

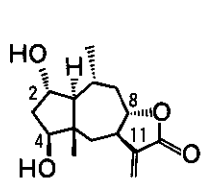
STRUCTURE-ACTIVITY RELATIONSHIPS OF PSEUDOGUAIANOLIDES
ISOLATED FROM GAILLARDIA PULCHELLA AND THEIR DERIVATIVES¹

Kenzo Harimaya[†] and Seiichi Inayama^{*}

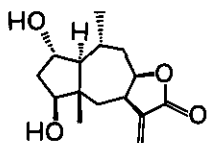
Pharmaceutical Institute, School of Medicine, Keio University,
Shinanomachi 35, Shinjuku-ku, Tokyo 160, Japan

Abstract—Three new pseudoguaianolides, 4-epipulchellin (1), 4-epineopulchellin (2) and pulcheloid D (3), were isolated from Gaillardia pulchella together with other eleven pseudoguaianolides and three guaianolides. Fifteen natural sesquiterpene lactones and their thirty one derivatives were tested for cytotoxic activity against KB cell line. Among them, twenty seven compounds were shown to be active ($ED_{50} < 4 \mu\text{g/ml}$). The structure-activity relationships of these sesquiterpene lactones are discussed.

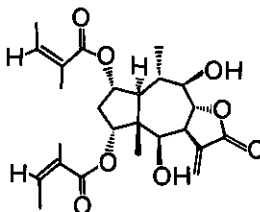
In the course of our medico-chemical studies on bio-active constituents of Gaillardia pulchella (Compositae), sesquiterpene lactones were isolated from this plant. It is interesting from the chemotaxonomic point of view that there is a difference in their skeletal types depending on locale of collection or cultivation. Among them, pseudoguaianolides were found to be principal sesquiterpenolide constituents in the aerial parts of the plant collected in Florida^{2,3,4} and of the cultivated material in Tokyo²⁻⁸ and Berlin.⁹ In our continuous search of the Japanese cultivated species we have reported the isolation and structure elucidation of several pseudoguaianolides, such as pulchellin^{2,3} neopulchellin,⁴ pulcheloid A,⁵ B,⁵ and C⁶ as well as two lactone alkaloids, pulchellidine,³ neopulchellidine,^{4a} and a lactone degraded pseudo-twistane, pulchellon.⁷ A cytotoxic guaianolide, gaillardin^{8,10} was isolated from G. pulchella collected in Texas and a Tokyo cultivation together with a minor constituent, neogaillardin.⁸ Eudesmanolides were also found as the main sesquiterpene lactones in the collection in Arizona and eastern New Mexico.¹¹ Since a crude extract containing gaillardin was found to show a higher cytotoxic



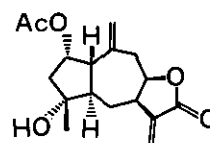
(1)



(2)



(3)



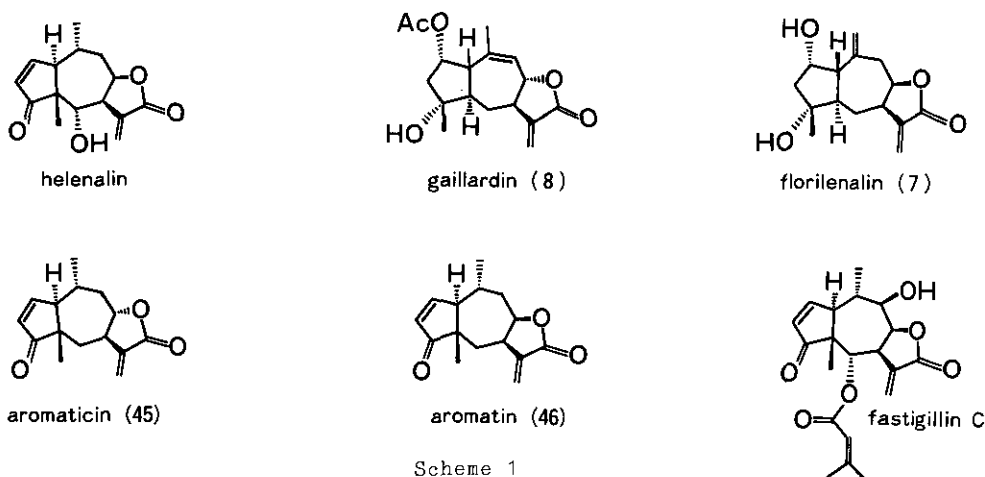
(4)

