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Studies on the Coloration Mechanism of Furostanol Derivatives with Ehrlich Reagent. II.[1] On the Reaction of Furostanol Glycoside with Ehrlich Reagent[2]

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The coloration mechanism in the Ehrlich reaction between furostanol glycoside and *p*-dimethylaminobenzaldehyde has been studied. From the reddish reaction mixture of proto-aspidistrin, one of the furostanol saponins, with Ehrlich reagent, colorless condensation products were isolated and characterized as methyl 23-(*p*-dimethylaminobenzylidenyl)-proto-aspidistrin (**5**), 3,26-dihydroxy-23-(*p*-dimethylaminobenzylidenyl)-furost-5,20(22)-diene 3-*O*-*β*-D-galactopyranosido-26-*O*-*β*-D-glucopyranoside (**9**), 23-(*p*-dimethylaminobenzylidenyl)-diosgenin (**6**), 3*β*-hydroxy-26,7′-epoxy-23-(*p*-dimethylaminobenzyl)-furost-5,20(22)-diene (**7**) and a stereoisomer of **7** (**8**). Among these products, compounds **5**, **9** and **6** showed red coloration under acidic conditions, while compounds **7** and **8**, which should not be able to form iminium cations under acidic conditions, did not show the red coloration. It was concluded that the Ehrlich reaction is initiated by condensation between *p*-dimethylaminobenzaldehyde and furostanol saponin to form 23-benzylidenyl derivatives, which are protonated to form iminium cations under acidic conditions.

Keywords——Ehrlich reaction; coloration mechanism; proto-aspidistrin; furostanol saponin; *p*-dimethylaminobenzaldehyde; ^{13}C-NMR

In the preceding paper[1] we have reported a study on the coloration mechanism of 3*β*,26-dimethoxy-furost-5,20(22)-diene (pseudo-diosgenin dimethyl ether, **1**) with the Ehrlich reagent, and it was clarified that the color reaction was initiated by condensation between *p*-dimethylaminobenzaldehyde and **1** to form 3*β*,26-dimethoxy-23-(*p*-dimethylaminobenzylidenyl)-furost-5,20(22)-diene (**2**), followed by protonation to form an iminium cation (**3**) under acidic conditions. As we pointed out in the preceding paper, the Ehrlich reaction had been applied as a screening test for detecting furostanol glycoside in Dioscoreaceous and Liliaceous plants by one of the authors, Kiyosawa, and co-workers.[3] The present paper deals mainly with a further study on the coloration mechanism of methyl proto-aspidistrin[4] (26-*O*-*β*-D-glucopyranosyl 22-methoxyfurost-5-en-3*β*,26-diol 3-*O*-*β*-lycotetraoside, **4**), one of the furostanol glycosides of *Aspidistra elatior* BLUME (Liliaceae), with Ehrlich reagent.

First, the Ehrlich reaction of methyl proto-aspidistrin (**4**) with *p*-dimethylaminobenzaldehyde was carried out under mild conditions. Compound **4** and *p*-dimethylaminobenzaldehyde were dissolved in ethanolic 5% hydrochloric acid, and the solution was left at room temperature for ten days. The reddish reaction mixture was purified by successive column chromatography on Sephadex LH-20 and Avicel to afford a white powder (**5**) and intact starting material (**4**). The infrared (IR) and the proton nuclear magnetic resonance (^{1}H-NMR) spectra of compound **5** did not show any signals assignable to pseudo-furostanol or spiroketal structure, but both spectra showed the partial structures of **4** and *p*-dimethylaminobenzaldehyde. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of

compound **5** revealed the presence of an $-N(CH_3)_2$ group (δ 41.0 ppm), aromatic ring carbons (δ 127.6, 131.0, 113.8, 150.8 ppm), 18-CH_3 (δ 17.1 ppm), 27-CH_3 (δ 17.7 ppm), 19-CH_3 (δ 20.0 ppm), 21-CH_3 (δ 14.3 ppm), and five anomeric carbons of saccharide moieties. Furthermore, the carbon signal corresponding to C_{23} of compound **4** at δ 31.2 ppm could not be found in the ^{13}C-NMR spectrum of compound **5**, but a new carbon signal was found at δ 135.5 ppm. Based on the slight shifts of the C_{22} and C_{24} signals to lower field, the disappearance of the aldehyde carbon signal of *p*-dimethylaminobenzaldehyde at δ 189.9 ppm and the appearance of a new olefinic carbon signal at δ 130.9 ppm, compound **5** was suggested to be formed by condensation between the aldehyde group of *p*-dimethylaminobenzaldehyde and the C_{23} methylene moiety of compound **4**. The structure of **5** was concluded to be methyl 23-(*p*-dimethylaminobenzylidenyl)-proto-aspidistrin.

