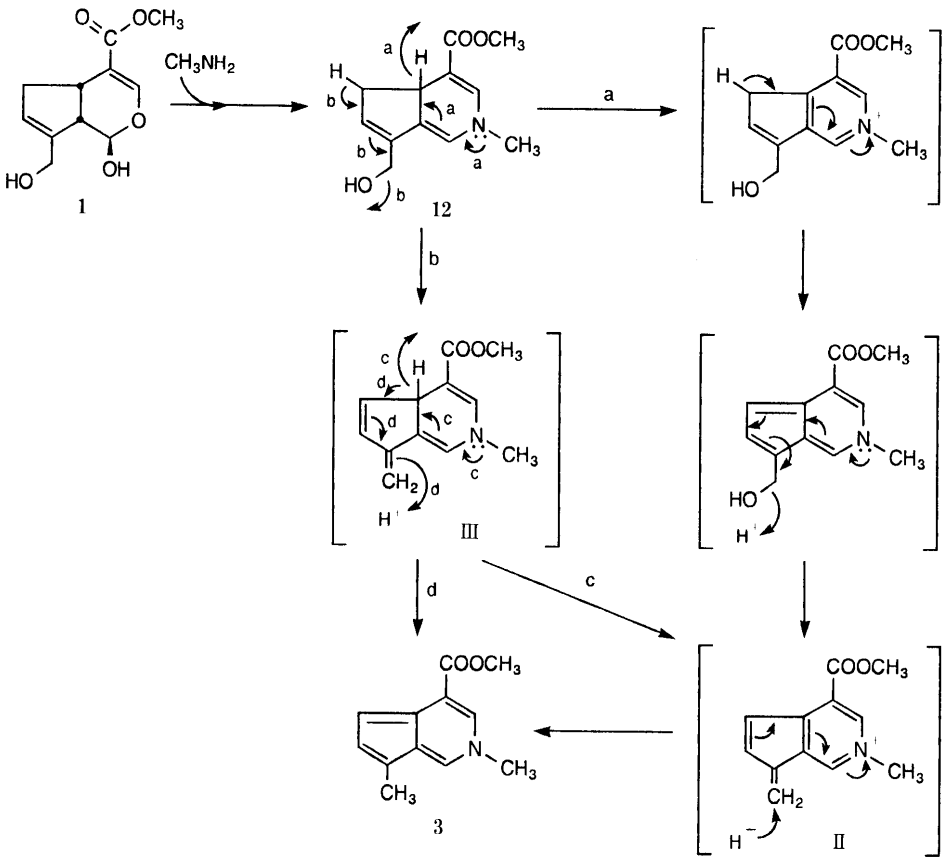


Chart 2. Presumed Mechanism for the Formation of the Pigment (3) from Genipin (1) and Methylamine

and E (7) The brownish-red pigment B (**4**) is unusual in having an extra methyl group at C-6 in the carbon skeleton of **1** and **12**. To investigate its mechanism of formation, we examined the origin of the methyl group at C-6 first, and also that of the corresponding group in the dimeric brownish-red pigment E (**7**). The possibility that the reaction medium might be the source of the methyl group could be easily excluded because a mixture of McIlvanine buffer and EtOH was used as the reaction medium. Considering the structure of genepin (**1**), the hydroxymethyl group at C-8 seemed the most probable candidate as the origin of the C_1 unit, although the methyl groups of the carbomethoxyl group and MA might also be candidates. The above presumption is supported by the structures of **6**, **7** and **9**, which are characterized by lack of the carbon atom of the hydroxymethyl group at C-8 in the 2-methyl-4-carbomethoxy-2-pyrindine portion. To examine these three possibilities, essentially the same reaction was first conducted using ethylamine instead of MA to give the *N*-ethyl derivative (**17**) corresponding to the structure **4**. This excluded the aforementioned possibility. The possibility that the extra methyl group originated from genepin (**1**) was then examined. When [$^2H_1$-carbomethoxy]genipin (**18**) was exposed to the conditions employed for the formation of the brownish-red pigments, the isolated pigment [$^2H_1$]-**4** contained one deuterium in the methyl group of the carbomethoxy group, but not in the methyl group at C-6, as determined from the ^{1}H-NMR spectrum. [10-$^2H_1$]Genipin (**19**) was then used for the formation of the brownish-red pigments and deuterated pigments A—F (**3**—**8**) were isolated. Their ^{1}H-NMR spectra showed the presence of one deuterium in the methyl (or methylene) group at C-8 in all cases, based on the integration of the corresponding signals. In addition, one deuterium was detected in the methyl group at C-6 in $^2H_2$-**4** and $^2H_2$-**7**. These results clearly demonstrated that the extra methyl group at C-6 in **4** and **7** originated from the hydroxymethyl group at C-8 of genipin (**1**). Thus, compound **12** was a precursor and the methyl group at C-6 originated from the hydroxymethyl group at C-8 of genipin (**1**) in the formation of brownish-red pigment B (**4**). These results, together with the presumed formation mechanism of brownish-red pigment A (**3**), suggested the formation mechanism of brownish-red pigment B (**4**) to be as shown in Chart 3. Namely, intermediate (IV), which might be formed by reaction of pigment A (**3**) with II derived from **12** (see Chart 2), suffers heterolytic carbon–carbon bond cleavage to give pigment B (**4**) and V. Compound (V) might have an important role in the formation of oligomeric brownish-red pigments as described later, although this compound was not isolated in the present study.