activity than does gaillardin itself,^{10a} we focused our attention on the other minor components in this plant which can be expected to exhibit potent antitumor activity. We now describe further isolation of three new pseudoguaianolides, 4-epipulchellin (1),¹² 4-epineopulchellin (2)¹² and pulchelloid D (3), together with eleven pseudoguaianolides and three guaianolides, from three cultivations mentioned below.

Fifteen naturally occurring sesquiterpenolides and their thirty one derivatives were tested for antitumor activity against KB cells. Among these forty six compounds, twenty seven compounds exhibit appreciable activity ($ED_{50} < 4 \mu\text{g/ml}$). The structure-activity relationship of these sesquiterpene lactones is discussed below.

The methanol extract of the aerial part of *G. pulchella* cultivated in Japan afforded a mixture of seventeen sesquiterpene lactones. After solvent partitions, separation of the mixture by liquid chromatography (silica gel) and high performance liquid chromatography (hplc) (silica gel and ODS) gave 2-acetylflorilenalin (4),¹³ pulchellin (5),^{2,8} neopulchellin (6),^{4,8} florilenalin (7),¹⁴ gaillardin (8),^{8,10} 6-acetylpulchelloid A (9),⁹ 6-acetylpulchelloid B (10),⁹ spathulin (11),¹⁵ 4-angeroyl-6 α -hydroxyneopulchellin (12),⁹ pulchelloid C (13),⁶ 4-acetylpulchelloid A (14),⁹ pulchelloid A (15),⁵ pulchelloid B (16)⁵ and 6 α -hydroxyneopulchellin (17).⁹ Compounds (1), (2) and (3) are all new pseudoguaianolides, and compound (4) is the guaianolide isolated from this plant for the first time.

4-Epipulchellin (1) was isolated as colorless oil and the molecular formula was determined as $C_{15}H_{22}O_4$ based on high resolution mass spectrometry (hrms). From its ir and ^1H -nmr spectroscopic data, **1** seemed to contain an exomethylene- δ -butyrolactone moiety and two hydroxyl groups. The ^1H -nmr spectrum was very similar to that of pulchellin (5) with an exception of the chemical shift and multiplicity of the proton at C(4). The partial structure from C(5) to C(10) was assigned by the proton decoupling experiments, but the complete assignment of whole structure was unsuccessful because of overlapping of proton signals of C(2) and C(4). In order to disclose the whole structure, we synthesized 4-epipulchellin (1) from pulchellin (5),^{2,8} which is the most abundant component of this plant, as follows. Sodium



Scheme 1

borohydride (NaBH_4) reduction of 2-acetyldehydropulchellin (18) derived from pulchellin (5)^{3c} (Cf. Table 1) afforded stereospecifically 2-acetyl-4-epipulchellin (19) in almost quantitative yield. The configuration of C(4)-hydroxyl group was assigned by the coupling constants ($J = 8.6, 10.7 \text{ Hz}$) of C(4)-H. Monoacetate (19) was further acetylated to give diacetyl-4-epipulchellin (20). The stereostructures of 19 and 20 were unequivocally determined by comparison of their ^1H -nmr data with those of naturally occurring 2-acetyl-4-epipulchellin and diacetyl-4-epipulchellin isolated previously from *Geigeria burkey*.¹⁶ Alkaline hydrolysis of 20 gave 4-epipulchellin (1) (see Experimental).