Next, the reaction of methyl proto-aspidistrin (**4**) with the Ehrlich reagent prepared from ethanolic 1% *p*-dimethylaminobenzaldehyde solution and ethanolic 10% hydrochloric acid solution was performed by heating. The reddish reaction mixture was neutralized with sodium carbonate and the precipitate was removed by filtration. The filtrate was concentrated to dryness *in vacuo* at 40 °C. The residue was separated into two fractions (Fr. I and Fr. II) by column chromatography on Avicel. Fr. I was further chromatographed on a silica gel column to afford *p*-dimethylaminobenzaldehyde, diosgenin and three other products (**6**, **7** and **8**), while another product **9** was obtained from Fr. II by successive column chromatography on Avicel, silica gel and Sephadex LH-20.

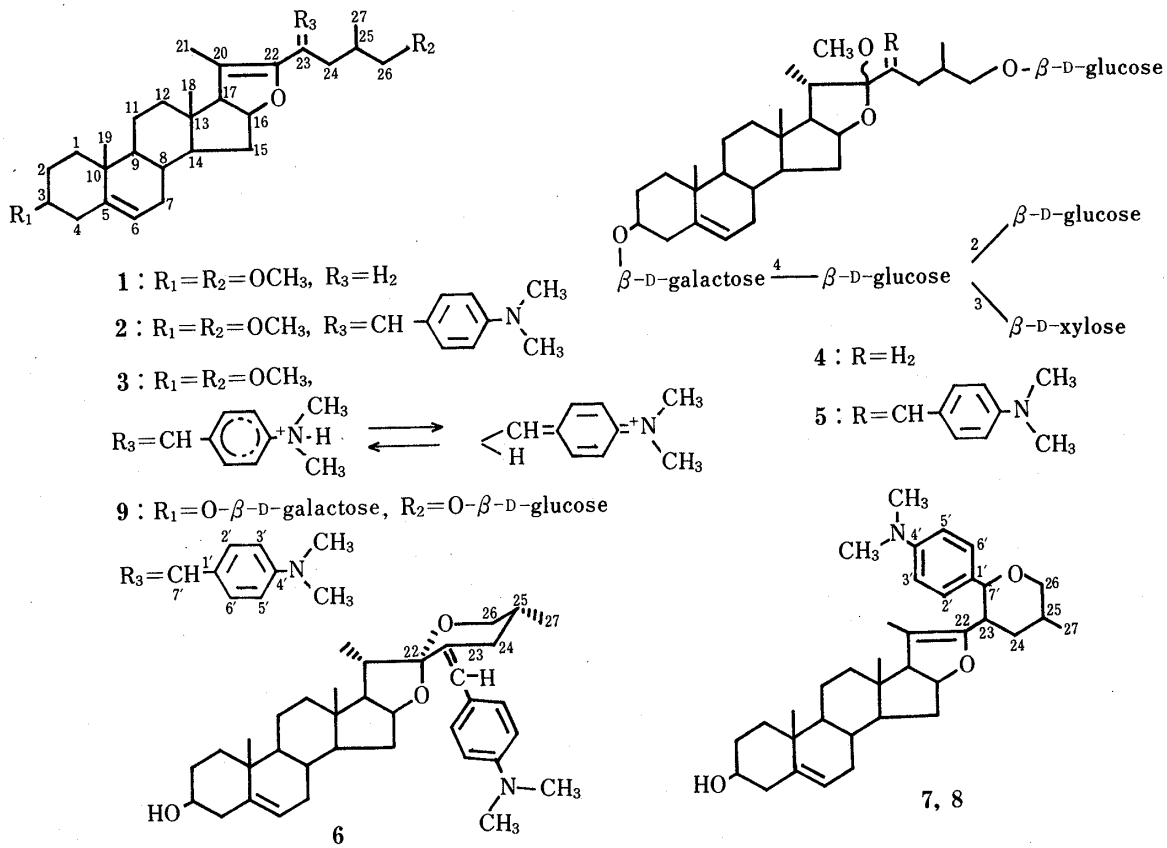

Chart 1

The IR and ^{1}H-NMR spectra of compound **9** revealed the presence of a *p*-dimethylaminobenzylidenyl group and a pseudo-furostanol type double bond between C-20 and C-22 by comparison with the signals of 3,26-dimethoxy-23-(*p*-dimethylaminobenzylidenyl)-furost-5,20(22)-diene (**2**).[1] The IR spectrum of **9** showed a strong hydroxyl

