Studies on the Blue Pigments Produced from Genipin and Methylamine. I. Structures of the Brownish-Red Pigments, Intermediates Leading to the Blue Pigments[1)]

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During the course of studies on the blue pigment formation by the reaction of genipin with methylamine, nine red to brownish-red intermediary pigments were obtained under conditions excluding oxygen. They were identified as monomer, dimer, trimer and tetramer of 2-methyl-4-carbomethoxy-2-pyrindine derivatives on the basis of spectroscopic evidence.

Keywords genipin; iridoid; methylamine; pigment; 2-methyl-2-pyrindine; pseudoazulene

Genipin (**1**), an iridoid constituent of *Genipa americana* fruit, is reported to produce an indelible bluish-violet color on the skin and to form blue pigments readily upon spontaneous reaction with amino acids (*e.g.* glycine, leucine and glutamic acid).[4,5)] It was further found by our group that primary amines, including even proteins, can undergo this reaction, but that neither secondary nor tertiary amines are involved, and that oxygen is indispensable for the blue color formation.[6)] This spontaneous reaction is practically applied in the food industry for the production of a coloring agent[7)]: in brief, a food dye is produced from the aqueous extract of *Gardenia jasminoides* fruits (which contain a large amount of geniposide, **2**,[8,9)] a β-glucoside of **1**), cellulase of *Trichoderma viride* and L-arginine as the primary amine. In spite of its importance, however, the structure and the formation mechanism of the pigment have not been clarified. This situation prompted us to undertake basic studies on this pigment.

At first we tried to react genipin (**1**) with methylamine, the simplest primary amine, in aqueous ethanol under an oxygen atmosphere. The blue pigment formed was found to be an intractable mixture of high-molecular polymers on the basis of its chromatographic behavior, unanalyzable ^{13}C-NMR spectrum and by molecular weight measurement, which will be described later. Thus, we carried out the same reaction under a nitrogen atmosphere. The reaction mixture at first turned yellow and then brownish-red and finally changed to blue when oxygen was passed into the solution.

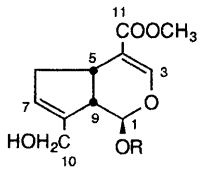
It was also found that the brownish-red reaction mixture consisted of various pigments, which were supposed to be intermediates leading to the blue pigment.

In the present paper, we describe the preparation and structural elucidation of these brownish-red intermediates as well as some characterization of the blue pigments.

Preparation of Intermediary Brownish-Red Pigments The intermediary pigments were prepared by reacting genipin (**1**) (1 mol eq) with methylamine hydrochloride (MA·HCl) (3 mol eq) in a mixed solution of McIlvaine buffer (pH 7.2) and ethanol under stirring for 2 h at 25 °C in an atmosphere of argon. The brownish-red reaction products, being extremely unstable and prone to turn blue, were subjected to alumina column chromatography followed by repeated preparative silica gel layer chromatography (PLC), giving nine pigments A—I (**3**—**11**). Reaction of the same mixture at 70 °C gave almost the same pigments in different yields. In the reaction at 70 °C under UV-irradiation for 1 h, these pigments were still formed, though substantial blue pigment formation was also observed.

Structures of Intermediary Brownish-Red Pigments A—I (3—11) Pigment A (**3**) was obtained as red needles of mp 148—149.5 °C. Its molecular formula, $C_{12}H_{13}NO_2$, determined by high-resolution (HR) electron impact (EI) MS, corresponded to that derived from the molecule of genipin (**1**) by elimination of two moles of H_2O and replacement of an oxygen atom by an N–Me group. It showed absorption bands at 1710 (ester) and 1630 (double bond) cm^{-1}, whereas no hydroxyl band was observed in the IR spectrum. Its ^{1}H- and ^{13}C-NMR spectra showed the presence of aromatic C–CH_3, N–CH_3 and $COOCH_3$ groups, four aromatic carbon atoms each bearing hydrogen and four aromatic quaternary carbon atoms, whereas no sp^3 carbon signals were observed. The NMR signals assigned on the basis of precise spin–spin decoupling and nuclear Overhauser effect (NOE) experiments [H_3-10/H-7 (5%), H_3-10/H-1 (10%), H_3-2/H-3 (15%) and H_3-2/H-1 (11%)] are listed in Tables I and II. On the basis of the above spectral evidence, the characteristic pseudoazulenic 2-methyl-2-pyrindine structure **3** was assigned to pigment A. This structure was confirmed by the observation of long-range couplings between the following signals: H-7/H_3-10 (1.0 Hz), H-6/H-1 (0.5 Hz) and H-3/H-1 (1.5 Hz). Thus, pigment A was formulated as 2-methyl-4-carbomethoxy-8-methyl-2-pyrindine (**3**).[10)]