4-Epineopulchellin (2), $\text{C}_{15}\text{H}_{22}\text{O}_4$, was isolated as colorless oil and also has an exomethylene- γ -butyrolactone moiety and hydroxyl groups as shown by its ir and ^1H -nmr data; ir (KBr) $\nu \text{ cm}^{-1}$: 3380 (OH), 1764 (γ -lactone), ^1H -nmr (acetone- d_6) $\delta \text{ ppm}$: 6.08, d, $J = 2.5 \text{ Hz}$ (H-13'), 5.62 d, $J = 2.2 \text{ Hz}$ (H-13). The nmr spectrum is very similar to that of neopulchellin (16). The only striking difference between 2 and 6 is chemical shifts and coupling constants of their H-4 protons [$\delta 4.01$, dd, $J = 8.8, 8.8 \text{ Hz}$ for 2; $\delta 3.68 \text{ ppm}$, d, $J = 5.0 \text{ Hz}$ for 6]. The above data suggest that compound (2) is a 4-epimer of 6. In order to prove this assignment, chemical transformation from 6 into 2 was performed in a similar manner as described for 4-epipulchellin (1). Acetylation of 6 with Ac_2O /pyridine gave a mixture (1:1) of 2-acetate (21) and diacetate (22). Keto acetate (23) obtained from the 2-acetate (21) by Jones oxidation was then reduced cautiously with NaBH_4 (1.3 eq. mol), thereby yielding almost quantitatively 2-acetyl-4-epineopulchellin (24) in a stereospecific manner. Alkaline hydrolysis of the acetate (24) gave rise to formation of 4-epineo-

pulchellin (2) in 94.2 % yield, whose spectroscopic data were in accord with those of natural 4-epineopulchellin (2) in every respect. Furthermore, acetylation of this compound 2 afforded the corresponding diacetate (25)¹⁶(see Experimental).

Pulchelloid D (3) obtained as colorless oil exhibited a prominent ion peak at m/z 463 (MH^+) in CI-ms. The ir and 1H -nmr spectra of 3 showed the presence of an exomethylene- γ -butyrolactone moiety, two hydroxyl and two angeloyl ester groups as follows; ir (KBr) ν cm^{-1} : 3440 (OH), 1772 (γ -lactone), 1716 (ester carbonyl), 1H -nmr ($CDCl_3$) δ ppm: 6.43, d, $J = 3.5$ Hz (H-13), 5.55, d, $J = 3.1$ Hz (H-13'), 6.16, qq, $J = 1.5, 7.3$ Hz (H-3' or H-3"), 6.08, qq, $J = 1.5, 7.3$ Hz (H-3' or H-3"), 2.02, 3H, dq, $J = 1.5, 7.3$ Hz (H-4' or H-4"), 1.98, 3H, dq, $J = 1.5, 7.3$ Hz (H-4' or H-4"), 1.84, 3H, dq, $J = 1.5, 1.5$ Hz. Coupling constants of its protons on the framework are approximate to those of pulchelloid A (15),⁵ which is a pseudoguaianolide having a 2-angeloyl group. The difference between compound (3) and 15 appeared in the chemical shift of H-4. The above observation and comparison of 1H -nmr data of (3) with other analogous pseudoguaianolides, such as spathulin¹⁵ and pulchelloid B,⁵ led us to the conclusion that pulchelloid D (3) is 4-angeloylpulchelloid A (or 6,9-desacetyl-2,4-diangeloylspathulin).

2-Acetylflorilenalin (4), $[\alpha]_D^{30} +98.8^\circ$ ($c = 0.15$, $CHCl_3$), mp $139-140^\circ C$, was obtained as colorless prisms with the molecular formula $C_{15}H_{22}O_5$ based on the HR-, CI- and EI-ms spectroscopies. Compound (4) seemed to possess an exomethylene- γ -lactone moiety, one hydroxyl group and one acetoxyl group from the following data; ir (KBr) ν cm^{-1} : 3530 (OH), 1767 (γ -lactone), 1723 (acetyl carbonyl), 1H -nmr ($CDCl_3$) δ ppm: 6.31, 1H, d, $J = 2.8$ Hz (H-13), 5.68, 1H, d, $J = 2.4$ Hz (H-13'), 5.68, d, $J = 2.4$ Hz (H-13'), 5.68, 1H, d, $J = 2.4$ Hz (H-13), 2.07, 3H (acetyl CH_3). Acetylation of 4 with Ac_2O /pyridine/DMAP at room temperature overnight afforded the corresponding acetate (26) ($C_{19}H_{26}O_7$, mp $127-129^\circ C$). Comparison of the 1H -nmr data of 26 with those of florilenalin diacetate obtained from florilenalin (7) under specified conditions of acetylation (Ac_2O /pyridine/DMAP at $80^\circ C$ for 18 h) revealed that compound (26) to be identical with florilenalin diacetate.¹⁵ Thus, the monoacetate (4) is a trans-fused guaianolide possessing a cis-fused α -methylene- γ -butyrolactone group.