absorption band at 3300—3500 cm^{-1} and the ^{13}C-NMR spectrum of **9** showed two anomeric carbon signals at δ 104.8 and 104.6 ppm, the C-26 methylene carbon signal at δ 75.8 ppm and signals assignable to galactose at the C-3 hydroxyl group and to glucose at the C-26 hydroxyl group by comparison with the signals of methyl proto-aspidistrin. Furthermore, the signals of the double bond between the C-20 and C-22 carbons at δ 107.2 and 151.6 ppm, and those of C-23 and the benzylidenyl group at δ 129.5 and 133.0 ppm supported the presence of pseudo-type and benzylidenyl partial structures in **9**. Based on comparisons of the IR, ^1H- and ^{13}C-NMR spectra of **9** with those of methyl proto-aspidistrin (**4**) and 3,26-dimethoxy-23-(*p*-dimethylaminobenzylidenyl)-furost-5,20(22)-diene (**2**), **9** was concluded to be 3,26-dihydroxy-23-(*p*-dimethylaminobenzylidenyl)-furost-5,20(22)-

TABLE I. ^{13}C-NMR Chemical Shifts of **2**, **4**, **5**, **6**, Diosgenin, **7**, **8**, and **9**[a]

Carbon No.	**2**[1]	**4**[4]	**5**	**6**	Diosgenin[6]	**7**	**8**	**9**	Carbon No.		**4**	**5**	**9**
1	37.2	38.5	38.5	37.3	37.2	37.3	37.3	38.4	Galactose	1	102.8	102.8	104.8[e]
2	28.0	30.6	30.6	31.7	31.6	31.7	31.7	30.6		2	73.1	73.2	72.2
3	80.2	78.6	—	71.8	71.3	71.7	71.7	—		3	75.1	75.2	75.0
4	38.7	39.8	39.7	42.3	42.2	42.3	42.3	39.7		4	80.0	80.0	71.4
5	140.8	142.0	142.0	140.8	140.8	140.8	140.8	142.2		5	75.8	76.4	77.0
										6	61.2	61.1	62.6
6	121.3	122.4	122.6	121.4	121.1	121.4	121.4	122.0					
7	32.3	32.8	32.8[b]	32.1	32.0	32.2	32.2	33.2[c]					
8	31.3	31.5	32.2[b]	31.4	31.4	31.3	31.0	32.3[c]	Glucose	1	104.6[d]	104.8	
9	50.2	50.9	51.2	50.1	50.0	50.1	50.1	51.4	(Inner)	2	80.9	81.0	
10	37.0	38.0	38.0	36.7	36.5	36.6	36.7	37.6		3	87.8	87.8	
										4	70.9	70.9	
11	21.0	22.0	21.5	21.0	20.8	21.0	20.9	21.9		5	77.7	77.8	
12	39.6	40.8	40.8	37.3	39.7	39.5	39.7	40.6		6	62.9	62.8	
13	44.0	41.8	41.7	40.1	40.2	43.0	42.8	45.0					
14	55.2	57.8	57.3	56.5	56.4	55.2	54.8	56.3					
15	34.3	33.1	33.1	31.7	31.8	34.1	34.1	35.2	Glucose	1	104.6[d]	104.8	
										2	75.1	75.2	
16	84.0	82.4	82.5	80.3	80.7	81.6	81.8	85.1		3	78.2	78.1	
17	65.2	65.1	65.9	61.6	62.0	63.8	63.9	65.6		4	70.5	70.6	
18	14.1	16.8	17.1	16.5	16.2	13.9	12.8	14.4		5	77.4	77.3	
19	19.4	19.9	20.0	19.4	19.3	19.4	19.4	19.9		6	62.9	62.8	
20	106.1	41.4	43.0	42.3	41.5	104.8	104.1	107.2					
21	13.2	16.2	14.3	14.9	14.5	11.0	11.4	12.8	Xylose	1	104.1	104.3	
22	153.3	113.9	116.0	111.0	109.1	151.2	151.9	151.6		2	75.6	75.8	
23	129.5	31.2	135.5	123.0	31.4	41.1	37.6	129.5		3	78.2	78.1	
24	32.6	28.8	32.6[b]	33.0	28.7	36.6	34.5	32.4[c]		4	71.6	71.6	
25	32.3	35.0	32.6[b]	33.0	30.2	30.9	31.0	32.3		5	67.1	67.1	
26	78.1	—	—	66.1	66.7	74.9	75.0	75.8					
27	17.2	17.6	17.7	17.3	17.1	17.1	17.0	17.5	Glucose	1	104.8[d]	104.8	104.6[e]
OMe	55.5, 58.6	47.8							(C_{26}–O–)	2	75.1	75.2	75.0
										3	78.2	78.1	77.9
1′	125.6		127.6	125.3		129.7	129.3	128.4		4	71.8	71.6	71.4
2′, 6′	130.0		131.0	130.1		127.6	128.4	130.5		5	78.0	77.8	77.6
3′, 5′	112.0		113.8	112.1		112.4	113.2	113.3		6	62.9	62.8	62.6
4′	149.2		150.8	—		150.1	150.9	150.7					
7′	131.8		130.9	134.4		84.2	84.6	133.0					
$N(CH_3)_2$	40.4		41.0	40.5		40.9	41.2	40.9					

a) Chemical shifts of **4**, **5** and **9** were measured in CD_3OD and the others were measured in $CDCl_3$ at room temperature.
b—*e*) Assignments may be reversed.

diene 3-*O*-*β*-D-galactopyranosido-26-*O*-*β*-D-glucopyranoside.

