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Iridoids of Apocynaceae. III.[1] Minor Iridoids from *Allamanda neriifolia*[2]

FUMIKO ABE, TOMOJIRO MŌRI, and TATSUO YAMAUCHI*

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 8–19–1, Jonan-ku, Fukuoka 814–01, Japan

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New iridoids, including isoallamandicin, allamcin, allamancin, 3-*O*-methyl derivatives of allamcin and allamancin, allamcidin, allamcidin glucoside, 13-*O*-acetylplumieride, plumiepoxide, and protoplumericin B, were isolated in addition to ten known iridoids from the stem and leaves of *Allamanda neriifolia* HOOK. Their structures were determined on the basis of spectral and chemical evidence.

Gardenoside and 10-dehydrogardenoside, two ordinal-type iridoids, were also obtained.

Keywords——iridoid; plumericin; plumieride; protoplumericin; plumericin monohydrate; plumericin dihydrate; epoxyplumieride; dihydroplumieride; 13-*O*-acylplumieride; *Allamanda neriifolia*; Apocynaceae

Characteristic iridoids with a five-membered lactone ring at C-8 have been found in three genera of Apocynaceae, *Plumeria*,[3] *Allamanda*,[2,4] and *Nerium*.[5] Among these compounds, one group with a tetracyclic framework in which the five-membered lactone moiety is conjugated with an ethylidene residue at C-11, such as plumericin, isoplumericin, and allamandin, was found to be active against KB-cell culture by Kupchan *et al.*[4a] Algicidal activity of plumericin and isoplumericin was also reported.[4c]

In the preceding paper of this series, we described the isolation and the structure determination of protoplumericin A,[6] a major iridoid from the stem of *Allamanda neriifolia* HOOK, as 13-*O*-(β-D-glucopyranosyl-*p*-coumaroyl)plumieride.[2] This paper deals with the companion iridoids in the leaves and stem of the same plant.

The isolation of each iridoid from the plant materials was conducted principally in the same manner as described in the preceding paper,[2] and ten new compounds (**1**—**10**) were obtained in addition to known iridoids including plumericin (**11**), isoplumericin (**12**), allamandin (**13**), allamandicin (**14**),[4a] deglucosyl-plumieride (**15**), 13-*O*-*p*-coumaroyl-plumieride (**16**), plumieride (**17**), and protoplumericin A (**18**),[2] and two ordinal-type iridoids, gardenoside (**19**)[7] and 10-dehydrogardenoside (**20**).[8]

Isoallamandicin (**1**), mp 170—173 °C, $[\alpha]_D$ +187.5 °, was obtained from the stem extract after **11** on column chromatography, and showed the same *Rf* value as **14**. The mass spectrum (MS) showed a peak at *m/z* 308 (M^+), the same as that of **14**. In the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, however, C-11 and C-13 were observed at δ 51.6 and 64.4, respectively, upfield from the corresponding signals of **14**, at δ 56.1 and 66.2. A marked difference of coupling constants between H-10 and H-11 of **1** (J=6 Hz) and **14** (br s) suggested that **1** is an isomer of **14** at C-11. In order to confirm the structure, **1** was reacted with $POCl_3$ in pyridine as described in the case of **14**.[4a] The product was identified as **11** accompanied by a small amount of **12**. Since **11** seems to have arisen by *trans* elimination between H-11 and the hydroxyl group at C-13, the stereochemistry at C-13 is assigned as *R*.

Allamcin (**2**), mp 198—210 °C (dec.), $[\alpha]_D$ +65.6 °, *m/z* 250 (M^+, $C_{13}H_{14}O_5$) showed no carbomethoxy group in the ^{1}H- and ^{13}C-NMR spectra. From the proton nuclear magnetic