Pigment B (**4**) was obtained as red needles of mp 138—139 °C. The molecular formula for **4** was determined to be $C_{13}H_{15}NO_2$ on the basis of its HREIMS, indicating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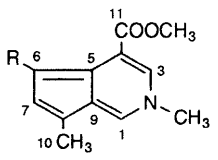
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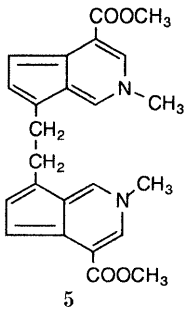
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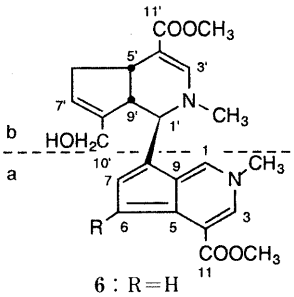
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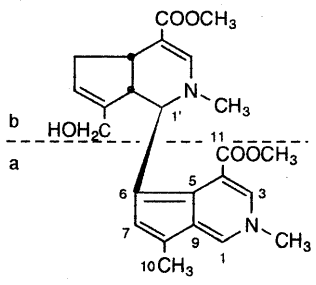
7 : R=CH3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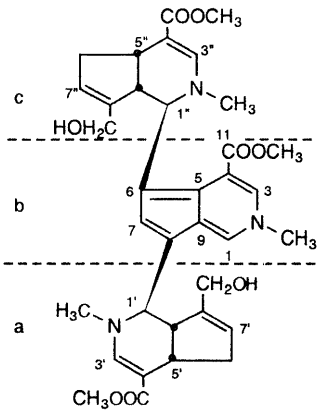
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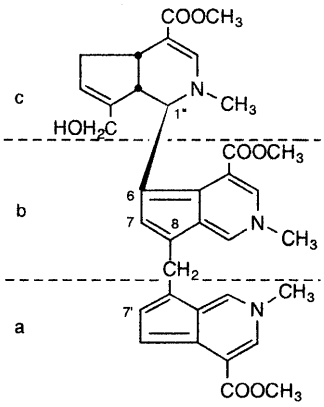
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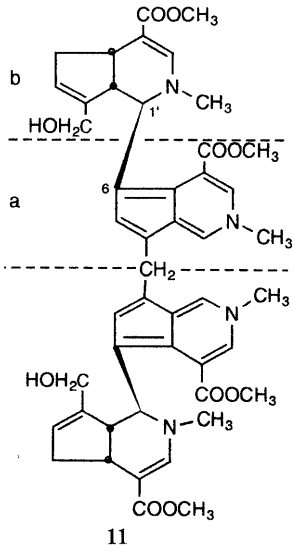
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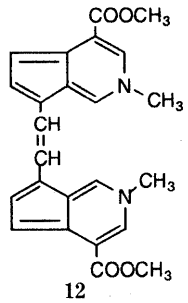
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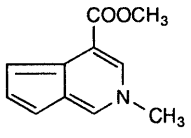
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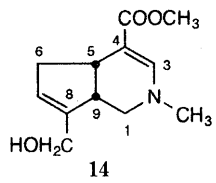
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it to possess one more methyl group than pigment A (**3**). Its IR spectrum showed the presence of an ester group and a double bond (1715 and 1630 cm^{-1}), but no hydroxyl group. The ^1H- and ^{13}C-NMR spectra of **4** are very similar to those of **3** except for the absence of signals of one aromatic carbon bearing hydrogen, and the appearance of signals due to an aromatic methyl group and an aromatic quaternary carbon atom. The NMR signals assigned by precise analyses are listed in Tables I and II. These spectral findings indicated that pigment B had the structure **4**. The validity of this structure was supported by the following NOEs: H_3-2/H-3 (16%), H_3-2/H-1 (11%) and H-1/H_3-10 (12%) as well as long-range couplings: H-1/H-3 (1.5 Hz) and H-7/H_3-10 (J was not determined). Thus, the structure of pigment B was established to be **4**. The origin of the methyl group at C-6 and the possible formation mechanism will be discussed in the following paper.

Pigment C (**5**) , a brownish-red amorphous compound, was formulated as $C_{24}H_{24}N_2O_4$ on the basis of EIMS, which exhibited the molecular ion peak at m/z 404. It showed similar UV and IR spectra to those of **3**. The ^{13}C-NMR spectrum (Table II) also resembled that of **3**, showing twelve carbon signals, corresponding to half the number of the signals which its molecular formula required. In accord with this, the MS of pigment C (**5**) showed a strong ion peak at m/z 202 with just half the size of the parent ion peak. These facts, in addition to the molecular formula of **5** with two hydrogens less than twice that of **3** , suggested that **5** is a dimer of **3**. The major differences in the ^1H- and ^{13}C-NMR spectra of **5** and **3** are the absence of the 8-methyl signals, which appeared in the spectra of **3**, and instead the appearance of methylene signals in the spectra of **5**. Thus, it was concluded that pigment C has the symmetrical dimeric structure (**5**) in which two pigment A (**3**) units are linked at the C-10 positions. This structure was confirmed by the formation of the blue colored dehydrogenation product **12** when oxygen was passed into an aqueous methanolic solution of **5**.