Compound **6**, $C_{36}H_{51}NO_3$, showed the absorption bands of a spiroketal group at 998, 962, 936, 910, 870 cm^{-1},[5] and those of an aromatic tertiary amine at 1610, 1515 and 1340 cm^{-1}. The ^{1}H-NMR spectrum of **6** showed four methyl signals at δ 0.83 (d, 27-CH_3), 0.94 (s, 18-CH_3), 1.03 (s, 19-CH_3) and 1.10 (d, 21-CH_3) ppm, one methylene signal assignable to C_{26}-methylene at δ 3.51 ppm and one trisubstituted olefinic methine proton signal at δ 6.58 ppm. Consequently, **6** was suggested to be *p*-dimethylaminobenzylidenyldiosgenin, and the position of the *p*-dimethylaminobenzylidenyl group on diosgenin was confirmed to be C-23 by comparing the ^{13}C-NMR spectra of diosgenin, *p*-dimethylaminobenzaldehyde and **6**. The C_{23}-methylene signal of diosgenin at δ 31.4 ppm and the aldehyde carbon signal of *p*-dimethylaminobenzaldehyde at δ 189.9 ppm were not observed in the ^{13}C-NMR spectrum of **6** and new olefinic carbon signals assignable to C_{23} and $C_{7'}$ were found at δ 123.0 and 134.4 ppm, respectively. Furthermore, besides the carbon signals assignable to the *p*-dimethylaminophenyl group, the C_{22}, C_{24} and C_{25} carbon signals were shifted slightly to lower field in the ^{13}C-NMR spectrum of **6** as compared with that of diosgenin. Based on the results described above, **6** was concluded to 23-(*p*-dimethylaminobenzylidenyl)-diosgenin.

Compound **7**, $C_{36}H_{51}NO_3$, $[\alpha]_D$ +17.9° (MeOH), was obtained as a white powder and the IR spectrum showed the characteristic absorptions of a pseudo-type steroid structure at 1685 cm^{-1} and a tertiary aromatic amine at 1615, 1520 and 1345 cm^{-1}. The ^{1}H-NMR spectrum of **7** was compared with that of **6**; the $C_{7'}$-olefinic methine proton signal of **6** at δ 6.58 ppm was no longer detectable in **7**, but a double methine proton signal assignable to the etheric α-methine proton was found at δ 4.24 ppm (d, J = 10 Hz). Furthermore, the ^{1}H-NMR spectrum of **7** showed methylene signals at δ 3.23 (1H, d, J = 11 Hz) and 3.93 (1H, br d, J = 11 Hz) corresponding to C_{26}-methylene. Based on the above results, compound **7** was suggested to be a pseudo-type steroid having a pyran ring formed by ring closure between the $C_{7'}$ double bond and C_{26}-hydroxyl group. The ^{13}C-NMR spectrum of **7** did not show any carbon signals corresponding to olefinic C_{23} and $C_{7'}$, but there were two methine carbon signals at δ 41.1 and 84.2 ppm, supporting the partial pyran structure discussed above. The coupling constants (J = 10 Hz) of the $C_{7'}$-H and C_{23}-H methine protons suggested a 1,2-diaxial relation of these protons. Therefore, compound **7** was characterized as 3-hydroxy-26,7′-epoxy-23-(*p*-dimethylaminobenzyl)-25(*R*)-furost-5,20(22)-diene. The mass spectrum (MS), which showed the molecular ion peak at *m/z* 545, a fragment ion derived from the partial structure of *p*-dimethylaminobenzene and methylpyran ring at *m/z* 217 and a *p*-dimethylaminobenzaldehyde ion at *m/z* 149, also supported the proposed structure of **7**.