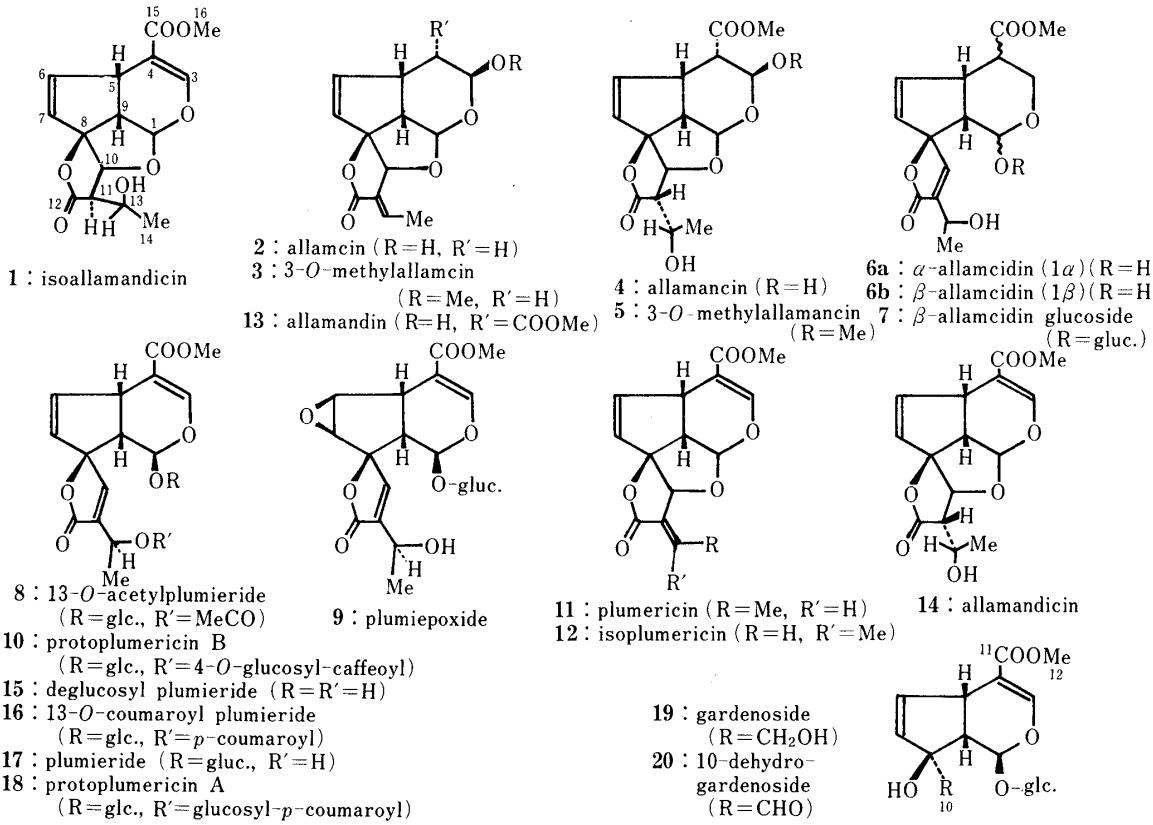

Chart 1

resonance (^{1}H-NMR) spectrum, however, **2** seemed to be a Δ^6-iridoid with an ethylidene moiety at C-11 with the same geometry as **11**, based on the characteristic doublet of quartets at δ 6.94, ascribable to the olefinic proton at C-13, and a pair of doublets of doublets at δ 6.04 (H-6) and δ 5.82 (H-7). Upon treatment of **2** with methanolic hydrochloric acid at room temperature, the acetal hydroxyl group was methylated and the methylate was identical with **3** (mp 138—140 °C, *m/z* 264, $C_{14}H_{16}O_5$) in terms of the NMR spectra. A resonance at δ 4.94, doublet of doublets (J=5, 8 Hz), in the spectrum of **2** was considered to be due to a proton attached to the acetal carbon, which was supposed to be C-3. On double irradiation of two multiplets at δ 1.55 and 1.98, the peak at δ 4.94 collapsed to a singlet, indicating it to be assignable to H-3, and the two multiplets, to H-4a and H-4b. The orientation of the hydroxyl group at C-3 is tentatively assigned to be β, as in allamandin (**13**).[4a)]

In the ^{1}H-NMR spectrum of allamancin (**4**), olefinic protons due to Δ^6 were observed at δ 5.93 as a multiplet. The chemical shifts of the peaks due to the lactone ring moiety with the C-11 side chain were identical with those of **14** in the ^{1}H- and ^{13}C-NMR spectra. The signal at δ 5.40, ascribable to a proton on the acetal carbon at C-3, was observed as a doublet (J=8 Hz) coupled with a methine proton at δ 2.78 (J=8, 5 Hz), and this feature was also seen in **13**. H-11 was observed at δ 2.73 as a triplet of J=2 Hz, which was collapsed to a doublet (J=2 Hz) by irradiation of the doublet at δ 4.83 (H-10), suggesting that the configuration of the C-11 side chain is α as in **14**. Thus, **4** was considered to be the 13-hydroxy derivative of 11,13-dihydroallamandin. Since dehydration with $POCl_3$–pyridine produced **11**, the stereochemistry at C-13 is assigned as *S*, as in the case of **14**.[4a)]

In the NMR spectra, **5** showed almost the same pattern as **4**, except for one methoxy group together with deshielding of C-3 and shielding of C-4, suggesting that **5** is 3-*O*-methyl-**4**. When **4** was reacted with methanolic HCl, the product was identified as **5**.

Although allamcidin (**6**) was obtained as prisms, the ^{1}H-NMR spectrum of **6** revealed it

TABLE I. ^{13}C-Chemical Shifts of Iridoids from *Allamanda neriifolia*, δ (ppm) from TMS[a]

	1	**14**	**2**[b]	**3**	**13**	**4**	**5**	**6a-1**	**6b-1**	**7**	**17**	**8**	**9**	**9-2**	**10**	**19**	**20**
C-1	102.0	102.3	98.5	98.3[c]	99.1	98.7	98.6	93.6	89.6	98.1	94.0	93.6	93.0	198.5	93.7	94.3	93.5
C-3	152.8	153.3	89.6	99.0[c]	92.0	91.6	99.1	62.0	57.2	56.0	152.0	152.2	153.5	156.9	152.1	151.2	151.4
C-4	108.1	109.2	30.0	28.8	47.1[c]	47.0[c]	45.2[c]	44.8[c]	44.4	44.3	109.5	109.3	106.7	112.7	109.4	110.6	111.1
C-5	38.1	38.5	38.4	39.1	43.1[c]	43.0[c]	42.6[c]	44.6[c]	38.3	38.3	40.1	40.4	32.3	42.6[c]	40.3	38.2	38.4
C-6	126.2	128.2	129.1	130.2	131.5	132.4	132.6	131.4	131.5	131.7	129.1	128.3	57.1[c]	60.2	128.4	133.9	132.3
C-7	141.4	140.3	142.0	142.2	139.2	138.2	137.9	139.5	139.5	141.3	140.9	141.9	58.7[c]	81.6	141.6	137.0	136.8
C-8	105.9	107.3	103.3	103.6	103.0	105.0	104.8	97.2	97.5	97.5	96.4	96.7	91.3	94.1	96.7	85.7	90.5
C-9	53.5	54.0	51.2	53.3	54.1	53.7	53.6	49.8	48.3	50.0	49.9	50.3	43.1	42.2[c]	50.3	52.2	54.6
C-10	84.0	84.2	78.7	80.2	80.3	83.4	83.7	149.1	149.3	148.3	149.1	150.6	146.0	146.4	148.8	67.0	201.1
C-11	51.6	56.1	128.3	129.3	129.2	56.1	56.1	135.1	135.1	140.0	138.7	133.3	141.9	143.6	133.6	167.1	166.9
C-12	175.9	177.4	167.9	168.6	172.8[d]	177.4	177.3	171.6[d]	171.6[c]	171.8[c]	171.3	170.2	170.7	171.2	170.2	51.0	51.2
C-13	64.4	66.2	143.2	143.7	143.9	66.1	66.2	65.5	65.5	62.9	62.7	65.2	62.8	63.2	65.1		
C-14	22.2	22.3	15.5	15.7	15.8	22.3	22.3	19.4	19.6	23.3	23.0	19.1	22.9	23.1	19.4		
C-15	166.7	167.0		(3-OMe)	171.6[d]	172.8	172.1	170.4[d]	170.3[c]	170.9[c]	166.7	166.7	166.3	164.7	166.6[c]		
C-16	51.3	51.6		55.0	52.1	52.1	52.2	52.2	52.2	51.9	51.2	51.3	51.3	51.0	51.2	51.0	51.2
C-1′							(3-OMe)	(–OAc)	(–OAc)	104.7	100.8	100.3	100.8		100.5	100.5	100.6
C-2′							55.5	20.7	20.5	75.0	74.7	74.7	74.4		74.7	74.6	74.5
C-3′								20.8	20.8	78.8	78.7	78.8	78.5		79.0	78.5	78.6
C-4′								169.2[d]	169.2[c]	71.0	70.7	71.3	70.7		71.0	71.1	71.1
C-5′								169.8[d]	169.8[c]	78.6	78.1	78.0	78.2		78.2	78.3	78.2
C-6′										62.4	62.1	62.3	61.8		62.1	62.2	62.3
C-α[e]												(–OAc)			116.6		
C-β[e]												21.0			145.8		
C-1′′[f]												170.2			130.2		
C-2′′															116.6		
C-3′′															149.4		
C-4′′															149.4		
C-5′′															118.1		
C-6′′															121.1		
C-1′′′[g]															103.4		
C-2′′′															74.7		
C-3′′′															79.0		
C-4′′′															71.3		
C-5′′′															78.3		
C-6′′′															62.4		
															166.2[c] (ester CO)		