Pigment D (**6**), $[\alpha]_{650}$ +43.0° (MeOH), was obtained as a brownish-red amorphous compound. The molecular formula, $C_{23}H_{26}N_2O_5$, having 6 mass units more than that of **5**, suggested that pigment D might also be a dimer consisting of 2-methyl-2-pyrindine derivatives. The IR spectrum showed absorption bands at 3450 (hydroxyl) and 1670 (double bond), in addition to those at 1705 (ester) and 1620 (double bond) cm^{-1}, which were observed in **3**, **4** and **5**. The ^{13}C-NMR spectrum (Table II) suggested that pigment D contains a 2-methyl-4-carbomethoxy-2-pyrin-

TABLE I. ^{1}H-NMR Data (δ) for Genipin and Pigments A—I (**3**—**11**)[a]

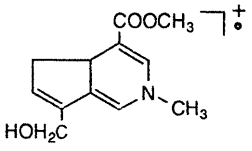
		H-1	H-3	H-5	H-6a or C_6-CH_3	H-6b	H-7	H-9	H_3-10 or H_2-10	OCH_3	NCH_3
Genipin (**1**)		4.78 d (7.8)	7.52 s	3.14 m	2.82 br dd (16.6, 9.0)	2.02 ddt (16.6, 9.0, 5.8)	5.82 m	2.48 t (7.8)	4.22 br ABq (14.6)	3.70 s	—
A (**3**)		7.76 m	7.73 d (1.5)	—	6.84 dd (2.9, 0.5)	—	7.14 dq (2.9, 1.0)	—	2.47 d (1.0)	3.97 s	3.95 s
B (**4**)		7.57 d (1.5)	7.38 d (1.5)	—	2.46 s	—	6.90 br s	—	2.41 br s	3.92 s	3.80 s
C (**5**)		7.57 m	7.65 d (1.5)	—	6.85 br d (2.9)	—	7.23 d (2.9)	—	4.51 br s	3.96 s	3.76 s
D (**6**)	a	7.95 m	7.84 d (1.5)	—	6.95 dd (3.2, 0.7)	—	7.33 d (2.9)	—	—	4.02 s	4.00 s
	b	4.04 d (9.0)	7.59 s	3.22 br q (7.5)	2.88 ddm (16.3, 7.3)	2.15 ddm (16.3, 7.3)	5.73 m	3.00 br dd (9.0, 7.2)	3.34 m	3.73 s	2.58 s
E (**7**)	a	7.82 d (1.5)	7.48 d (1.5)	—	2.46 s	—	7.07 s	—	—	3.94 s	3.90 s
	b	4.02 d (9.0)	7.56 s	3.19 br q (7.6)	2.83 ddm (16.1, 7.6)	2.12 ddm (16.1, 7.6)	5.71 m	2.98 br dd (9.0, 7.8)	3.36 br s	3.71 s	2.59 s
F (**8**)	a	7.72 d (1.5)	7.54 d (1.5)	—	—	—	7.04 q (1.0)	—	2.42 d (1.0)	3.86 s	3.92 s
	b	4.97 d (6.6)	7.61 br s	3.13 br q (6.8)	2.72 ddqu (15.9, 7.3, 1.0)	2.23 ddqu (15.9, 6.3, 2.0)	5.67 m	3.01 td (6.8, 1.0)	3.65 br s	3.70 s	2.75 s
G (**9**)	a	3.97 d (9.5)	7.58 s	3.20 br q (8.1)	*ca.* 2.65 m	2.25—2.07 m	5.72 br s	2.89 br t (8.1)	3.33 ABq (14.9)	3.72 s	2.55 s
	b	7.95 d (1.5)	7.62 d (1.5)	—	—	—	7.16 s	—	—	3.88 s	3.97 s
	c	5.02 d (5.9)	7.54 s			2.25—2.07 m	5.72 br s		3.80 ABq (*ca.* 13.0)	3.68 s	2.78 s
H (**10**)	a	7.66 m	7.69 d (1.5)	—	6.84 dd (2.9, 0.7)	—	7.21 d (2.9)	—	—	3.96 s	3.85 s
	b	7.55 d (1.5)	7.46 d (1.5)	—	—	—	7.16 br s	—	4.49 br s	3.84 s	3.74 s
	c	4.95 d (7.1)	7.61 br s	3.11 br q (6.9)	2.99 ddm (16.5, 6.9)	2.23 ddm (16.5, 6.8)	5.68 m	3.02 br t (7.3)	3.96 br s	3.68 s	2.77 s
I (**11**)	a	7.65 d (1.5)	7.52 d (1.5)	—	—	—	7.16 br s	—	4.45 br s	3.82 s	3.85 s
	b	5.08 d (5.9)	7.62 s	3.15—2.88 m	2.70 br dd (17.3, 6.4)	2.28 br d (17.3)	5.70 br s	3.15—2.88 m	3.83 br s	3.68 s	2.73 s

a) All spectra were measured in $CDCl_3$ at 200 MHz, and *J* values (in Hz) are shown in parentheses. Abbreviations: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; qu, quintet; m, multiplet. a, b and c refer to the structural moieties shown in the formulae of pigments **6**—**11**.