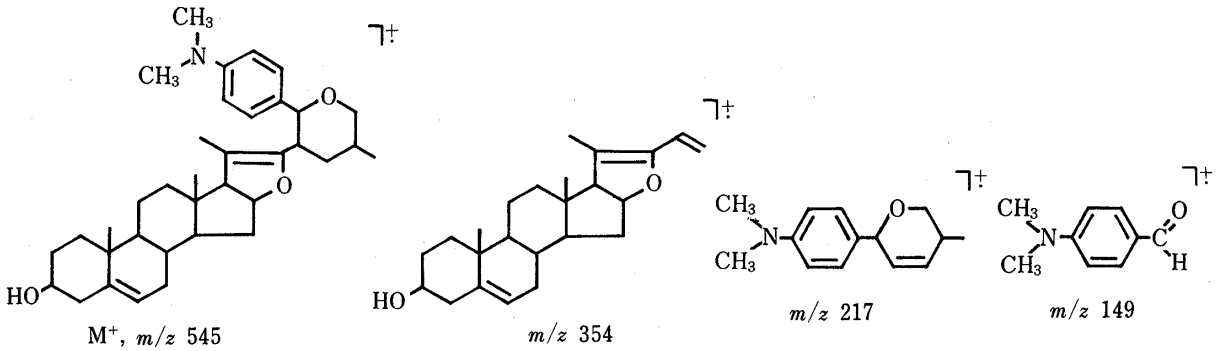

Chart 2. Mass Fragment Ions of **7** and **8**

Compound **8**, $C_{36}H_{51}NO_3$, $[\alpha]_D$ −29.0° (MeOH), was obtained as prisms from MeOH and the IR spectrum showed absorptions similar to those of **7**. As in the case of compound **7**, the ^{1}H-NMR spectrum of **8** showed the C_{26}-methylene protons at δ 3.22 (d, J = 11 Hz) and

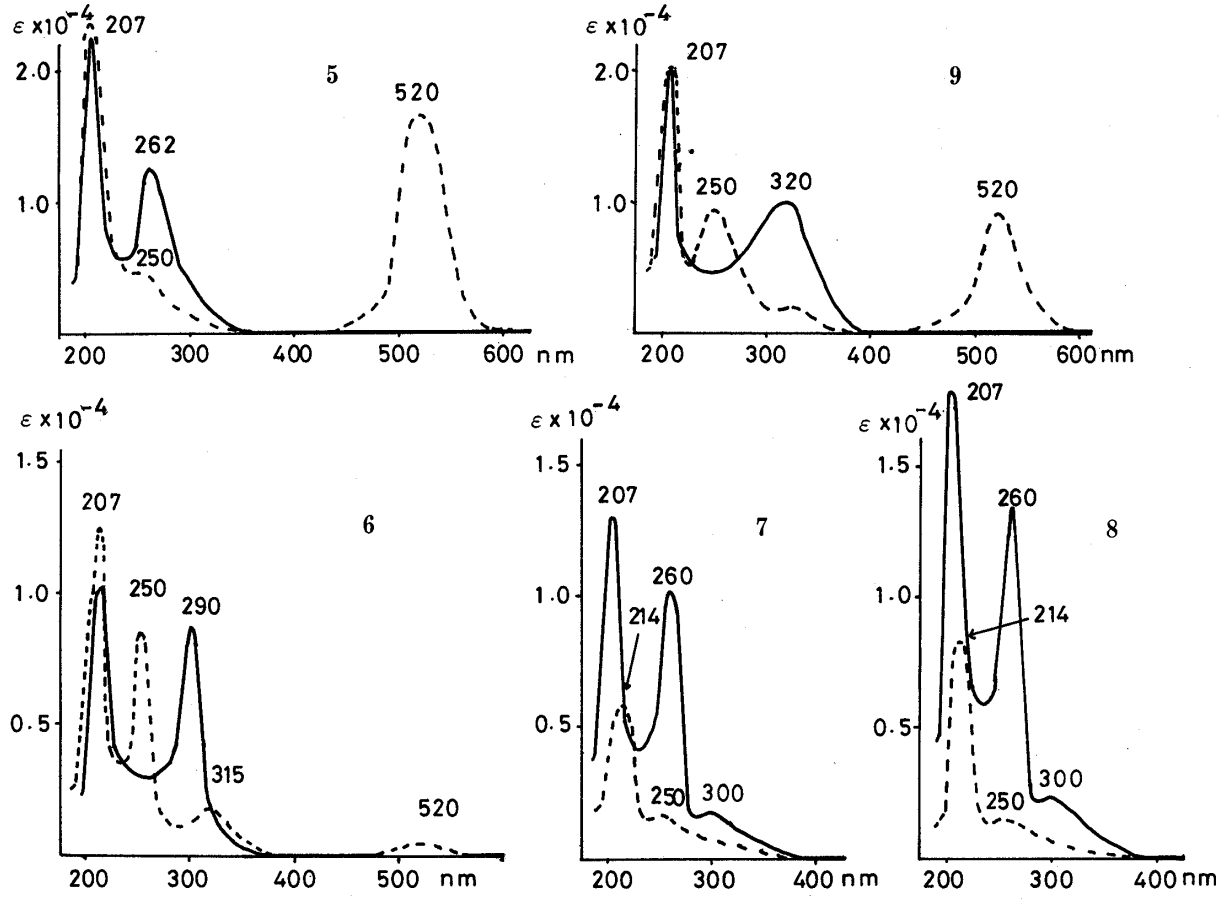

Fig. 1. Absorption Spectra of **5**, **6**, **7**, **8** and **9** in EtOH and 3% HCl–EtOH
——, neutral medium; – – – –, acidic medium.