The cytotoxic and/or antitumor pseudoguaianolides and guaianolides are commonly characterized by ornamentation with the α -methylene- γ -lactone moiety as exemplified in Scheme 1. Furthermore, an $\alpha,8$ -unsaturated carbonyl and an additional acyloxy or hydroxy group seem to enhance the cytotoxicity.¹⁷ Systematic studies on the

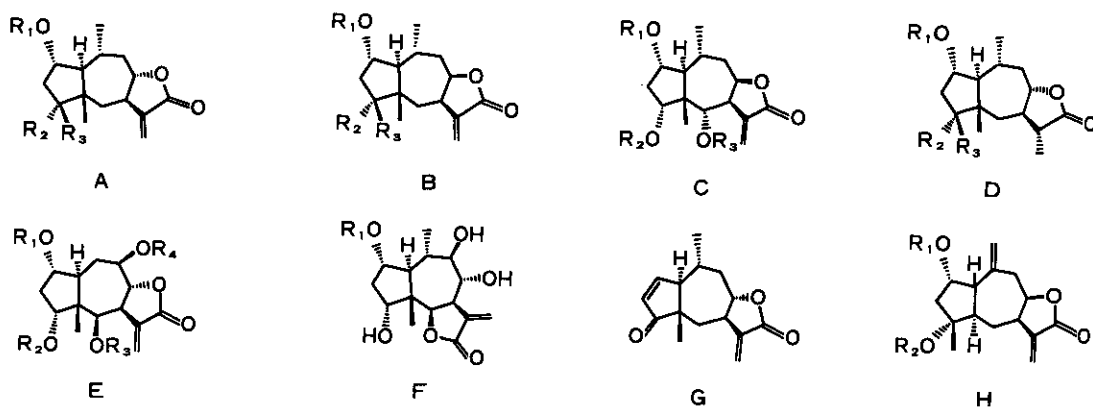


Table 1 Cytotoxic Activity of Pseudoguaianolides and Guaianolides against KB Cells

Compounds (No.) Categories	Substituents				ED ₅₀ (μg/ml)
	R ₁	R ₂	R ₃	R ₄	
A (trans-lactone diol)					
pulchellin (5)	H	OH	H		1.9
2-acetylpulchellin (27)	Ac	OH	H		3.5
diacetylpulchellin (29)	Ac	OAc	H		1.7
4-epipulchellin (1)	H	H	OH		21.5
2-acetyl-4-epipulchellin (19)	Ac	H	OH		40
pulchellidine (30)	H	OH	H		4.5
4-angeloylpulchellin (57)	(11βH,13-piperidino) H OAng H				29
B (cis-lactone diol)					
neopulchellin (6)	H	OH	H		2.6
2-acetylneopulchellin (21)	Ac	OH	H		4.2
diacetylneopulchellin (22)	Ac	OAc	OAc		2.2
4-epineopulchellin (2)	H	H	OH		7.5
2-acetyl-4-epineopulchellin (24)	Ac	H	OH		3.5
diacetyl-4-epineopulchellin (25)	Ac	H	OAc		5.4
neopulchellidine (31)	H OH H				> 30
	(11αH,13-piperidino)				
C (lactone triol)					
6α-hydroxyneopulchellin (17)	H	H	H		6.7
4-angeloyl-6α-hydroxyneopulchellin (12)	H	Ang	H		2.9
6α-hydroxyneopulchellin triacetate (35)	Ac	Ac	Ac		0.62
pulchelloid C (13)	H	Ang	H		1.2
		(8βH)			
2,6-diacetylpulchelloid C (36)	Ac	Ang	Ac		1.4
		(8βH)			
D (11,13-dihydrolactone diol)					
dihdropulchellin (32)	H	OH	H		> 100
2-acetyldihdropulchellin (33)	Ac	OH	H		> 100
diacetyldihdropulchellin (34)	Ac	OAc	H		> 100
4-epi-11βH-dihydroneopulchellin (37)	H	H	OH		> 100
	(8α,11βH)				
4-epi-11αH-dihydroneopulchellin (38)	H	H	OH		> 100
	(8α,11αH)				