Formation Mechanism of Oligomeric Brownish-Red Pigments C—I (5—11) As previously mentioned, the formation of oligomeric brownish-red pigments *via* precursor (**12**) was experimentally demonstrated. The tetrahydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine portion in the structures of all the oligomeric pigments D—I (**6**—**11**) corresponds to the dihydro derivative of the precursor **12** and is connected at C-1 with C-6 or C-8 of the 2-methyl-4-carbomethoxy-2-pyrindine portion (structural unit a in the structures **6**—**11**). These

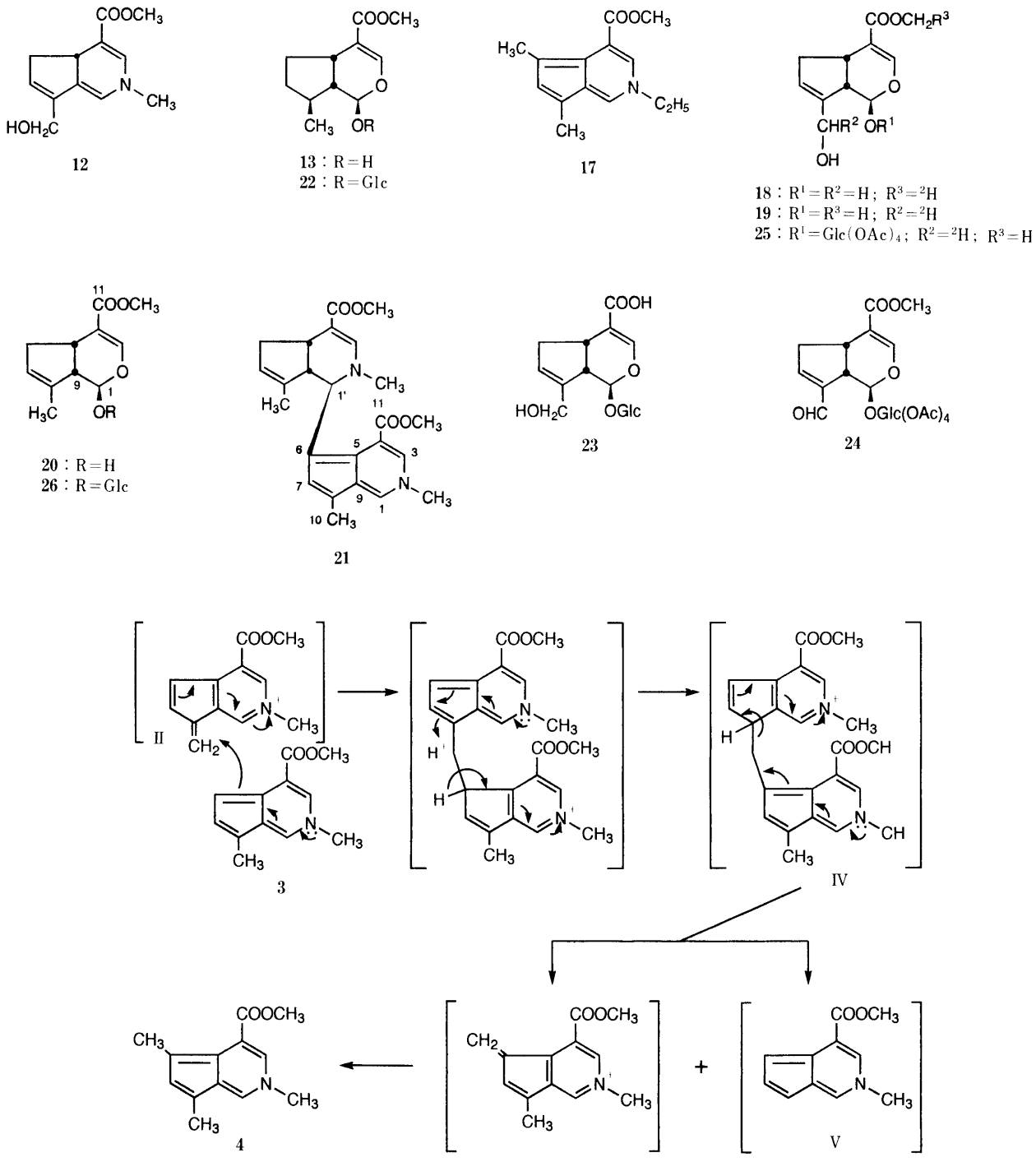
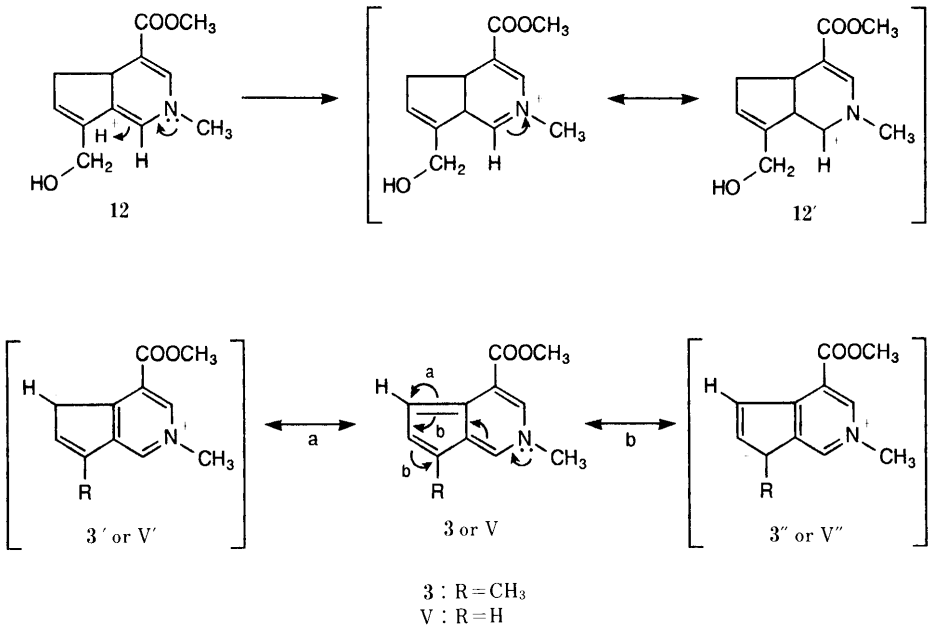


Chart 3. Presumed Mechanism for the Formation of Brownish-Red Pigment (**4**)

structural features could be reasonably explained by bond formation between C-1 of the former and C-6 or C-8 of the latter, because C-1 in **12** will be an electron deficient position and C-6 or C-8 in **3** and V will be electron rich, as shown in Chart 4.