a) Dissolved in pyridine-d_5, unless otherwise mentioned.
b) Dissolved in DMSO-d_6.
c, *d*) Assignments marked *c*) or *d*) in any column may be reversed.
e) C-α and C-β mean the α and β carbons, respectively, of caffeic acid.
f) C-1′′—C-6′′ mean the aromatic carbons of caffeic acid.
g) C-1′′′—C-6′′′ mean carbons of the second glucose attached to caffeic acid. The chemical shift assignment of each carbon may be interchanged with that of C-1′—C-6′

TABLE II. ^{1}H-Chemical Shifts of Iridoids from *Allamanda neriifolia*[a)]

	1-H	3-H	5-H	6-H	7-H	9-H	10-H	11-H	13-H	14-H	-COOMe	Others[d)]
1	5.58 (6)	7.42 (s)	3.97 (dt, 9, 2)	6.12 (5, 2)	5.66 (5, 2)	3.43 (9, 6)	4.58 (6)	2.78 (9, 6)	4.27 (m)	1.39 (6)	3.78	3.86 (br s, 13-OH)
14	5.48 (6)	7.34 (s)	3.93 (dt, 9, 2)	5.90 (5, 2)	5.72 (5, 2)	3.37 (9, 6)	4.72 (br s)	2.75 (3)	4.42 (dq, 3, 7)	1.37 (7)	3.73	2.59 (br s, 13-OH)
2[b)]	5.54 (5)	4.94 (5, 8)	3.28 (m)	6.04 (6, 2)	5.82 (6, 2)	2.90 (5, 8)	5.03 (br s)		6.94 (dq, 2, 7)	1.97 (7)		1.55 (m, 4-Ha), 1.98 (m, 4-Hb) 3.30 (br s, 3-OH)
3[c)]	5.71 (4)	4.77 (6, 8)	3.17 (m)	5.98 (5, 2)	5.88 (5, 2)	2.98 (4, 9)	5.29 (br s)		7.07 (dq, 2, 7)	1.88 (7)		1.50—2.30 (2H, m, 4-H_2) 3.37 (3H, s, 3-OMe)
4	5.67 (4)	5.40 (8)	3.58 (m)	5.93 (2H, m)		3.07 (8, 4)	4.83 (2)	2.73 (t, 2)	4.45 (m)	1.38 (6)	3.79	2.78 (8, 5, 4-H)
5	5.54 (4)	4.99 (8)	3.52 (m)	5.90 (2H, m)		3.04 (8, 4)	4.83 (2)	2.77 (2)	4.48 (m)	1.39 (6)	3.79	2.74 (8, 4, 4-H) 3.43 (3H, s, 3-OMe)
6a-1	5.80 (9)	3.80 (2H, m)	3.32 (m)	6.17 (6, 2)	5.54 (6, 1)	2.44 (9, 11)	7.03 (2)		5.58 (dq, 2, 6)	1.49 (6)	3.74	2.08, 2.10 (3H, s, -OAc) 2.80 (m, 4-H)
6b-1	6.42 (3)	3.49 (13, 1) 3.94 (13, 4)	3.56 (m)	6.38 (6, 2)	5.61 (6, 1)	2.48 (3, 11)	7.01 (2)		5.64 (dq, 2, 6)	1.48 (6)	3.73	2.09 (6H, s, 2×-OAc) 2.82 (m, 4-H)
7[c)]	5.83 (3)	3.47 (13, 1) 4.58 (13, 4)		6.42 (6, 2)	5.63 (6, 1)	2.80 (3, 10)	7.74 (2)		5.05 (dq, 2, 6)	1.67 (6)	3.83	2.69 (m, 4-H) 5.02 (8, 1′-H)
17[c)]	5.58 (6)	7.60 (2)		6.44 (6, 2)	5.40 (6, 2)	3.06 (6, 8)	7.90 (2)		4.96 (dq, 2, 6)	1.61 (6)	3.63	5.32 (7, 1′-H)