continued

E (trans-lactone tetraol)					
pulchelloid A (15)	Ang	H	H	H	12
pulchelloid B (16)	iVal	H	H	H	1.3
pulchelloid D (3)	Ang	Ang	H	H	0.47
spathulin (11)	H	H	Ac	Ac	4.6
4-acetylpulchelloid A (14)	Ang	Ac	H	H	0.38
6-acetylpulchelloid A (9)	Ang	H	Ac	H	0.5
pulchelloid A triacetate (39)	Ang	Ac	Ac	Ac	1.3
pulchelloid B triacetate (40)	iVal	Ac	Ac	Ac	1.0
diacetylspathulin (42)	Ac	Ac	Ac	Ac	3.2
6,9-desacetylspathulin (41)	H	H	H	H	> 100
F (6-fused cis-lactone tetraol)					
isopulchelloid A (44)	Ang				3.1
6,9-desacetylisospathulin (43)	H				> 100
G (cyclopentenone derivatives)					
aromaticin (45)					1.05
aromatin (46)		(8 α H)			> 0.3
2 β ,3 β -epoxyaromaticin (47)		2 β ,3 β -epoxy			1.1
2 β ,3 β -epoxyaromatin (48)		2 β ,3 β -epoxy (8 α H)			0.62
4-anhydro-2-dehydropulchellin (49)					1.5
4-anhydro-2-dehydrodihydropulchellin (50)		(11 β H)			> 100
2-dehydropulchellin (51)					1.9
H (guaianolide)					
2-acetylflorilenalin (4)	Ac	H			1.8
gaillardin (8)	Ac	H	($\Delta^9,10,8\beta$ H)		0.36
gaillardin acetate (52)	Ac	H	($\Delta^9,10,8\beta$ H)		1.5

structure-activity relationship of these sesquiterpenolides are very few except for helenalin and its related derivatives.¹⁸ It has generally been said that the α -methylene- γ -butyrolactone moiety acts as an *in vivo* alkylating agent which undergoes a Michael type reaction with biological cellular nucleophiles^{3,17} such as L-cysteine, glutathione or sulfhydryl-containing enzymes such as phosphofructokinase, glycogen synthetase and DNA polymerase.¹⁸ In order to clarify the structure-activity relationship of sesquiterpene lactones in *G. pulchella*, the above-mentioned fifteen naturally occurring sesquiterpenolides and their thirty one derivatives were screened for an antitumor activity by using KB cell culture. Among them, cytotoxic aromaticin (45) and aromatin (46) containing cyclopentenone moiety were derived from pulchellin (5) and neopulchellin (6), respectively, for the comparison of cytotoxicities.^{3c} The structures and cytotoxic activities against KB cells (ED₅₀ μ g/ml) are shown in Table 1. Pseudoguaianolides and guaianolides tested are classified on the basis of structures into seven categories of pseudoguaianolides (A - G) and guaianolide (H) groups. As expected, gaillardin (8), aromaticin (45) and aromatin (46) showed potent cytotoxic activities and none of the 11,13-dihydro derivatives such as dihydro-