Based on these considerations, we propose the formation mechanism of the pigments D (**6**), E (**7**), F (**8**) and G (**9**) to be as shown in Chart 5. Namely, reaction of **12**′ with **3**′ and V″ gives dimeric pigments F (**8**) and D (**6**) *via* intermediates VI and VII. Pigment D (**6**) further reacts with another molecule of **12**′ to give trimeric pigment G (**9**) *via* intermediate VIII, while it may also react with another molecule of **12** to form an intermediate IX, which undergoes heterolytic bond cleavage as described for the formation of pigment B (**4**) (see Chart 3) to give the dimeric pigment E (**7**).

In the brownish-red pigments C (**5**), H (**10**) and I (**11**), C-10 of one monomer unit is bound to C-10 or C-8 of another monomer unit. These bond formations could be ascribed to the intervention of **12**, III or V. Thus, the presence of a hydroxyl group at C-10 or an equivalent functional group could be considered as an essential structural requirement for the formation of the above-mentioned bonds. To test this presumption, 10-deoxy-genipin (**20**) was exposed to the conditions employed for the brownish-red pigment formation. In this case, compound **21** was obtained together with pigment A (**3**) and the formation of **5**, **10** and **11** was not detected. The re-



3 : R=CH_3
V : R=H

Chart 4. Presumed Resonance Structures of **3**, **12** and V

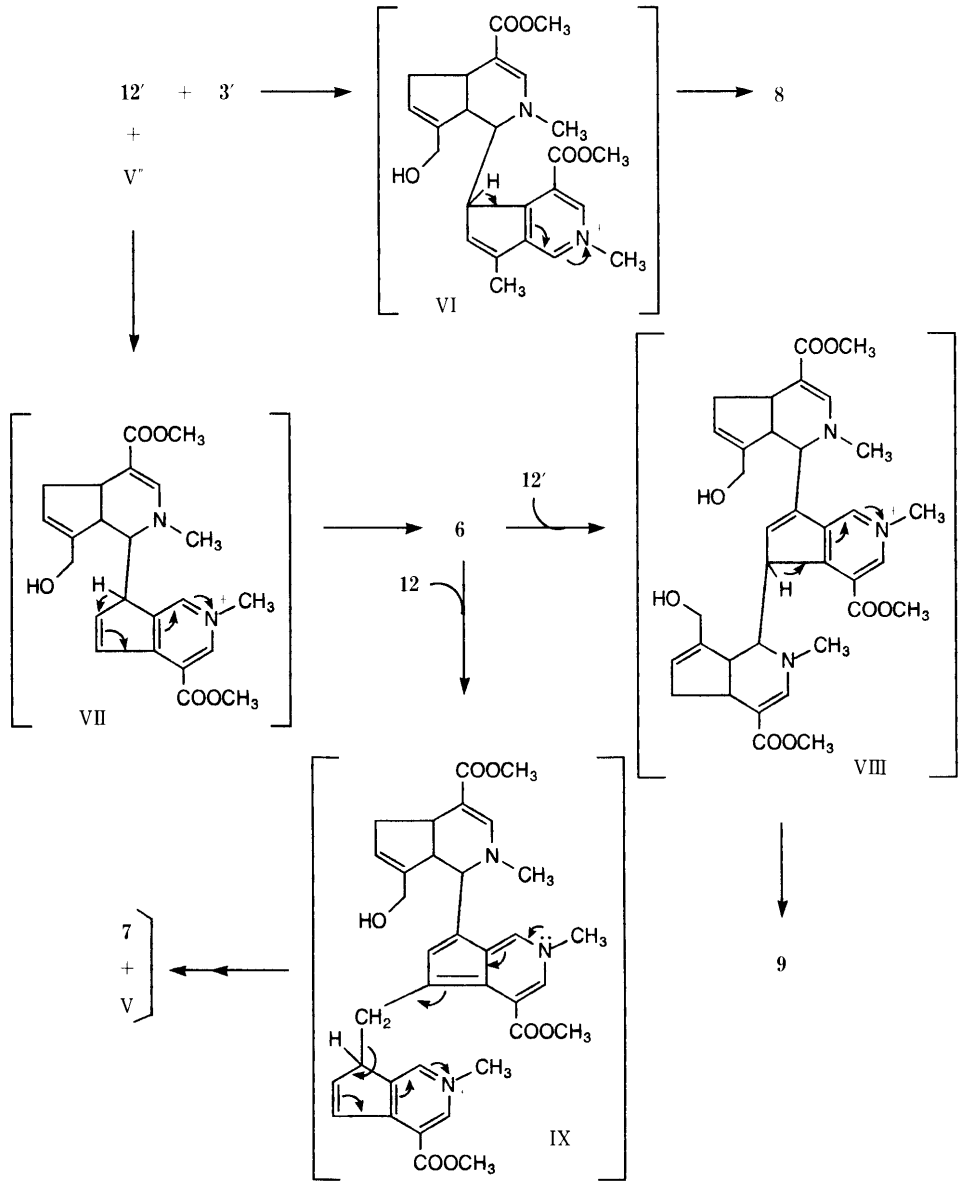


Chart 5. Presumed Mechanism for the Formation of Brownish-Red Pigments (**6**, **7**, **8**, **9**)