8[c]	5.57 (6)	7.61 (2)		6.43 (5, 2)	5.37 (5, 2)	2.99 (t, 6)	7.91 (2)	5.88 (dq, 2, 7)	1.50 (7)	3.64	2.06 (3H, s, -OAc) 5.37 (8, 1′-H)
9[c]	5.87 (br s)	7.71 (1)		3.47 (2)	4.24 (2)	3.22 (9, 1)	7.48 (2)	4.95 (dq, 2, 6)	1.59 (6)	3.60	5.18 (8, 1′-H)
9-1	5.00 (2)	7.46 (2)	3.36 (8, 2)	3.26 (2)	4.02 (2)	2.91 (8, 2)	6.82 (2)	5.61 (dq, 2, 7)	1.50 (7)	3.78	1.90, 1.96, 1.98, 2.04 2.07 (-OAc)
9-2[c]	9.98 (2)	7.52 (2)	4.50 (m)	5.59 (6, 11)	4.77 (6)	3.53 (7, 2)	7.84 (2)	5.03 (dq, 2, 6)	1.67 (6)	3.63	
10[c]	5.60 (6)	7.60 (2)		6.43 (6, 2)	5.40 (6, 2)	3.04 (6, 9)	7.97 (1)	6.06 (dq, 1, 6)	1.62 (6)	3.64	5.36, 5.55 (8, 1′- and 1‴-H) 6.68 (16, α-H), 7.07 (8, 2, 6″-H) 7.47 (8, 5″-H), 7.54 (2, 2″-H) 7.94 (16, β-H)
10-1		6.94 (2)		6.41 (6, 3)	5.43 (6, 1)	3.14 (2, 9)	7.36 (1)	5.74 (dq, 1, 6)	1.57 (6)	3.74	1.90—2.28 (9 × -OAc), 6.31 (16, α-H) 7.32 (8, 5″-H), 7.26 (2, 2″-H) 7.61 (16, β-H)
10-2[c]		7.62 (2)		6.43 (6, 2)	5.38 (6, 2)	3.01 (t, 6)	8.02 (1)	6.09 (dq, 1, 6)	1.63 (6)	3.65	3.84 (3H, s, 3″-OMe), 6.77 (16, α-H) 7.43 (8, 2, 6″-H), 7.94 (16, β-H)
19[c]	6.48 (2)	7.63 (2)		6.37 (6, 2)	6.16 (6, 2)	3.25 (9, 2)				3.58	5.33 (7, 1′-H)
20[c]	6.10 (3)	7.59 (2)		6.61 (6, 3)	5.82 (6, 2)	3.32 (8, 3)	9.95 (s)			3.60	5.27 (8, 1′-H)

a) δ (ppm) in $CDCl_3$, unless otherwise mentioned (*J*/Hz values in parentheses).
b) Dissolved in DMSO-d_6. *c*) Dissolved in pyridine-d_5.
d) For numbering in **10**, **10-1**, and **10-2**, see footnotes *e*—*g*) in Table I.

to be a mixture. Upon acetylation of **6**, two acetates **6a-1** and **6b-1** were obtained, and fractionated by column chromatography. In both compounds, peaks due to the five-membered lactone moiety were almost the same in both the ^{1}H- and ^{13}C-NMR spectra. An olefinic proton at H-10 (δ 7.03, d, $J=2$ Hz in **6a-1**; 7.01, d, $J=2$ Hz in **6b-1**) showed a long-range coupling with H-13. Carbomethoxy and Δ^6 functions were observed in both compounds. On the basis of a triplet carbon signal assignable to C-3 in the ^{13}C-NMR spectra of **6a-1** (δ 62.0) and **6b-1** (δ 57.2), **6a** and **6b** were both considered to be 3,4-dihydro derivatives of **15**, and were named α-, and β-allamcidin, respectively. Among them, **6a** was assigned as the 1α-, and **6b** as the 1β-hydroxy compound, based on the coupling constants between H-1 and H-9 (9 Hz and 3 Hz, respectively). The shielding of C-3 and C-5 of **6b-1** in the ^{13}C-NMR spectrum seemed to be due to a 1,3-diaxial relation between the 1β-hydroxyl group and H-3, or H-5. The configuration at C-13 is tentatively assigned as *S* as in **17** and **18**, the major iridoids in this plant.

In the ^{1}H-NMR spectrum of **7**, protons due to 14-CH_3 (δ 1.67), a carbomethoxy group (δ 3.83) and the olefinic linkage at C-6 and C-7 (δ 6.42 and 5.63) were observed. The ^{13}C-NMR spectrum of **7** indicated the presence of a glucose moiety (δ 104.7, 78.8, 78.6, 75.0, 71.0, and 62.4). The evidence from ^{1}H- and ^{13}C-NMR as well as the field desorption (FD)-MS ($M^+ + 1$, *m/z* 473, $C_{21}H_{28}O_{12}$, $M^+ -$ glc., 292) indicated **7** to be an iridoid glucoside. The carbons due to the aglycone moiety of **7** were comparable to those of **17**, except for C-3 and C-4. Therefore, **7** was considered to be a glucoside of **6**. Since the chemical shifts of C-13 in **7** and **17** were almost the same, and the coupling constants of H-1 and of the anomeric proton were 3 Hz and 8 Hz, respectively, **7** was assigned as the 1-*O*-β-D-glucoside of **6b**, that is, 3,4-dihydroplumieride. Upon hydrolysis of **7** with β-glucosidase followed by acetylation, the product was identified as **6b-1** on thin layer chromatography (TLC).

Plumieride 13-*O*-acetate (**8**), $[\alpha]_D$ $-37.8°$, *m/z* 513 ($M^+ + 1$, $C_{23}H_{28}O_{13}$), was obtained as a solid from the leaves and stem in rather good yield. The presence of one acetyl residue was seen in the ^{1}H- and ^{13}C-NMR spectra, in addition to the other functions observed in the spectra of **17**, suggesting that **8** is plumieride monoacetate. Since C-13 was deshielded by +2.5 ppm, and C-11 and C-14 were shielded by -5.4 ppm and -3.9 ppm, respectively, the acetyl group was assigned to the 13-hydroxyl group. Upon hydrolysis with β-glucosidase, **8** afforded **11**, as in the case of **18**. The acetylation of **8** gave **17**-pentaacetate.