TABLE II. ^{13}C-NMR Data (δ) for Genipin (**1**) and Pigments A—I (**3**—**11**)[a]

		C-1	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	OCH_3	NCH_3	6-CH_3
Genipin (**1**)		97.67 d	154.30 d	111.60 s	37.44 d	39.86 t	128.41 d	145.36 s	48.27 d	61.46 t	169.86 s	51.73 q	—	—
A (**3**)		129.43 d	126.78 d	113.65 s	128.02 s	104.45 d	132.98 d	122.74 s	115.57 s	11.97 q	167.72 s	51.76 q	44.56 q	—
B (**4**)		128.80 d	125.76 d	115.23 s	128.63 s	116.62 s	136.93 d	113.48 s	115.30 s	11.63 q	167.87 s	51.62 q	43.98 q	15.13 q
C (**5**)		130.57 d	126.93 d	113.56 s	127.13 s	103.80 d	132.62 d	123.49 s	120.99 s	26.79 t	167.69 s	51.79 q	44.64 q	—
D (**6**)	a	130.36 d	127.51 d	114.47 s	126.25 s	104.79 d	134.23 d	125.06 s	117.05 s	—	167.16 s	51.93 q	45.07 q	—
	b	57.19 d	147.77 d	100.22 s	37.90 d	39.50 t	127.10 d	146.97 s	49.07 d	61.23 t	169.35 s	50.52 q	40.55 q	—
E (**7**)	a	129.48 d	126.03 d	116.32 s	126.88 s	118.83 s	137.73 d	113.89 s	116.49 s	—	167.53 s	51.89 q	44.56 q	15.23 q
	b	56.77 d	147.77 d	100.10 s	37.73 d	39.48 t	127.20 d	146.83 s	49.45 d	61.32 t	169.38 s	50.52 q	40.62 q	—
F (**8**)	a	129.56 d	127.51 d	114.57 s	128.66 s	117.54 s	132.11 d	116.32 s	118.51 s	11.89 q	167.32 s	52.13 q	44.34 q	—
	b	54.61 d	148.19 d	98.44 s	36.58 d	38.96 t	127.63 d	146.43 s	49.79 d	60.96 t	169.38 s	50.30 q	40.91 q	—
G (**9**)	a	57.75 d	147.56 d	100.54 s	37.97 d	39.46 t	126.20 d	146.90 s	49.33 d	61.08 t	169.33 s	50.57 q	40.57 q	—
	b	130.48 d	127.80 d	115.71 s	125.44 s	120.58 s	133.84 d	116.15 s	118.95 s	—	167.50 s	52.35 q	44.81 q	—
	c	54.97 d	148.16 d	98.66 s	36.70 d	38.90 d	128.73 d	146.14 s	49.53 d	60.76 t	169.38 s	50.40 q	40.99 q	—
H (**10**)	a	130.65 d	127.49 d	114.74 s	127.34 s	103.65 s	132.64 d	122.99 s	115.42 s	—	167.75 s	51.76 q	44.59 q	—
	b	130.33 d	127.17 d	113.70 s	127.71 s	120.58 d	131.45 d	119.29 s	123.84 s	27.00 t	167.60 s	52.13 q	44.42 q	—
	c	54.71 d	148.19 d	98.42 s	36.78 d	38.99 t	128.00 d	146.32 s	49.20 d	60.98 t	169.25 s	50.30 q	40.94 q	—
I (**11**)	a	130.66 d	127.73 d	114.20 s	127.15 s	114.96 s	131.09 d	119.06 s	121.64 s	27.07 t	167.69 s	52.16 q	44.24 q	—
	b	54.43 d	148.01 d	97.73 s	36.27 d	38.54 t	127.85 d	145.60 s	49.80 d	60.67 t	168.75 s	50.16 q	40.96 q	—

a) All spectra were measured in $CDCl_3$ at 50 MHz. The assignments were based on proton noise decoupling, off-resonance decoupling, selective proton decoupling, long-range selective proton decoupling, analyses of ^{13}C–^{15}N coupling and mutual comparisons. Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet. a, b and c refer to the structural moieties shown in the formulae of pigments **6**—**11**.