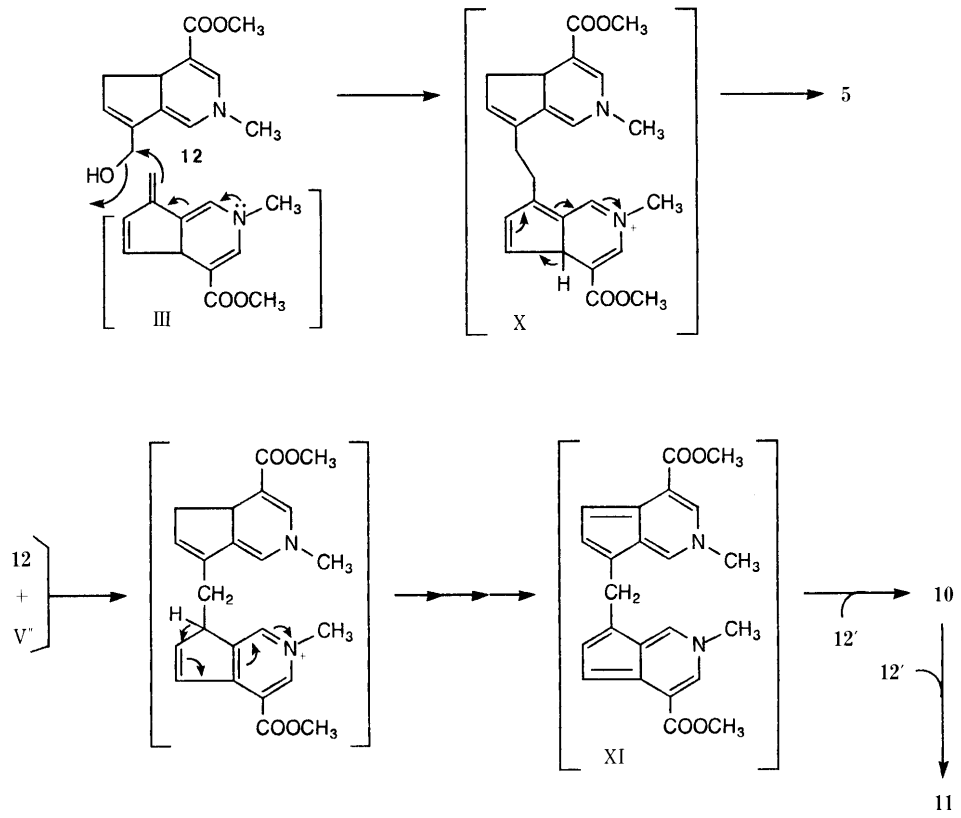


Chart 6. Presumed Mechanism for the Formation of Brownish-Red Pigments (**5**, **10**, **11**)

TABLE I. Mutual Transformation of Pigments **3**, **4**, **6**, **7**, **8** and **9** under an Inert Gas[a] Atmosphere

Pigment [mg]	Temperature	Time (h)	Products [mg]
3 [50]	Reflux	3	—[c]
4 [50]	Reflux	3	—[c]
6 [53][b]	Reflux	3	**3**[d], **9**[d]
7 [67.3]	Stirring at 40 °C	1	**3**[e]
8 [74.2]	Reflux	4	**3** [7], **4** [trace], **9** [4]
9 [100]	Reflux	3	**3** [6], **4** [10], **6** [25], **8** [5]

a) Reactions were carried out in 50% aqueous EtOH under an argon atmosphere unless otherwise noted. *b*) Reaction performed under a nitrogen atmosphere. *c*) Only the starting material was recovered. *d*) Yields not determined. *e*) Formation detected only by TLC.

sults clearly showed that bond formation through C-10 and the introduction of the carbon atom at C-10 into C-6 cannot take place if a hydroxyl group is absent at C-10 in the substrate. We therefore presumed the formation mechanism of dimeric pigment C (**5**), trimeric pigment H (**10**) and tetrameric pigment I (**11**) to be as shown in Chart 6. Symmetrical dimeric pigment C (**5**) could be formed by dehydrogenation of X which is formed by the attack of C-10 of III on C-10 of **12** with elimination of the hydroxyl group. On the other hand, an attack by V'' on C-10 of **12** in the same manner would yield an intermediate XI, which would react with **12'** at the electron-rich C-6 position to form the trimeric brownish-red pigment G (**10**). The pigment (**10**) would further condense with another molecule of **12'** to give the tetrameric brownish-red pigment I (**11**).

Mutual Conversion of Brownish-Red Pigments Finally, to examine the sequence of formation of the brownish-red pigments, pigments **3**, **4**, **6**, **7**, **8** and **9** were each heated in 50% aqueous EtOH under an atmosphere of inert gas. The results are summarized in Table I. Pigments **3** and **4** never changed, while pigments **6** and **7** gave **3** and **9**, and a trace amount of **3**, respectively. Pigment **8** gave **3**, **9** and a trace amount of **4**, and pigment **9** gave **3**, **4**, **6** and **8**. The results support the conclusion that the presence of a hydroxyl group at C-10 is essential for the formation of polymeric (oligomeric) brownish-red pigment having a connection through C-10. They also suggest that other routes besides those shown in the Charts, including degradation of the polymeric (oligomeric) pigments and re-combination of the degradation products, exist for the formation of the brownish-red pigments.