Plumiepoxide (**9**) was obtained as crystals, mp 224—225 °C, $[\alpha]_D$ $-129.5°$, showing similar *Rf* values to **7** or **17** on TLC, and provided a pentaacetate (**9-1**) upon acetylation. In the ^{1}H-NMR spectrum of **9**, no olefinic protons ascribable to Δ^6 were observed, whereas a pair of doublets at δ 3.47 and 4.24 coupled with each other ($J=2$ Hz) was observed in addition to other signals due to H-3, H-10, H-13, and an anomeric proton (δ 5.18, d, $J=8$ Hz). The ^{13}C-NMR spectrum of **9** also showed two doublets at δ 57.1 and 58.7 but no resonances due to Δ^6. The evidence from the NMR spectra and FD-MS ($M^+ + 1$, *m/z* 487, $C_{21}H_{26}O_{13}$) suggested that **9** was a 6,7-epoxide of **17**. In order to confirm this structure, epoxidation of **17** was attempted. From the reaction mixture of **17**-acetate with *m*-chloroperbenzoic acid in $CHCl_3$, three products were isolated. The major product (oxide 2) and one of the minor compounds (oxide 3) were considered to be the 3,4- and 10,11-epoxides, respectively, on the basis of ^{1}H-NMR evidence. The third product (oxide 1), which was isolated as a solid after repeated column chromatographies, provided a peak at *m/z* 697 ($M^+ + 1$). The ^{1}H-NMR spectrum showed no olefinic protons due to Δ^6, suggesting that this product was the 6,7-epoxide of 17-acetate. Since the ^{1}H-NMR spectrum was identical with that of **9-1**, **9** was determined to be the 6,7-epoxide of **17**. The orientation of the epoxide ring was assigned as β, since the approach of the oxidation reagent seems to be easier on the β-side than on the α-side. The fact that no coupling was observed between H-5 and H-6 in **9-1** is consistent with this assignment. Although the chemical shifts of H-13 in the 13-*R* and 13-*S* configurations have almost the

same values,[8a] the stereochemistry at C-13 in **9** is tentatively assigned as *S*, as in the cases of **17** and **18**.

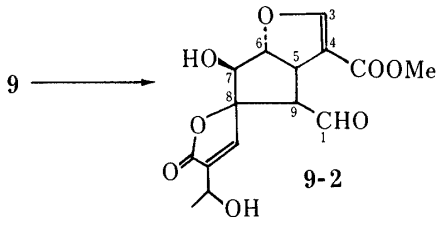

Chart 2

When **9** was subjected to enzymatic hydrolysis, the aglycone (**9-2**) no longer showed the two methine peaks due to H-6 and H-7 in the ^{1}H-NMR spectrum. Instead, one formyl (δ 9.98, d) and two carbinyl (δ 4.77, d, and δ 5.59, dd) protons were found in addition to the carbinyl proton attached to C-13 and two olefinic protons, indicating the opening of the pyran ring. Each proton on the cyclopentane ring was assigned by means of decoupling experiments involving the irradiation of the formyl proton, followed by H-9, H-5, H-6 and H-7. On irradiation of the methine proton at δ 5.03, assignable to H-13, the 14-methyl protons at δ 1.67 and one olefinic proton at δ 7.84 (d, H-10) were each transformed into a singlet. The five-membered lactone ring attached at C-8 was therefore considered to be retained. In the ^{13}C-NMR spectrum, a remarkable downfield shift of C-1 (+105.5 ppm) was observed in addition to the deshielding of C-6 and C-7. The evidence from NMR and high resolution MS ($C_{15}H_{16}O_8$) suggests the structure to be as represented in Chart 2.

Protoplumericin B (**10**), the most polar compound among the iridoids described in this series, was obtained as a solid in a small amount by successive column chromatographies and droplet counter current chromatographies (DCCC). On TLC and column chromatographies, **10** showed a polarity quite similar to that of **18**. Upon acetylation, **10** afforded a nonaacetate (**10-1**), while **18** gave an octaacetate. The presence of a phenyl propenoyl moiety in **10** was suggested not only by the ultraviolet (UV) maxima at 288 and 320 nm, but also by a pair of doublets at δ 6.68 and 7.94 that are coupled with each other (J = 16 Hz), and phenyl proton signals at δ 7.07, 7.47, and 7.54 in the ^{1}H-NMR spectrum. The plumieride moiety was confirmed by ^{13}C-NMR. Since **10** showed a brown color with $FeCl_3$ reagent on TLC and gave a monomethylate (**10-2**) with CH_2N_2 in methanolic solution, **10** was considered to be 13-*O*-(glucosyl-caffeoyl)plumieride.

In order to identify the hydroxyl group carrying the second glucose moiety, **10-2** was subjected to hydrolysis with $NaOCH_3$ to give a glucoside of methyl monomethylcaffeate, which was further cleaved to glucose and methyl ferulate upon acid hydrolysis. Based on the coupling constant of the anomeric proton, the linkage of the glucosyl residue was considered to be β. The structure of **10** was hence elucidated as 13-*O*-(*p*-β-D-glucopyranosyl-caffeoyl)plumieride.

Compounds **19** and **20** appeared to be ordinal C_{10}-iridoids because the staining with H_2SO_4 reagent on TLC was quite different from that of plumieride-type iridoids. The NMR spectra of **19** suggested the presence of $-COOCH_3$ and $-\overset{|}{C}-CH_2OH$, in addition to Δ^3 and Δ^6 functions, and a glucose moiety at the C-1 hydroxyl group. On the bases of spectral and biogenetical considerations, **19** was considered to be gardenoside, and this was finally confirmed by comparison of the NMR spectra and TLC behavior with those of an authentic sample of gardenoside isolated from the fruits of *Gardenia jasminoides*.[7]

From the NMR spectra, it was clear that **20** also has a glucosyl moiety and a formyl group, along with a carbomethoxy residue and Δ^3, and Δ^6 functions, but no primary alcohol. Since **20** was transformed into **19** on reduction with $NaBH_4$ in MeOH, **20** was proved to be

10-dehydrogardenoside.[8)]

In addition to the known compounds, eight new iridoids and two 3-*O*-methyl derivatives were isolated from the leaves and stem of *Allamanda neriifolia*. Among them, four compounds, protoplumericins A and B, and 13-*O*-acetyl- and 13-*O*-coumaroylplumieride, are characterized as "protoplumericins" on the basis of their facile conversion into plumericin on enzymatic hydrolysis.