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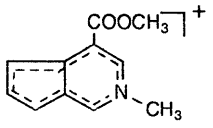

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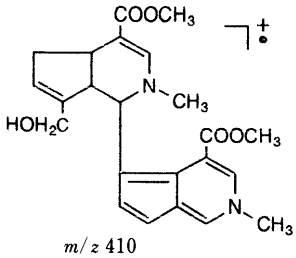

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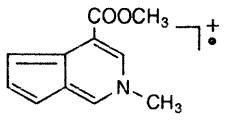

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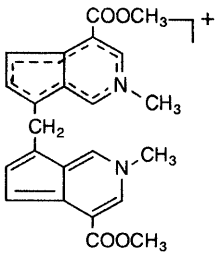

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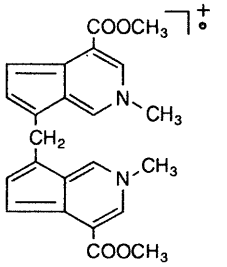

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dine (**13**) moiety (part a) and a 1,9,5,6-tetrahydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine moiety (**14**) (part b) in the structure, which was supported by the observation of ion peaks at *m/z* 221 (**15**) and 188 (**16**) in the MS. Precise analyses of its ^{1}H- and ^{13}C-NMR spectra led to the assignments of signals as shown in Tables I and II, suggesting that pigment D had the structure **6**. The linking position of both parts was confirmed by NOE [H-1/H-1′ (5%)] and the stereochemistry at C-1′, C-9′ and C-5′ was assigned as shown on the basis of the $J_{1',9'}$ and $J_{9',5'}$ values and the signal patterns of the corresponding protons, presuming that the configuration at C-5 of genipin (**1**) was retained during the reaction. Thus, the structure of pigment D was concluded to be **6**.

Pigment E (**7**), $[\alpha]_{650}$ +22.0° (MeOH), was obtained as a brownish-red amorphous compound. The molecular formula of **7** was determined to be $C_{24}H_{28}N_2O_5$ on the basis of HREIMS, suggesting it to have one more methyl group than pigment D (**6**). Pigment E showed absorption bands at 3450 (hydroxyl), 1715 (ester), 1665 and 1615 (double bonds) cm^{-1} in the IR spectrum. Its ^{1}H- and ^{13}C-NMR spectra (Tables I and II) were very similar to those of **6** except for similar discrepancies to those observed between **3** and **4**. Based on the afore-mentioned facts, the structure of pigment E including the stereochemistry was elucidated to be **7**, the 6-methyl derivative of pigment D (**6**). The linkage positions of both parts were also supported by the NOE observation [H-1/H-1′ (7%)].

Pigment F (**8**), a brownish-red amorphous compound, $[\alpha]_{650}$ +241° (MeOH), was formulated as $C_{24}H_{28}N_2O_5$, the same as that of **7**, on the basis of HREIMS. This pigment showed similar UV and IR spectra to those of **7**. Its ^{1}H- and ^{13}C-NMR spectra (Tables I and II) also resembled those of **7** , indicating that **8** also consisted of 2-methyl-4-carbomethoxy-2-pyridine (**13**) and tetrahydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine (**14**) moieties. The major differences in the ^{1}H- and ^{13}C-NMR spectra of **8** and **7** were the absence of the 6-methyl signals, which appeared in the spectra of **7**, and instead the appearance of 8-methyl signals in the spectra of **8**. Thus the structure of pigment F was elucidated to be **8**.

Pigment G (**9**), a brownish-red amorphous compound, $[\alpha]_{650}$ +202° (MeOH), was formulated as $C_{35}H_{41}N_3O_8$ on the basis of HREIMS, which exhibited a molecular ion peak at *m/z* 631. Besides this molecular ion peak, **9** showed fragment ion peaks at *m/z* 410 (**17**) , 221 (**15**) and 189 (**18**), suggesting that **9** possessed one 2-methyl-4-carbomethoxy-2-pyridine (**13**) and two tetrahydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine (**14**) moieties in its molecule. The presence of these three component structures was also verified by the ^{1}H- and ^{13}C-NMR findings (Tables I and II). The linkages of the three parts and the stereochemistry of the tetrahydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine moiety (**14**) were concluded to be shown in the structure **9** from a comparison of the NMR spectra of this compound with those of pigments **6**—**8**, and in particular from the absence of NMR signals due to the methyl group of the central 2-methyl-4-carbomethoxy-2-pyridine (**13**) moiety. Thus, the structure of pigment G was formulated as **9**.

Pigment H (**10**), a brownish-red amorphous compound, was found to possess the molecular formula $C_{35}H_{37}N_3O_7$ on the basis of HREIMS. Detailed studies of its ^{1}H- and ^{13}C-NMR spectra (Tables I and II) disclosed that this compound (**10**) consisted of one tetrahydro-2-methyl-4-carbomethoxy-8-hydroxymethyl-2-pyrindine (**14**) and two 2-methyl-4-carbomethoxy-2-pyridine (**13**) moieties. The linking position of the former to one of the latter was elucidated to be C-1″ to C-6 by comparing the ^{1}H- and ^{13}C-NMR spectra of this compound (**10**) with those of **8**. The 2-methyl-2-pyridine moieties were concluded to be linked at C-8 and C-8′ through an intermediate methylene group in view of the observed long-range couplings (H_2-10/H-7 and H-7′). Thus, the structure of pigment H was elucidated as **10**, which was supported by the ion peaks observed at *m/z* 389 (**19**), 390 (**20**) and 221 (**15**) in the MS of **10**.