Experimental

General procedures used throughout this work were essentially the same as described in the preceding paper.[1]

Isolation of a Yellow Intermediate (12) A solution of genipin (**1**) (904 mg) and MA·HCl (808 mg) in a mixture of McIlvaine buffer (pH 7.2) (35 ml) and EtOH (10 ml) was stirred for 2 h at room temperature (25 °C) under an atmosphere of argon. The mixture was concentrated *in vacuo* to remove EtOH, and the aqueous solution was extracted with $CHCl_3$ (50 ml × 3); the extract was washed with H_2O, dried, and concentrated *in vacuo* to give a residue (1.21 g), which was chromatographed over aluminum oxide (80 g). Elution was conducted with $CHCl_3$ (450 ml), $CHCl_3$–EtOH (49 : 1, 150 ml) and $CHCl_3$–EtOH (19 : 1, 100 ml) successively. The eluates were collected as 50 ml fractions. The residue (331 mg) from fraction Nos. 1—6 was separated by preparative layer chromatography (PLC) (silica gel, solvent: Et_2O). A very unstable yellow substance (**12**) (24 mg) was isolated from the second most polar band among the five bands. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.12 (1H, d, J = 1.2 Hz, H-3), 6.01 (1H, d, J = 1.2 Hz, H-1), 5.80 (1H, m, H-7), 4.37 (2H, br s, H_2-10), 3.70 (3H, s, $-COOCH_3$), 3.30 (1H, m, H-5), 3.15 (3H, s, N–CH_3), 3.00 (1H, br dd, J = 16.8, 8.3 Hz, H_1-6), 2.25 (1H, br dd, J = 16.8, 9.0 Hz, H_1-6).

Formation of Brownish-Red Pigments (3—9) from Yellow Intermediate (12) Yellow intermediate (**12**) (70 mg) was dissolved in aqueous EtOH (1 : 1, 40 ml) and the solution was heated at 75 °C for 3 h under an atmosphere of argon. The reaction mixture was worked up and separated as described in the preceding paper[1] to give brownish-red pigments **3** (8 mg), **4** (15 mg), **5** (2 mg), **6** (12 mg), **7** (5 mg) and **9** (12 mg). When the yellow intermediate (**12**) (20 mg) was similarly treated, but at 25 °C, it gave brownish-red pigment **8** (5 mg) together with the brownish-red pigments **3**, **4** and **5**.

Deoxyloganin Aglycone (13) Deoxyloganin (**22**)[5] (24.0 g) was dissolved in citrate buffer (0.1 M, pH 5.0) (450 ml). The solution was incubated with cellulase (Amano Medicine Co., Ltd.) (360 mg) at 45 °C for 70 h. After removal of the precipitates by filtration, the filtrate was extracted with $CHCl_3$ (300 ml × 3); the extract was washed with saturated NaCl aqueous solution, dried, and evaporated *in vacuo* to give an oily residue (12.2 g), which was subjected to silica gel (200 g) chromatography with a mixture of toluene–Et_2O (1 : 1). Fractions of 100 ml were collected. Fraction Nos. 4—6 were combined and the solvent was removed *in vacuo* to give deoxyloganin aglycone (**13**) (8.731 g) as a colorless oil. IR ν_{max} ($CHCl_3$) cm^{-1}: 3350, 1695, 1630, 1605 (sh), 1445. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.43 (1H, d, J=1.2 Hz, H-3), 4.90 (1H, d, J=6.8 Hz, H-1), 3.72 (3H, s, $COOCH_3$), 2.87 (1H, br q, J=8.1 Hz, H-5), 2.25 (1H, m, H_1-6), 2.09—1.80 (2H, m, H_1-6, H_1-7), 1.65 (1H, ddd, J=8.3, 7.1, 4.1 Hz, H-9), 1.40—1.10 (2H, m, H_1-7, H-8), 1.11 (3H, d, J=6.6 Hz, H_3-10). HREIMS m/z: 212.1045 $(M)^+$. Calcd for $C_{11}H_{16}O_4$: 212.1049.

Reaction of Deoxyloganin Aglycone (13) with MA · HCl i) A solution of MA·HCl (3.16 g) in H_2O (10 ml) was added to a solution of deoxyloganin aglycone (**13**) (5.13 g) in a mixture of McIlvaine buffer (pH 7.2) (130 ml) and EtOH (50 ml), and the mixture was heated at 70 °C for 2.5 h under an atmosphere of nitrogen. The mixture was concentrated *in vacuo* to remove EtOH and the aqueous solution was extracted with $CHCl_3$ (150 ml × 3); the extract was washed with H_2O, dried, and evaporated *in vacuo* to give a yellow residue (2.99 g), which was purified by silica gel (60 g) chromatography. Elution was conducted with Et_2O–petroleum ether (2 : 3, 200 ml) and Et_2O–petroleum ether (1 : 1, 200 ml) successively. The eluate with the latter solvent was concentrated *in vacuo* to give **14** (1.76 g) as an oil. The aqueous layer (pH 6.1) was concentrated *in vacuo* and the residue was separated into $CHCl_3$-soluble and insoluble fractions. The $CHCl_3$-soluble fraction was concentrated *in vacuo* to give a residue (520 mg), and aliquot (260 mg) of which was separated by PLC [silica gel, solvent: $CHCl_3$–MeOH (7 : 3)] to give **15** (93 mg) as an amorphous powder, which showed an *Rf* value of 0.35.