The framework of the iridoids with the five-membered lactone ring at C-8 (such as **17**) is considered to be produced biogenetically from **20** with an additional four-carbon unit. Following the biogenetic hypothesis, **17** was chemically synthesized by Inoue *et al.*[8a)] from geniposide *via* **20**. This report, however, is the first to show that **19** and **20** exist in a plant containing plumieride.

Experimental

^{1}H- and ^{13}C-NMR, and UV spectral measurements were taken with the same machines in the same manner as described in the preceding paper.[2)] MS and infrared (IR) spectra were recorded on a JEOL JMS DX-300 mass spectrometer and a JASCO A-100 infrared spectrophotometer, respectively. Melting points were measured on Kofler block and are uncorrected. For column, thin layer, and DCC chromatographies, the following solvent systems were applied; solv. 1: $CHCl_3$–MeOH–H_2O (bottom layer), solv. 2: benzene–acetone, solv. 3: EtOAc–MeOH–H_2O (top layer), solv. 4: EtOAc–hexane, solv. 5: $CHCl_3$–MeOH–H_2O (5:6:4, for DCCC, ascending), solv. 6: *n*-BuOH–AcOH–H_2O (4:1:5, top layer) for paper chromatography (PC). In order to detect spots on TLC, 10% H_2SO_4 was sprayed and the plate was charred in an oven.

Extraction and Fractionation——The plant was cultivated at Ibusuki Experimental Station of Kyushu University, and was harvested in March, 1982. The dried powdered stem (2.6 kg) and leaves (6.7 kg) were separately percolated with MeOH. The MeOH percolates were concentrated *in vacuo* and partitioned with benzene, $CHCl_3$, and then with *n*-BuOH, in the same manner as described in the preceding paper.[2)] Each fraction was subjected to column chromatographies with MCI CHP20P (Mitsubishi Chem. Ind. Ltd.)/H_2O–MeOH, silica gel/solv. 1—4, Sephadex LH20/$CHCl_3$–MeOH, and DCCC, and **1**—**10** were obtained in addition to the known compounds **11**—**20**. Among the new compounds, **1**—**6** were principally obtained from the benzene fraction, **8**, from the $CHCl_3$ fraction, and **7**, **9**, and **10**, from the BuOH fraction.

Isoallamandicin (1)——On crystallization from MeOH, **1** was obtained as prisms (yield: 10 mg from stem), mp 170—173 °C, $[\alpha]_D^{18}$ +187.5° (c=0.16, MeOH), MS m/z: 308.089 (Calcd for $C_{15}H_{16}O_7$: 308.089). A small amount of $POCl_3$ was added to a solution of **1** (5 mg) in 2 ml of pyridine. The mixture was allowed to stand at 0 °C for 1 h, then diluted with ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness and the residue was crystallized from hexane–EtOAc to give prisms, mp 195—198 °C. On admixture with authentic **11**, no melting point depression was observed, and the IR spectra of the two samples were identical (ν_{max}^{KBr} cm^{-1}: 3100, 2960, 2920, 1760, 1710, 1690, 1650, 1620).

Allamcin (2)——On crystallization from MeOH, **2** was obtained as needles (230 mg from leaves), mp 198—210 °C (dec.), $[\alpha]_D^{20}$ +65.6° (c=0.50, pyridine), *Anal.* Calcd for $C_{13}H_{14}O_5$: C, 62.40; H, 5.64. Found: C, 62.47; H, 5.54. UV λ_{max}^{MeOH} nm (ε): 214 (22700). MS m/z: 250 (M^+), 232, 211, 153, 136, 98. Acetylation of **2** was carried out in the usual manner with Ac_2O and pyridine to give **2**-acetate (**2-1**) as a solid. A solution of **2** (20 mg) in methanolic HCl (2 N) was allowed to stand at room temp. for 5 h. The solution was deacidified with Amberlite IR-410 and the MeOH was evaporated off *in vacuo*. The residue was crystallized from MeOH to give needles, mp 136—138 °C; this product was identified as **3** by mixed melting point determination and TLC (solv. 2, 4:1; solv. 4, 3:1).

3-*O*-Methylallamcin (3)——On crystallization from MeOH, **3** was obtained as needles (30 mg from leaves), mp 138—140 °C, $[\alpha]_D^{18}$ +48.4° (c=0.29, MeOH). UV λ_{max}^{MeOH} nm (ε): 215 (15800). MS m/z: 264.100 (Calcd for $C_{14}H_{16}O_5$: 264.100).

Allamancin (4)——From the stem, **4** was obtained as a solid (102 mg), $[\alpha]_D^{18}$ +74.5° (c=0.80, $CHCl_3$). MS m/z: 326 (M^+, $C_{15}H_{18}O_8$), 308, 280, 264, 221, 160, 110, 103. After methylation of **4** with methanolic HCl (2 N) for 5 h at room temp., the reaction mixture was deacidified. The product was purified on a silica gel column. The ^{1}H-NMR spectrum and *Rf* values on TLC (solv. 2, 4:1; solv. 4, 3:1) were in good agreement with those of **5**. A small amount of $POCl_3$ was added to a solution of **4** (10 mg) in pyridine at 0 °C. The mixture was allowed to stand at room temp. for 1 h, then diluted with ice-water, followed by extraction with $CHCl_3$. The $CHCl_3$ extract was crystallized from MeOH to give prisms, mp 209—212 °C. On admixture with authentic **11**, no melting point depression was observed and the IR spectra of the two samples were in good agreement.

3-*O*-Methylallamancin (5)——Compound **5** was obtained as a solid from the leaves (41 mg), $[\alpha]_D^{18}$ +112.3° (c=1.64, $CHCl_3$). MS m/z: 340 (M^+, $C_{16}H_{20}O_8$), 309, 280, 221, 159, 134.

Allamcidin (6)——Compound **6** was isolated from the leaves and crystallized from EtOH to give prisms (125 mg). MS *m/z*: 310 (M^+, $C_{15}H_{18}O_7$), 292, 246, 187, 160.