Pigment I (**11**), a brownish-red amorphous compound, was found to possess the molecular formula $C_{47}H_{52}N_4O_{10}$ on the basis of EIMS. Its ^{13}C-NMR spectrum, however, showed only twenty-four carbon signals (Table II), suggesting **11** to have a tetrameric symmetrical molecular structure consisting of two identical dimeric portions having an extra methylene carbon in between. Furthermore, the ^{1}H- and ^{13}C-NMR spectra (Tables I and II) of **11** showed a close similarity to those of pigment F (**8**) except for the absence of the methylene signals, and the appearance of methyl signals. Thus, pigment I was formulated as **11**, which was further confirmed by the presence of the ion peaks at *m/z* 611 (M^+ of **10**, 100%), *m/z* 424 (M^+ of **8**, 17%), *m/z* 410 (**17**, 69%), *m/z* 221 (**15**, 40%) in its MS.

Nature of the Blue Pigment The above-described pigments, especially pigments C—I (**5**—**11**) are very unstable and rapidly turn blue in the presence of oxygen, even at room temperature, while pigments **3** and **4** discolor rather gradually. The blue pigment obtained by the method described in Experimental is a mixture of high-molecular polymers which is soluble in water, methanol and ethanol, but insoluble in other organic solvents such as chloroform. It was found to have an average molecular weight of 8970 ± 600 on the basis of osmotic pressure measurement.[11] Since the molecular weights of the monomers **3**, **4** and **14** are 203, 217 and 223, the blue pigment is considered to be a mixture of polymers consisting on average of 40—44 monomer units.

Considering the formation of the blue pigment i) from the intermediary products upon passing oxygen into the reaction solution, and ii) from genipin (**1**) and methylamine in an aqueous ethanolic solution (even under exclusion of oxygen) by UV irradiation, this blue pigment would presumably be formed through oxygen radical-induced polymerization and dehydrogenation of several intermediary pigments such as those described above. The nature of the blue pigment is now under detailed investigation.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV and IR spectra were recorded on a Hitachi model 200-20 spectrometer and a Hitachi 215 grating infrared spectrometer, respectively. Optical rotations were taken with a JASCO ORD/UV-5 spectrometer. ^{1}H-NMR spectra were taken with a Hitachi R-22 or a JEOL JNM-FX 200 spectrometer and ^{13}C-NMR spectra were taken with a Varian XL-200 or a JEOL JNM-FX 200 spectrometer. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts are given in δ (ppm) values. MS were determined with a JEOL JMS-01SG-2 spectrometer. Neutral aluminum oxide (M. Woelm, activity grade II) was used for column chromatography, Silica gel 60 GF_{254} (Merck) and precoated Silica gel GF_{254} plates (Merck, 0.25 mm in thickness) were utilized for thin layer chromatography (TLC), and Silica gel 60 PF_{254} (Merck) for PLC. The spots or bands were 7 detected under UV illumination.

Reaction of Genipin (1) with MA·HCl) under Argon Expt. 1: A solution of genipin (**1**) (904 mg) and MA·HCl (808 mg) dissolved 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) with a mixture of $CHCl_3$ and EtOH containing an increasing EtOH content. $CHCl_3$ (300 ml), $CHCl_3$–EtOH (49 : 1, 150 ml) and $CHCl_3$–EtOH (19 : 1, 250 ml) were passed successively and the eluates were collected in 50 ml fractions. The residue (331 mg) from fraction nos. 1—6 was further separated by PLC (solvent: Et_2O) to give pigments B (**4**) (52 mg), A (**3**) (29 mg) and C (**5**) (15 mg) in increasing order of polarity. The residue (407 mg) from fraction nos. 7—9 was separated by PLC [solvent: Et_2O–EtOH (49 : 1), developed 5 times]. The faster-moving zone gave pigment E (**7**) (13 mg) and the slower-moving zone gave pigment F (**8**) (51 mg). Though a zone of pigment D (**6**) was found in TLC (Et_2O–EtOH, 49 : 1), it was not isolated in this experiment. The residue (274 mg) from fraction nos. 10—14 was separated by PLC [solvent: Et_2O–EtOH (19 : 1), developed 8 times] to give pigments H (**10**) (8 mg), G (**9**) (36 mg) and I (**11**) (23 mg).

Expt. 2: The reaction was carried out under exactly the same conditions as in Expt. 1 except for a higher temperature (70 °C). The same work-up as above gave a residue (900 mg), which was chromatographed over aluminum oxide (50 g) with $CHCl_3$–EtOH containing an increasing EtOH content. $CHCl_3$ (150 ml), $CHCl_3$–EtOH (99 : 1) (300 ml), $CHCl_3$–EtOH (49 : 1) (100 ml), and $CHCl_3$–EtOH (19 : 1) (200 ml) were passed successively. The residue (191 mg) from the $CHCl_3$ eluate was separated by PLC (solvent: Et_2O) to give pigments B (**4**) (45 mg) and A (**3**) (33 mg). The spot of **5** was also detected on TLC of this portion. The residue (437 mg) from the eluate with $CHCl_3$–EtOH (99 : 1) was separated by PLC [solvent: Et_2O–EtOH (49 : 1), developed 5 times] to give pigments E (**7**) (23 mg) and D (**6**) (31 mg). The residue (121 mg) from the eluate with $CHCl_3$–EtOH (49 : 1 and 19 : 1) was separated by PLC [solvent: Et_2O–EtOH (19 : 1), developed 9 times] to give pigments G (**9**) (15 mg) and I (**11**) (4 mg).