14: UV λ_{max} (EtOH) 295 nm (log ε: 4.24). IR ν_{max} ($CHCl_3$) cm^{-1}: 1680, 1620. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.32 (1H, br s, H-3), 3.67 (3H, s, $COOCH_3$), 2.94 (3H, s, N–CH_3), 2.92 (1H, ddd, J=12.2, 5.4, 1.0 Hz, H-1β), 2.78 (1H, br dt, J=9.0, 7.3 Hz, H-5), 2.63 (1H, dd, J=12.2, 9.7, H-1α), 2.29—2.19 (1H, m, H_1-6), 1.95—1.82 (1H, m, H_1-7), 1.80—1.60 (2H, m, H-8, H-9), 1.30—1.11 (2H, m, H_1-6, H_1-7), 1.06 (3H, d, J=6.6 Hz, H_3-10). ^{13}C-NMR (50.1 MHz, $CDCl_3$) δ: 169.33 (s, C-11), 146.17 (d, C-3), 99.49 (s, C-4), 50.38 (q, OCH_3), 49.36 (t, C-1), 43.25 (d, C-9), 42.67 (q, N–CH_3), 36.93 (d, C-5), 34.61 (d, C-8), 33.84 (t, C-7), 32.40 (t, C-6), 21.58 (q, C-10). HREIMS m/z: 209.1415 $(M)^+$. Calcd for $C_{12}H_{19}N_1O_2$: 209.2884.

15: UV λ_{max} (EtOH) nm (log ε): 272 (3.34), 280 (3.30). IR ν_{max} ($CHCl_3$) cm^{-1}: 1730, 1645. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 9.92 (1H, br s, $W_{1/2}$=3.3, H-3), 9.45 (1H, br s, $W_{1/2}$=2.8 Hz, H-1), 4.84 (3H, s, N-CH_3), 4.02 (3H, s, $COOCH_3$), 3.59 (1H, ddd, J=20.3, 8.8, 4.4 Hz, H-6eq), 3.58 (1H, tq, J=8.4, 7.0 Hz, H-8), 3.36 (1H, ddd, J=20.3, 8.8, 8.4 Hz, H-6ax), 2.56 (1H, dtd, J=12.8, 8.4, 4.4 Hz, H-7eq), 1.84 (1H, dq, J=12.8, 8.4 Hz, H-7ax), 1.51 (3H, d, J=7.0 Hz, H_3-10). ^{13}C-NMR (50.1 MHz, $CDCl_3$) δ: 165.73 (s, C-11), 162.42 (s, C-5), 151.57 (s, C-9), 145.07 (d, C-1), 143.79 (d, C-3), 125.81 (s, C-4), 53.44 (q, $COOCH_3$), 48.82 (q, N–CH_3), 38.31 (d, C-8), 33.62 (t, C-6 and 7), 19.68 (q, C-10).

ii) A solution of MA·HCl (942 mg) in H_2O (5 ml) was added to a solution of deoxyloganin aglycone (**13**) (2.484 g) in a mixture of EtOH–H_2O (1 : 1) (70 ml), and the mixture was heated at 70 °C for 10 min under an atmosphere of nitrogen. After addition of $NaHCO_3$ (1.07 g) the mixture was further reacted under the same conditions for 45 min, then concentrated *in vacuo* to remove EtOH. The aqueous solution was extracted with $CHCl_3$ (70 ml × 3); the extract was washed with saturated NaCl aqueous solution, dried, and evaporated *in vacuo* to give a yellow residue (2.62 g), an aliquot (270 mg) of which was separated by PLC [silica gel, solvent: Et_2O–petroleum ether–NH_4OH (10 : 10 : 0.1)] to give crude **16** (83 mg) (*Rf* 0.1). The crude **16** was further purified by Lobar column (Li Chroprep RP-18 size A) chromatography with CH_3CN–H_2O (1 : 1) to give pure **16** (19 mg) as a colorless oil. UV λ_{max} (EtOH) 283 nm (log ε: 4.34). IR λ_{max} ($CHCl_3$) cm^{-1}: 3400, 1675, 1625, 1610 (sh), 1535, 1505. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.12 (1H, br s, H-3), 4.38 (1H, br s, H-1), 3.66 (3H, s, $COOCH_3$), 3.02 (3H, s, N–CH_3), 2.91 (1H, m, H-5), 2.11 (1H, m, H_1-6), 1.90—1.53 (3H, m, H_1-7, H-8, H-9), 1.25—1.00 (2H, m, H_1-7, H_1-6), 1.04 (3H, d, J=6.1 Hz, H_3-10). ^{13}C-NMR (50.1 MHz, $CDCl_3$) δ: 168.62 (s, C-11), 143.69 (d, C-3), 102.07 (s, C-4), 81.21 (d, C-1), 52.98 (d, C-9), 50.60 (q, $COOCH_3$), 39.94 (q, N–CH_3), 36.17 (d, C-5), 32.47 (t, C-6), 32.47 (d, C-8), 31.62 (t, C-7), 20.07 (q, C-10).

Reaction of Genipin (1) with Ethylamine Hydrochloride A solution of genipin (**1**) (904 mg) and ethylamine hydrochloride (450 mg) in a mixture of McIlvaine buffer (pH 7.2) (30 ml) and EtOH (30 ml) was stirred for 2 h at 70 °C under an atmosphere of nitrogen. The mixture was concentrated *in vacuo* to remove EtOH and the aqueous solutionwas extracted with $CHCl_3$ (50 ml × 3); the $CHCl_3$ extract was washed with H_2O, dried, and evaporated *in vacuo* to give a brownish-red residue (580 mg), which was subjected to silica gel (15 g) chromagography. The column was washed with $CHCl_3$ (500 ml). The material (180 mg) eluted with $CHCl_3$ was further purified by PLC (silica gel, solvent: Et_2O) to give **17** (95 mg). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 7.56 (1H, d, J=2.0 Hz, H-1), 7.38 (1H, d, J=2.0 Hz, H-3), 6.90 (1H, m, H-7), 3.89 (3H, s, –$COOCH_3$), 3.87 (2H, q, J=8.0 Hz, –N$\underline{CH_2}CH_3$), 2.48 (3H, s, CH_3-6), 2.40 (3H, d, J=1.0 Hz, H_3-10), 1.37 (3H, t, J=8.0 Hz, –NCH$_2\underline{CH_3}$). EIMS m/z: 231 $(M)^+$.