Acetates of 6a (Allamcidin A) (6a-1) and 6b (Allamcidin B) (6b-1)——Diacetates, **6a-1** and **6b-1**, were obtained by usual acetylation of **6** (60 mg) with Ac_2O and pyridine at room temp., followed by chromatography on a silica gel column with solv. 2 (15 : 1). **6a-1** (solid): $[\alpha]_D^{18}$ +35.0° (*c*=0.60, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 213 (20000). MS *m/z*: 335 (M^+ −AcO, M^+: $C_{19}H_{22}O_9$: 394), 292, 274, 173, 160. **6b-1**: mp 161—163 °C (crystallized from EtOAc–hexane), $[\alpha]_D^{18}$ +38.0° (*c*=0.30, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 213 (18000). MS *m/z*: 335 (M^+ −AcO, M^+: $C_{19}H_{22}O_9$: 394), 274, 173, 160.

Allamcidin B β-D-Glucoside (7)——Compound **7** was isolated from the stem by successive chromatographies on silica gel/solv. 1 (7 : 2 : 1.4) and DCCC (solv. 5), and obtained as a crystalline powder (17 mg), mp 135—138 °C, $[\alpha]_D^{16}$ +49.4° (*c*=0.75, MeOH). *Anal.* Calcd for $C_{21}H_{28}O_{12}\cdot 2H_2O$: C, 49.61; H, 6.34. Found: C, 49.96; H, 6.76. UV λ_{max}^{MeOH} nm (ε): 212 (25000). FD-MS *m/z*: 473 (M^++1, $C_{21}H_{28}O_{12}$), 292 (M^+ −glucose). Upon usual acetylation, it gave a pentaacetate (**7-1**) as a solid. An aqueous solution of **7** (5 mg/1 ml) was shaken overnight with 30 mg of Cellulase (Sigma; preparative type II) at 38 °C. The mixture was extracted with *n*-BuOH. The BuOH extract was acetylated and the product showed the same *Rf* values as **6b-1** on TLC (solv. 1, 7 : 2 : 1, solv. 4, 1 : 1).

Plumieride 13-*O*-Acetate (8)——Compound **8** was obtained as a solid from the stem (760 mg) and the leaves (*ca.* 2 g), $[\alpha]_D^{16}$ −37.8° (*c*=2.07, MeOH). UV λ_{max}^{MeOH} nm (ε): 210 (20500), 235 (sh) (13700). FD-MS *m/z*: 513 (M^++1, $C_{23}H_{28}O_{13}$). A solution of **8** in 20% EtOH (50 mg/2 ml) was shaken overnight with 200 mg of Cellulase. The mixture was extracted with *n*-BuOH. The BuOH extract was subjected to column chromatography on silica gel with solv. 1 (20 : 1), followed by crystallization from MeOH to give prisms, mp 215—217 °C. On admixture with authentic **11**, no melting point depression was observed and the IR spectra of the two samples were in good agreement. Acetylation of **8** (50 mg) was conducted in the usual manner. The ^{1}H-NMR spectrum and TLC behavior (solv 4, 2 : 1) of the acetate (solid) (**8-1**) were identical with those of **17**-pentaacetate.

Plumiepoxide (9)——Compound **9**, showing a slightly more polar *Rf* value than that of **8** on TLC, was obtained by successive column chromatographies on silica gel (solv. 1, 7 : 2 : 1.2) and DCCC (solv. 5), and crystallized from EtOH to give needles (7 mg from stem, 374 mg from leaves), mp 224—225 °C, $[\alpha]_D^{20}$ −129.5° (*c*=0.28, MeOH). *Anal.* Calcd for $C_{21}H_{26}O_{13}\cdot 1/2H_2O$: C, 50.91; H, 5.49. Found: C, 50.74; H, 5.54. UV λ_{max}^{MeOH} nm (ε): 222 (27000). FD-MS *m/z*: 487 (M^++1). The pentaacetate of **9** (**9-1**) was obtained as a solid by usual acetylation.

A solution of **9** in 20% EtOH (50 mg/2 ml) was shaken overnight with Cellulase (200 mg). The mixture was extracted with *n*-BuOH, and the BuOH extract was subjected to column chromatography on a silica gel column. The major product (**9-2**) was obtained as a solid, MS *m/z*: 324.085 (Calcd for $C_{15}H_{16}O_8$: 324.085).

Conversion of 17-Acetate (17-1) into 9-Acetate (9-1)——A solution of **17-1** (250 mg) in $CHCl_3$ (10 ml) was treated with *m*-chloroperbenzoic acid (600 mg). The mixture was allowed to stand at room temp. for 27 h and then diluted with $CHCl_3$. The solution was washed with H_2O, and the $CHCl_3$ was evaporated off *in vacuo*. The residue was subjected to chromatography on a silica gel column with solv. 2 (10 : 1—4 : 1). From the first fraction, **17-1** was recovered (120 mg). From the second fraction, after successive column chromatographies with solv. 2 (10 : 1—3 : 1) and solv. 4 (1.5 : 1), oxide 1 (3 mg) was isolated as a solid. FD-MS *m/z*: 697 (M^++1). The ^{1}H-NMR spectrum of oxide 1 was identical with that of **9-1**. From the third fraction, oxide 2 (50 mg) was isolated and crystallized from hexane–EtOAc to give prisms, mp 170—171 °C, which showed no olefinic proton signal ascribable to Δ^3: ^{1}H-NMR ($CDCl_3$) δ: 1.54 (3H, d, *J*=7 Hz, 14-CH_3), 1.99 (6H, s, 2×OAc), 2.02, 2.07, 2.09 (3H each, s, OAc), 3.84 (3H, s, –$COOCH_3$), 4.63 (1H, s, H-3), 5.58 (1H, dd, *J*=5, 2 Hz, H-7), 5.66 (1H, dq, *J*=2, 7 Hz, H-13), 6.18 (1H, dd, *J*=5, 2 Hz, H-6), 7.37 (1H, d, *J*=2 Hz, H-10). From the fourth fraction, oxide 3 (10 mg) was obtained as a solid, which exhibited no olefinic proton signal ascribable to Δ^{10}: ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, d, *J*=7 Hz, 14-CH_3), 2.00 (6H, s, 2×OAc), 2.05 (9H, s, 3×OAc), 3.87 (3H, s, –$COOCH_3$), 7.49 (1H, d, *J*=2 Hz, H-3).