The physical data of pigments A—I (**3**—**11**) are as follows.

Pigment A (**3**): Red needles, mp 148—149.5 °C. UV λ_{max} (EtOH) nm (log ε): 282, 333, 483 (4.33, 3.42, 3.02). IR ν_{max} (KBr) cm^{-1}: 1710, 1630, 1440. ^{1}H- and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 203.0951 $(M)^+$. Calcd for $C_{12}H_{13}NO_2$: 203.0943.

Pigment B (**4**): Red needles, mp 138—139 °C. UV λ_{max} (EtOH) nm (log ε): 283, 347, 488 (4.30, 3.46, 3.02). IR ν_{max} (KBr) cm^{-1}: 1715, 1630, 1440. ^{1}H- and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 217.1099 $(M)^+$. Calcd for $C_{13}H_{15}NO_2$: 217.1101 .

Pigment C (**5**): Brownish-red amorphous powder. UV λ_{max} (EtOH) nm (log ε): 279 (3.85). IR ν_{max} (KBr) cm^{-1}: 1700, 1610, 1440. ^{1}H- and ^{13}C-NMR (see Tables I and II). EIMS *m/z*: 404 (M^+, 0.2%), 203 (22.2%), 202 (31.9%), 189 (59.0%), 131 (100%), 130 (52.8%).

Pigment D (**6**): Brownish-red amorphous powder, $[\alpha]_{650}^{25}$ +43.0° ($c = 0.18$, MeOH). UV λ_{max} (EtOH) nm (log ε): 294, 472 (4.61, 3.27). IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 1720 (sh), 1705, 1670, 1620, 1600 (sh), 1435. ^{1}H and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 410.1842 $(M)^+$. Calcd for $C_{23}H_{26}N_2O_5$: 410.1842.

Pigment E (**7**): Brownish-red amorphous powder, $[\alpha]_{650}^{25}$ +22.0° ($c = 0.20$, MeOH). UV λ_{max} (EtOH) nm (log ε): 291, 479 (4.42, 3.07). IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 1715, 1665, 1615, 1600 (sh), 1435. ^{1}H- and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 424.2002 $(M)^+$. Calcd for $C_{24}H_{28}N_2O_5$: 424.1998.

Pigment F (**8**): Brownish-red amorphous powder, $[\alpha]_{650}^{25}$ +241° ($c = 0.20$, MeOH). UV λ_{max} (EtOH) nm (log ε): 298, 467 (4.49, 2.82). IR ν_{max} ($CHCl_3$) cm^{-1}: 3460, 1715 (sh), 1660, 1645 (sh), 1630, 1600 (sh), 1430. ^{1}H- and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 424.1998 $(M)^+$. Calcd for $C_{24}H_{28}N_2O_5$: 424.1988.

Pigment G (**9**): Brownish-red amorphous powder, $[\alpha]_{650}^{25}$ +202° ($c = 0.06$, MeOH). UV λ_{max} (EtOH) nm (log ε): 295, 451 (4.76, 3.32). IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 1710, 1670, 1620, 1440. ^{1}H and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 631.2876 $(M)^+$ Calcd for $C_{35}H_{41}N_3O_8$: 631.2881.

Pigment H (**10**): Brownish-red amorphous powder, ^{1}H- and ^{13}C-NMR (see Tables I and II). HREIMS *m/z*: 611.2632 $(M)^+$. Calcd for $C_{35}H_{37}N_3O_7$: 611.2632.

Pigment I (**11**): Brownish-red amorphous powder, IR ν_{max} ($CHCl_3$) cm^{-1}: 3480, 1720 (sh), 1705, 1675 (sh), 1670 (sh), 1660, 1650 (sh), 1615, 1600 (sh), 1440. ^{1}H- and ^{13}C-NMR (see Tables I and II). EIMS *m/z*: 832 (M^+, 27%), 611 (100%), 424 (17%), 410 (69%), 221 (40%).