[*carbomethoxy*-$^2H_1$]Genipin (18) Geniposide (**2**) (1.5 g) was dissolved in 0.5 N NaOH aqueous solution (30 ml) and the solution was stirred for 3 h at room temperature, then neutralized with Amberlite IR 120 (H^+-form). The ion exchange resin was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was treated with active charcoal (2 g) in MeOH. After removal of the active charcoal by filtration, the filtrate was evaporated *in vacuo* to give geniposidic acid (**23**)[6] (1.32 g). A solution of **23** (500 mg) in a mixture of D_2O (2 ml) and anhydrous dioxane (3 ml) was treated dropwise with diazomethane ethereal solution until a yellow color persisted. After 20 min, a few drops of AcOH was added and the solution was concentrated *in vacuo* to give a residue (500 mg), which was purified by PLC [silica gel, solvent: $CHCl_3$–MeOH (8 : 2); *Rf*=0.4] to give [*carbomethoxy*-$^2H_1$]geniposide (**2**) (390 mg). A solution of [*carbomethoxy*-$^2H_1$]-**2** (1.5 g) in acetate buffer (0.2 M, pH 4.8) (20 ml) was incubated with β-glucosidase (P. L. Chemicals Inc.) (100 mg) at 37 °C overnight. The reaction mixture was extracted with Et_2O (30 ml × 3); the extract was washed with saturated NaCl aqueous solution, and concentrated *in vacuo* to give a residue, which was recrystallized from a mixture of Et_2O and petroleum ether to give [*carbomethoxy*-$^2H_1$]genipin (**18**) (480 mg) as colorless needles, mp 120—121 °C. The ^{1}H-NMR spectrum of this substance was identical with that of authentic sample except for the intensity of the signal of the carbomethoxy group [δ 3.70 (2H, s)]. EIMS m/z: 227 $(M)^+$.

[10-$^2H_1$]Genipin (19) A solution of NaB^2H_4 (257 mg) dissolved in H_2O (1.5 ml) was added dropwise to a solution of 10-dehydro-geniposide tetraacetate (**24**)[7] (1.3 g) dissolved in dioxane (86 ml) under ice cooling, and the mixture was stirred for 30 min at room temperature. After addition of a few drops of AcOH, the resulting precipitates were filtered off and the filtrate was concentrated *in vacuo* to give a residue, which was extracted with $CHCl_3$ (50 ml × 3). The extract was washed with H_2O, dried, and evaporated *in vacuo* to give a residue, which was recrystallized from a mixture of Et_2O and petroleum ether to give [10-$^2H_1$]geniposide 2′,3′,4′,6′-tetraacetate (**25**) (900 mg) as colorless needles, mp 118—119 °C. A solution of **25** (900 mg) in anhydrous MeOH (10 ml) was treated with 0.1 N MeONa methanolic solution (2.0 ml) and the mixture was refluxed for 15 min then neutralized with Amberlite IRC 50 (H^+-form). The ion exchange resin was removed by filtration, and the filtrate was concentrated *in vacuo* to give [10-$^2H_1$]geniposide (**2**) (750 mg), which was treated with β-glucosidase as described above to give [10-$^2H_1$]genipin (**19**) (252 mg) as colorless needles, mp 119—121 °C. The ^{1}H-NMR spectrum of this compound was identical with that of an authentic sample except for the intensity of the signal of the hydroxymethyl group at C-8 [δ 4.22 (1H, br s)]. EIMS m/z: 227 $(M)^+$.

Reaction of [*carbomethoxy*-$^2H_1$]Genipin (18) with MA · HCl A solution of [*carbomethoxy*-$^2H_1$]genipin (**18**) (454 mg) and MA·HCl (134 mg) in a mixture of McIlvaine buffer (pH 7.2) (35 ml) and EtOH (10 ml) was stirred for 3 h at 25 °C under an atmosphere of argon. The mixture was concentrated *in vacuo* to remove EtOH and the aqueous solution was extracted with $CHCl_3$ (50 ml × 3); the extract was washed

with H_2O, dried, and evaporated *in vacuo* to give a residue (450 mg), which was chromatographed over aluminum oxide (40 g) with mixtures of $CHCl_3$ and EtOH containing increasing amounts of EtOH. Based on the results of TLC analyses, the fractions showing the same *Rf* value as authentic **4** were combined and evaporated *in vacuo* to give [*carbomethoxy*-$^2H_1$]-**4** (50 mg). This substance showed an identical ^{1}H-NMR ($CDCl_3$) spectrum with that of an authentic sample except for the signal intensity of the carbomethoxy group [δ 3.92 (2H, s, $-COOCH_2{}^2H_1$)]. EIMS *m/z*: 218 $(M)^+$.

Reaction of [10-$^2H_1$]Genipin (19) with MA·HCl A solution of [10-$^2H_1$]genipin (**19**) (1.480 g) and MA·HCl (450 mg) in McIlvaine buffer (pH 7.2) (50 ml) and EtOH (50 ml) was stirred at 70 °C for 2 h under an atmosphere of argon. The reaction mixture was treated essentially in the same way as above to give a mixture of brownish-red pigments (1.410 g) which was separated as described in the preceding paper[1] to give [$^2H_1$]-**3** (38 mg), [$^2H_2$]-**4** (58 mg), [$^2H_2$]-**5** (21 mg), [$^2H_1$]-**6** (12 mg), [$^2H_2$]-**7** (16 mg), [$^2H_2$]-**8** (56 mg). The ^{1}H-NMR spectra were essentially the same as those of the non-labelled compounds except for the signals described below.