Protoplumericin B (10)——Compound **10** was accompanied by a large amount of **18**. Its isolation from **18** was conducted by successive applications of DCCC (solv. 5), and 70 mg of **10** was obtained from the leaves as a solid, $[\alpha]_D^{16}$ −61.2° (*c*=1.41, MeOH). UV λ_{max}^{MeOH} nm (ε): 206 (33500), 230 (22000), 288 (13500), 320 (11800). FD-MS *m/z*: 817 (M^++Na, $C_{36}H_{42}O_{20}$: 794). A solution of **10** in H_2O (5 mg/1 ml) was shaken at 38 °C with 20 mg of Cellulase for 10 h. The mixture was extracted with *n*-BuOH and the BuOH extract was examined by TLC. The *Rf* values of the product were in good agreement with those of **11** in solv. 2 (10 : 1) and solv. 4 (1 : 2). The nonaacetate of **10** (**10-1**) was obtained as a solid by the usual acetylation with Ac_2O and pyridine, $[\alpha]_D^{16}$ −74.5° (*c*=2.20, MeOH). FD-MS *m/z*: 1173 (M^++1, $C_{54}H_{60}O_{29}$).

Monomethylate of 10 (10-2)——A solution of **10** (30 mg) in MeOH (2 ml) was treated with CH_2N_2 in ether. The mixture was concentrated *in vacuo* and the residue was purified by chromatography on a silica gel column. The monomethylate (**10-2**) was obtained as a solid, $[\alpha]_D^{16}$ −49.2° (*c*=1.18, MeOH). UV λ_{max}^{MeOH} nm (ε): 206 (34700), 230 (22000), 283 (9300), 317 (7700). FD-MS *m/z*: 831 (M^++Na, $C_{37}H_{44}O_{20}$).

Compound **10-2** (20 mg) was added to 2 ml of methanolic $NaOCH_3$ (Na in MeOH: 4 mg/2 ml), and the mixture was allowed to stand at room temp. for 4 h, then neutralized with IR-120B. The MeOH was evaporated off *in vacuo* and the residue was subjected to DCCC (solv. 5). Each fraction was monitored by TLC (solv. 1, 7 : 3 : 1). The first fraction was identified as **17**. The second fraction, showing a less polar spot than **17**, was considered to be a glucoside

of methyl monomethylcaffeate. This fraction was refluxed with 2 ml of 0.5 N H_2SO_4–50% EtOH for 2 h. The mixture was deacidified with IR-410, and the solution was extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated *in vacuo*. The residue showed one spot having an *Rf* value the same as that of methyl 3-*O*-methylcaffeate (methyl ferulate) but different from that of methyl 4-*O*-methylcaffeate (solv. 4, 1 : 1). The water layer, after extraction with $CHCl_3$, was concentrated *in vacuo* and examined by PC (solv. 6, $R_{glc.}$: 1.00).

Isolation of the Known Iridoids——The following iridoids were obtained principally from the benzene and the $CHCl_3$ extracts of the 50% MeOH-soluble fraction of the MeOH percolate; plumericin (**11**) (mp 211—217 °C, 78 mg from stem, 570 mg from leaves), isoplumericin (**12**) (mp 202—204 °C, 10 mg from stem),[9] allamandin (**13**) (mp 217—223 °C, 190 mg from leaves), allamandicin (**14**) (solid, 300 mg from stem), deglucosylplumieride (**15**) (mp 165—171 °C (dec.), 50 mg from stem), 13-*O*-coumaroylplumieride (**16**) (solid, 2.1 g from leaves).

The following iridoids were obtained principally from the *n*-BuOH extract of the 50% MeOH-soluble fraction: plumieride (**17**) (mp 228—230 °C, 9.2 g from stem, 10 g from leaves), protoplumericin A (**18**) (solid, 10 g from stem, 14.7 g from leaves).

Isolation of Gardenoside (19)——Gardenoside was isolated from the *n*-BuOH extract of the stem (20 mg) and leaves (40 mg) as a solid, after column chromatography with MCI Gel/H_2O–MeOH and DCCC (solv. 5). $[\alpha]_D^{19}$ −54.9° (c=1.86, MeOH). UV λ_{max}^{MeOH} nm (ε): 232 (12500). MS *m/z*: 225 (M^+ − glucose + 1). On TLC (solv. 1, 7 : 3 : 1; solv. 3, 4 : 1 : 0.5), **19** showed the same *Rf* values as authentic gardenoside obtained from the fruits of *Gardenia jasminoides*. The pentaacetate (**19-1**) and hexaacetate (**19-2**) of **19** were prepared by usual acetylation, followed by column chromatography on silica gel (solv. 2, 10 : 1). **19-1** (crystalline powder): mp 61—68 °C, $[\alpha]_D^{19}$ −96.8° (c=1.78, MeOH). UV λ_{max}^{MeOH} nm (ε): 233 (13000). MS *m/z*: 614.184 (Calcd for $C_{27}H_{34}O_{16}$: 614.185). **19-2** (solid): mp 66—68 °C, $[\alpha]_D^{19}$ −52.4° (c=2.06, MeOH). UV λ_{max}^{MeOH} nm (ε): 233 (10000).

Isolation of 10-Dehydrogardenoside (20)——10-Dehydrogardenoside was isolated as a solid from the BuOH extract of the leaves (100 mg) after successive chromatographies on MCI Gel/H_2O–MeOH, and DCCC (solv. 5), $[\alpha]_D^{19}$ −101.0° (c=0.20, MeOH). FD-MS *m/z*: 403 (M^+ + 1, $C_{17}H_{22}O_{11}$). A solution of **20** in EtOH (5 mg/2 ml) was treated portionwise with 10 mg of $NaBH_4$ at room temp. After stirring for 1 h, the product showed the same *Rf* values as **19** when run in parallel with **19** (solv. 1, 7 : 3 : 1; solv. 3, 8 : 2 : 1), and the same blue stainings were observed after heating of the TLC plate with H_2SO_4 reagent.

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