3.92 (br d, $J=11$ Hz) ppm and an etheric methine proton at δ 4.18 (d, $J=10$ Hz) ppm. The ^{13}C-NMR spectrum of **8** also showed two methine carbons corresponding to C_{23} and $C_{7'}$ at δ 37.6 and 84.6 ppm. Comparisons of the ^{1}H- and ^{13}C-NMR spectra suggested that the structures of **7** and **8** are closely similar, and that the relation between the $C_{7'}$- and C_{23}-methine protons of **8** is 1,2-diaxial as in the case of compound **7**, but the C_{18}-methyl protons of **8** were observed at δ −0.15 ppm, *i.e.*, at higher field than those of **7**. The difference of the chemical shifts and the high field shifts of the C_{21}-methyl protons in these compounds were assumed to result from the long-range shielding effects of the aromatic ring at $C_{7'}$.[6] It could therefore be presumed that **7** and **8** are stereoisomeric at C_{23} and $C_{7'}$, but the configurations have not been confirmed because the structures are difficult to characterize with regard to spatial correlations among the oxacyclohexanyl, *p*-dimethylaminophenyl, C_{18}- and C_{21}-methyl groups.

The process of Ehrlich reaction between a furostanol glycoside, methyl proto-aspidistrin for example, and *p*-dimethylaminobenzaldehyde was suggested to be initiated by condensation between *p*-dimethylaminobenzaldehyde and the furostanol glycoside at the C_{23}-methylene group, followed by dehydration (or elimination of methanol) and/or hydrolysis of the sugar moiety to form a pseudo-diosgenin type glycoside, which is further transformed by hydrolysis of the sugar moieties to a 23-(*p*-dimethylaminobenzylidenyl)-spirostanol derivative or 26,7′-epoxy-23-(*p*-dimethylaminobenzyl)-furost-5,20(22)-diene derivatives.

The ultraviolet (UV) spectra of **5**, **6**, **7**, **8** and **9** showed absorption maxima at 262, 290, 260, 260 and 320 nm, respectively. On addition of hydrochloric acid to each solution, and the absorption maxima of **5**, **6** and **9** shifted to 520 nm, but those of **7** and **8** did not show

any bathochromic shift. The coloration mechanism of compounds **5**, **6** and **9** can therefore be reasonably explained by the formation of the iminium cation under acidic conditions as discussed in the preceding paper.[1] The formation of the iminium cation of **5** proceeds more smoothly than that of **9**, but the formation of the iminium cation of **6** in acidic solution is assumed to be restricted. Compounds **7** and **8** should not be able to form iminium cations under acidic conditions and this supports the above conclusion regarding the coloration mechanism of furostanol derivatives with Ehrlich reagent.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. The IR spectra were recorded with a Shimadzu IR-27G spectrometer, UV spectra with a Shimadzu UV-200S spectrophotometer, ^{1}H-NMR spectra with a Varian CFT-20 spectrometer at 80 MHz and ^{13}C-NMR spectra with the same apparatus at 20 MHz and with a JEOL FX-100 spectrometer at 25 MHz. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet; br, broad). The carbon signals of ^{13}C-NMR spectra were assigned on the basis of the reported spectral data for diosgenin,[7] proto-aspidistrin[4] and 3,26-dimethoxy-23-(*p*-dimethylaminobenzylidenyl)-furost-5,20(22)-diene.[1] Off-resonance spectra of each compound were also taken in order to aid assignment. Thin layer chromatography (TLC) was performed on precoated silica gel plates and detection was achieved by spraying 10% H_2SO_4 followed by heating, or under irradiation with a UV lamp. Column chromatography was carried out on silica gel (Merck), Avicel (Funakoshi Co.) and Sephadex LH-20 (Pharmacia Co.).