[$^2H_1$]-**3**, ^{1}H-NMR δ: 2.47 (2H, m, $CH_2{}^2H_1$-10). EIMS *m/z*: 204 $(M)^+$.

[$^2H_2$]-**4**, ^{1}H-NMR δ: 2.41, 2.46 (each 2H, br s, $CH_2{}^2H_1$-6, $CH_2{}^2H_1$-10). EIMS *m/z*: 219 $(M)^+$.

[$^2H_2$]-**5**, ^{1}H-NMR δ: 4.51 (1H, br s, C^2H_1H-10). EIMS *m/z*: 406 $(M)^+$.

[$^2H_1$]-**6**, ^{1}H-NMR δ: 3.34 (1H, m, C^2H_1H-10′). EIMS *m/z*: 411 $(M)^+$.

[$^2H_2$]-**7**, ^{1}H-NMR δ: 3.36 (1H, m, C^2H_1H-10′), 2.46 (2H, br s, $CH_2{}^2H_1$-6). EIMS *m/z*: 426 $(M)^+$.

[$^2H_2$]-**8**, ^{1}H-NMR δ: 3.65 (1H, br s, C^2H_1H-10′), 2.42 (2H, br s, $CH_2{}^2H_1$-10). EIMS *m/z*: 426 $(M)^+$.

10-Deoxygenipin (20) 10-Deoxygeniposide (**26**)[8] (2 g) was treated with β-glucosidase as described above to give 10-deoxygenipin (**20**) (570 mg) as a colorless syrup. IR ν_{max} ($CHCl_3$) cm^{-1}: 3350, 2890, 1680, 1620, 1595 (sh), 1430. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.50 (1H, d, J=1.0 Hz, H-3), 5.53 (1H, m, H-7), 5.03 (1H, d, J=6.6 Hz, OH-1), 4.86 (1H, dd, J=8.1, 6.6 Hz, H-1), 3.72 (3H, s, $-COOCH_3$), 3.14 (1H, qd, J=8.3, 1.0 Hz, H-5), 2.77 (1H, m, H_1-6), 2.38 (1H, br t, J=7.6 Hz, H-9), 1.99 (1H, m, H_1-6). HREIMS *m/z*: 210.0886 $(M)^+$. Calcd for $C_{11}H_{14}O_4$: 210.0892.

Reaction of 10-Deoxygenipin (20) with MA·HCl A solution of 10-deoxygenipin (**20**) (66 mg) and MA·HCl (32 mg) in a mixture of McIlvanine buffer (pH 7.2) (7 ml) and EtOH (7 ml) was stirred at 70 °C for 2.6 h under an atmosphere of nitrogen. The mixture was concentrated *in vacuo* to remove EtOH and the aqueous solution was extracted with $CHCl_3$ (20 ml × 3); the extract was washed with H_2O, dried, and evaporated *in vacuo* to give a brownish-red residue (54 mg), which was chromatographed over aluminum oxide (5 g) with Et_2O to give **3** (28 mg) and **21** (17 mg).

21: UV λ_{max} (EtOH) 297 nm (log ε: 4.50). IR ν_{max} ($CHCl_3$) cm^{-1}: 1715, 1660, 1615, 1595 (sh). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.69 (1H, d, J=1.7 Hz, H-3), 7.62 (1H, s, H-3′), 7.47 (1H, d, J=1.7 Hz, H-1), 7.03 (1H, br d, J=1.0 Hz, H-7), 5.36 (1H, m, 7′-H), 4.91 (1H, d, J=7.1 Hz, H-1′), 3.90 (3H, s, $-C_{11}OOCH_3$), 3.85 (3H, s, $-C_{11'}OOCH_3$), 2.77 (3H, s, N_2-CH_3), 3.08 (1H, br q, J=6.8 Hz, H-5′), 2.77 (3H, s, $N_{2'}-CH_3$), 2.69 (1H, br t, J=7.3 Hz, H-9′), 2.70—2.55 (1H, m, H-6′), 2.42 (3H, d, J=1.0 Hz, H_3-10), 2.23—2.08 (1H, m, H-6′), 1.25 (3H, q-like, H_3-10′). ^{13}C-NMR (50.1 MHz, $CDCl_3$) δ: 169.50 (s, C-11′), 167.65 (s, C-11), 148.26 (d, C-3′), 142.62 (s, C-8′), 132.62 (d, C-7), 129.14 (d, C-1), 128.44 (s, C-5), 126.83 (d, C-3), 125.52 (d, C-7′), 118.27 (s, C-6, 9), 116.93 (s, C-4), 114.89 (s, 8-C), 98.32 (s, C-4′), 54.49 (d, C-1′), 52.42 (d, C-9′), 51.96 (q, $C_{11}OOCH_3$), 50.26 (q, $C_{11'}OOCH_3$), 44.22 (q, N_2-CH_3), 40.87 (q, $N_{2'}-CH_3$), 39.02 (t, C-6′), 36.54 (d, C-5′), 15.59 (q, C-10′), 11.94 (q, C-10). HREIMS *m/z*: 408.2048 $(M)^+$. Calcd for $C_{24}H_{28}N_2O_4$: 408.2048.

Mutual Conversion of Brownish-Red Intermediates Brownish-red pigments, **3** (50 mg), **4** (50 mg), **6** (53 mg), **7** (67 mg), **8** (74 mg) and **9** (100 mg), were each dissolved in 50% aqueous EtOH and held under an atmosphere of inert gas. The reaction conditions and the results are summarized in Table I.

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