Reaction of Genipin (1) with MA·HCl under UV Irradiation 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 irradiated by a high-pressure mercury lamp (200 W) through a quartz glass filter at 70 °C under an atmosphere of argon. After 30 min, the reaction mixture turned blue, and after 1 h dark blue. The reaction mixture was concentrated *in vacuo* to remove EtOH, and the aqueous solution was extracted with $CHCl_3$ (50 ml × 3). The $CHCl_3$ layer was washed with H_2O, dried and concentrated *in vacuo* to yield a blue-colored residue (1.05 g), which was chromatographed over aluminum oxide with $CHCl_3$–EtOH containing an increasing EtOH content. During the chromatographic process, the blue material was adsorbed on the uppermost part of the column. $CHCl_3$ (200 ml), $CHCl_3$–EtOH (99 : 1) (150 ml), $CHCl_3$–EtOH (49 : 1) (100 ml), and $CHCl_3$–EtOH (19 : 1) (300 ml) were passed successively. The residue (170 mg) from the $CHCl_3$ eluate showed the spots of **3**, **4** and **5** on TLC (solvent: Et_2O), but their isolation was not attempted in this experiment. The residue (437 mg) from the eluates with EtOH containing $CHCl_3$ was separated by PLC [solvent: Et_2O–EtOH (19 : 1), developed twice] to give **7** (17 mg), **6** (24 mg) and **10** (20 mg) in order of increasing polarity. The residue (84 mg) from the most polar band was further separated by PLC (solvent: same as above, developed 11 times) to give **9** (14 mg) and **11** (9 mg).

Dehydrogenation of Pigment C (5) Pigment C (**5**) (100 mg) was dissolved in a mixture of H_2O–MeOH (1 : 1) (50 ml) and air was passed

through the solution for 5 h at 50 °C. The reaction mixture was concentrated *in vacuo* to give a residue (95 mg), which was chromatographed over aluminum oxide (activity grade III, 30 g) with $CHCl_3$–EtOH containing an increasing EtOH content. The eluate from $CHCl_3$–EtOH (19 : 1) was concentrated *in vacuo* to give the blue pigment (**12**) (60 mg).

Blue Pigment (**12**): UV λ_{max} (MeOH) nm (log ε): 283, 599 (4.14, 4.73). IR ν_{max} (KBr) cm^{-1}: 1710, 1570, 1390. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 9.38 (2H, m, H-1 and 1′), 8.24 (6H, m, H-3, 3′, 6, 6′, 7 and 7′) 7.40 (2H, d, J=4.0 Hz, H-10 and 10′), 4.05 (6H, s, 2×–$COOCH_3$), 4.27 (6H, s, –NCH_3). EIMS m/z: 402 $(M)^+$.

Reaction of Genipin (1) and [^{15}N]MA·HCl Genipin (**1**) (230 mg) and [^{15}N]MA·HCl (40 mg) were dissolved in a mixture of McIlvaine buffer (pH 7.2) (20 ml) and EtOH (15 ml) and the solution was subjected to the conditions used in Exp. 1 above. Work-up and separation as above gave pigments B ([^{15}N]-**4**), D ([^{15}N]-**6**), E ([^{15}N]-**7**) and G ([^{15}N]-**9**). The ^{1}H-NMR spectra of these compounds were essentially the same as those of the non-labeled compounds. [^{15}N]-**4**, HREIMS m/z: 218.1072 $(M)^+$. Calcd for $C_{13}H_{15}{}^{15}NO_2$: 218.1073. [^{15}N]-**6**, HREIMS m/z: 412.1775 $(M)^+$. Calcd for $C_{23}H_{26}{}^{15}N_2O_5$: 412.1780. [^{15}N]-**7**, HREIMS m/z: 426.1988 $(M)^+$. Calcd for $C_{24}H_{28}{}^{15}N_2O_5$: 426.1996. [^{15}N]-**9**, HREIMS m/z: 634.2818 $(M)^+$. Calcd for $C_{35}H_{41}{}^{15}N_3O_8$: 634.2805.

Preparation of the Blue Pigment from Genipin (1) and Methylamine Usually blue pigment is prepared in a one-step reaction of genipin (**1**) and methylamine in the presence of oxygen, but the reaction here was carried out in two steps in the following way. A solution of genipin (**1**) (904 mg) and MA·HCl (452 mg) in a mixture of McIlvaine buffer (pH 7.2) (20 ml), H_2O (20 ml) and EtOH (20 ml) was stirred for 2.5 h at 50 °C under a nitrogen atmosphere. On cooling, the solution was extracted with $CHCl_3$, and the organic solution was dried over $MgSO_4$ and concentrated *in vacuo*. The brownish-red residue was dissolved in a mixture of MeOH (20 ml) and H_2O (20 ml), heated for 6 h at 80 °C while air was passed into the solution, and concentrated *in vacuo*. The residue was partitioned between $CHCl_3$ and H_2O. After removal of $CHCl_3$ by concentration *in vacuo*, the H_2O layer was dialyzed against flowing H_2O for 16 h, whereupon blue color was scarcely found in the dialyzate. After filtration of the remaining blue solution, the filtrate was lyophilized to give the blue pigment (510 mg) as an amorphous powder. This pigment showed an absorption maximum at 575 nm in the visible spectrum (solvent: H_2O).

Acknowledgement This work was supported in part by a Grant-in-Aid (No. 387148) from the Ministry of Education, Science and Culture, Japan.

References and Notes

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