Mild Ehrlich Reaction of Proto-Aspidistrin (4)——A mixture of proto-aspidistrin (**4**, 2.0 g) and Ehrlich reagent (solution A (30 ml), 1% *p*-dimethylaminobenzaldehyde in ethanol; solution B (20 ml), 5% hydrochloric acid in ethanol) was kept at room temperature for 10 d and the reaction mixture was concentrated *in vacuo* at 30 °C. The concentrate was taken up in 45 ml of methanol and the methanolic solution was passed through a Sephadex LH-20 column to afford a mixture of methyl proto-aspidistrin (**4**) and compound **5**. The mixture was repeatedly subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O (70 : 23 : 10, v/v, lower phase) to give compound **5** (450 mg) and the starting material, methyl proto-aspidistrin (**4**, 500 mg). Compound **5**: A hygroscopic white powder from ethanol, (mp 222—224 °C (dec.)), $[\alpha]_D^{30}$ −73.8° (c=1.1, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3300—3500 (OH), 1610, 1520 (aromatic ring), 1350 (tertiary amine). ^{1}H-NMR (CD_3OD) δ: 0.81 (3H, s, 18-CH_3), 0.92 (3H, d, J=7 Hz, 27-CH_3), 1.04 (3H, s, 19-CH_3), 1.25 (3H, br, 21-CH_3), 2.90 (6H, s, $N(CH_3)_2$), 5.36 (1H, br, C_6-H), 6.58 (1H, s, $C_{7'}$-H), 6.74, 7.24 (each 2H, d, J=9 Hz, aromatic H). UV λ_{max}^{EtOH} nm (ε): 207 (23500), 262 (13600). UV $\lambda_{max}^{EtOH+HCl}$ nm (ε): 207 (23800), 250 (4300), 520 (17300). *Anal.* Calcd for $C_{66}H_{103}NO_{28}\cdot 2H_2O$: C, 56.84; H, 7.73; N, 1.00. Found: C, 56.64; H, 7.70; N, 0.94.

Ehrlich Reaction of Methyl Proto-Aspidistrin (4) under Heating——A mixture of proto-aspidistrin (**4**, 1.2 g) and Ehrlich reagent (solution A (20 ml), 1% *p*-dimethylaminobenzaldehyde in ethanol; solution B (10 ml), 10% hydrochloric acid in ethanol) was heated at 60 °C for 4 h. The reaction mixture was neutralized with Na_2CO_3. After removal of the precipitate by filtration, the solution was evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O (10 : 1 : 1, v/v, lower phase, or 70 : 28 : 10, v/v, lower phase) to obtain two fractions, Fr. I (730 mg) and Fr. II (435 mg). Fr. I was further subjected to column chromatography on silica gel with hexane–AcOEt (5 : 1, v/v) to afford Fr. Ia and Fr. Ib. Fr. Ia (100 mg) was chromatographed on Sephadex LH-20 with MeOH then on silica gel with hexane–AcOEt (3 : 1, v/v) to give diosgenin (20 mg) and *p*-dimethylaminobenzaldehyde. Fr. Ib (520 mg) was repeatedly chromatographed on a silica gel column with benzene–acetone (8 : 1, v/v) or hexane–AcOEt (3 : 1, v/v) to afford compound **6** (20 mg), **7** (29 mg) and **8** (62 mg). Fr. II was successively chromatographed on an Avicel column with $CHCl_3$–MeOH–H_2O (9 : 1 : 1, v/v, lower phase), on a silica gel column with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1, v/v) and on a Sephadex LH-20 column with MeOH to afford compound **9** (72 mg). Compound **6**: Prisms from MeOH, mp 148—150 °C (dec.), $[\alpha]_D^{30}$ −30.2° (c=1.7, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1610, 1515 (aromatic ring), 1340 (tertiary amine), 998, 962, 936, 910, 870 (spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, d, J=7 Hz, 27-CH_3), 0.94 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.10 (3H, d, J=7 Hz, 21-CH_3), 2.95 (6H, s, $N(CH_3)_2$), 3.51 (2H, m, C_{26}-H), 4.40 (1H, m, C_{16}-H), 5.32 (1H, br, C_6-H), 6.58 (1H, s, $C_{7'}$-H), 6.68, 7.14 (each 2H, d, J=9 Hz, aromatic H). UV λ_{max}^{EtOH} nm (ε): 207 (10500), 290 (8300). $\lambda_{max}^{EtOH+HCl}$ nm (ε): 207 (12600), 250 (8200), 315 (1900), 520 (400). MS m/z: 545 (M^+), 355 ($C_{24}H_{35}O_2$), 270 ($C_{18}H_{24}NO$), 245 ($C_{15}H_{19}NO_2$), 135 ($C_9H_{13}N$). *Anal.* Calcd for $C_{36}H_{51}NO_3\cdot 1/2H_2O$: C, 77.93; H, 9.45. Found: C, 77.89; H, 9.71. Compound **7**: A white powder from aqueous MeOH, (mp 268—271 °C (dec.)), $[\alpha]_D^{30}$ +17.9° (c=1.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1685 (–O–C=C), 1615, 1520 (aromatic ring), 1345 (tertiary amine). ^{1}H-NMR ($CDCl_3$) δ: 0.63 (3H, s, 18-CH_3), 0.84 (3H, d, J=7 Hz, 27-CH_3), 1.00 (3H, s, 19-CH_3), 1.22 (3H, s, 21-CH_3), 2.89 (6H, s, $N(CH_3)_2$), 3.23 (1H, d, J=11 Hz, C_{26}-H), 3.93 (1H, br d, J=11 Hz, C_{26}-H), 4.24 (1H, d, J=10 Hz, $C_{7'}$-H), 4.60 (1H, m, C_{16}-H), 5.33 (1H, br, C_6-H), 6.63, 7.15 (each 2H, d, J=9 Hz, aromatic H). UV λ_{max}^{EtOH} nm (ε): 207 (13000), 260 (10300), 300

(1900). $\lambda_{max}^{EtOH+HCl}$ nm (ε): 214 (5900), 250 (1600). High-resolution MS *m/z*: Calcd for $C_{36}H_{51}NO_3$: 545.38662. Found: 545.3888. MS *m/z*: 545 (M^+), 527 ($M^+ - H_2O$), 354 ($C_{24}H_{34}O_2$), 336 ($C_{24}H_{32}O$), 271 ($C_{19}H_{27}O$), 217 ($C_{14}H_{19}NO$), 149 ($C_9H_{11}NO$), 134 ($C_9H_{12}N$). Compound **8**: Prisms from MeOH, mp 242—245 °C (dec.), $[\alpha]_D^{30}$ −29.0° (*c*=1.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (–O–C=C), 1615, 1520 (aromatic ring), 1340 (tertiary amine). ^{1}H-NMR ($CDCl_3$) δ: −0.15 (3H, s, 18-CH_3), 0.84 (3H, d, *J*=6 Hz, 27-CH_3), 0.95 (3H, s, 19-CH_3), 1.39 (3H, s, 21-CH_3), 2.86 (6H, s, $N(CH_3)_2$), 3.22 (1H, d, *J*=11 Hz, C_{26}-H), 3.92 (1H, br d, *J*=11 Hz, C_{26}-H), 4.18 (1H, d, *J*=10 Hz, $C_{7'}$-H), 4.65 (1H, m, C_{16}-H), 5.31 (1H, br, C_6-H), 6.65, 7.17 (each 2H, d, *J*=9 Hz, aromatic H). UV λ_{max}^{EtOH} nm (ε): 207 (18800), 260 (13300), 300 (2300). $\lambda_{max}^{EtOH+HCl}$ nm (ε): 214 (8400), 250 (1900). High-resolution MS *m/z*: Calcd for $C_{36}H_{51}NO_3$: 545.38662. Found: 545.3835. MS *m/z*: 545 (M^+), 527 ($M^+ - H_2O$), 354 ($C_{24}H_{34}O_2$), 336 ($C_{24}H_{32}O$), 271 ($C_{19}H_{27}O$), 217 ($C_{14}H_{19}NO$), 149 ($C_9H_{11}NO$), 134 ($C_9H_{12}N$). Compound **9**: A white powder, (mp 136—138 °C (dec.)), $[\alpha]_D^{30}$ −51.2° (*c*=1.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3300—3500 (OH), 1690 (–O–C=C), 1610, 1520 (aromatic ring), 1350 (tertiary amine). ^{1}H-NMR (CD_3OD) δ: 0.79 (3H, s, 18-CH_3), 0.92 (3H, d, *J*=7 Hz, 27-CH_3), 1.06 (3H, s, 19-CH_3), 1.95 (3H, s, 21-CH_3), 2.90 (6H, s, $N(CH_3)_2$), 4.62 (1H, br, $W_{1/2h}$=12 Hz, anomeric H), 4.95 (1H, d, *J*=7 Hz, anomeric H), 5.39 (1H, br, C_6-H), 6.50 (1H, s, $C_{7'}$-H), 6.74, 7.15 (each 2H, d, *J*=9 Hz, aromatic H). UV λ_{max}^{EtOH} nm (ε): 207 (20100), 320 (10300). $\lambda_{max}^{EtOH+HCl}$ nm (ε): 207 (20100), 250 (9500), 520 (9800). *Anal.* Calcd for $C_{48}H_{71}NO_{13}$: C, 66.26; H, 8.23; N, 1.61. Found: C, 66.51; H, 8.63; N, 1.55.

UV Spectra of 5, 6, 7, 8 and 9 in Neutral or Acidic Solution——a) In Neutral Solution: An EtOH solution of the sample (1 mg/5 ml) was prepared and the solution (1 ml) was diluted with EtOH to 10 ml. The absorption spectrum of the solution was recorded directly.

b) In Acidic Solution: An EtOH solution of the sample (1 mg/5 ml) was prepared and the solution (1 ml) was diluted with 3% HCl–EtOH to 10 ml. The absorption spectrum of the solution was recorded over five min. The results of procedures a) and b) are shown in Fig. 1.

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