pulchellin (32),² its 2-acetate (33),² 2, 4-diacetate (34),² 4-epi-11 β H- (37),¹⁹ 4-epi-11 α H-dihydroneopulchellin (38)¹⁹ in Category D as well as 4-anhydro-2-dehydrodihdropulchellin (50) in Category G exhibited cytotoxicities. This observation implicates that the α -exomethylene- γ -lactone moiety must be essential for the appearance of cytotoxic activities. In the case of lactone diols of Category A and B, 2 α , 4 α -hydroxyl and acyloxyl groups are effective for the activity of trans-lactone diol (5) and cis-lactone diol (6), whereas the corresponding epimers at C(4) of 5 and 6 demonstrated reduced activities as shown by compounds (1) and (2). Meanwhile, acetylation at C(2) of both the 4 α -diols (5) and (6) diminished their activity as shown by compounds (27) and (21). In the case of 2, 4-diacetates (29) and (22), each activity is comparable to that of diol (5) and (6), respectively. In the case of lactone triols in Category C, a cis-lactone triol, 6 α -hydroxyneopulchellin (17) shows a higher cytotoxic activity in the corresponding mono-angelate (12). trans-Lactone triol, pulcheloid C (13) showed a relatively higher activity than does the corresponding cis-lactone triol, 4-angeroyl-6 α -hydroxyneopulchellin (12), whose cycloheptane ring takes a twisted boat conformation as evidenced by X-ray crystallography.²⁰ Furthermore, in the case of lactone tetraols of Category E and F, a trans-lactone tetraol, 6, 9-desacetylspathulin (41) and a cis-lactone tetraol (6-fused), 6, 9-desacetylspathulin (43) have no cytotoxicity. However, a remarkable augmentation of the activity was observed by acylation of the two hydroxyl groups in the tetraol molecule. For example, diester diols such as pulcheloid D (3), 6-acetylpulcheloid A (9) and 4-acetylpulcheloid A (14) exhibit a highly potent activity ($ED_{50} < 1 \mu\text{g/ml}$). This fact seems to suggest that introduction of hydroxyl groups into the parent molecule not always contributes to their cytotoxic activity, but also to indicate adequate lipophilicity to be essential for enhancing the activity of these pseudoguaianolides. Guaianolides such as 2-acetylflorilenalin (4), gaillardin (8) and its acetate (52) in Category H reveal almost the same cytotoxicities as pseudoguaianolides in Category G. Pseudoguaianolides containing a cyclopentenone or epoxycyclopentanone moiety in category G exhibit the highest activity (ED_{50} 0.3-2 $\mu\text{g/ml}$). This fact concludes that the major cytotoxicity of the α -methylene- γ -butyrolactone moiety is considerably increased by enhancement effect of α , β -unsaturated ketone coexisting in the molecule. It is noteworthy to mention that 4-anhydrodihdropulchellin (49), for example, exhibited an appreciable activity, whereas its 11 β H-dihydro derivatives (50) containing a cyclopentenone moiety had virtually no activity.

Table 2 Hydrophobicity (log P) and Cytotoxicity (ED₅₀) of Sesquiterpene Lactones

Compounds	ED ₅₀ (μ g/ml)	log(1/C ₅₀) ^a	log P
pulchellin (5)	1.9	5.15	0.93
neopulchellin (6)	2.6	5.01	1.31
2-acetylneopulchellin (21)	4.2	4.86	1.32
aromaticin (45)	1.05	5.37	1.60
gaillardin (8)	0.63	5.92	1.80

a: Log of reciprocal half-maximal effective dose in moles/l
 $\log(1/C_{50}) = 0.902(\log P) + 4.0007$, $n = 5$, $r = 0.72$ (Eq. 1)

In connection with the aforementioned structure-activity relationship of cytotoxic sesquiterpene lactones, the estimation of hydrophobic parameter (log P) was taken in consideration substantially measured according to Hansch-Fujita's method.²¹ Partition coefficients were calculated based on measurements of the absorbance at absorption maxima (210-220 nm) attributable to the α -methylene- γ -lactone chromophore. The results were shown in Table 2. There observed an evident tendency that the more hydrophobicity (log P) was provided in the structure, the higher cytotoxic activity was secured in the molecule. A reasonable correlation of log 1/C₅₀ with log P for these five compounds was preliminary deduced by the given equation 1 to be a linear relationship between lipophilicity and cytotoxicity. A further investigation is now in progress.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter, and infrared (ir) spectra were obtained with a Hitachi EPI-G3 spectrometer. ¹H-Nmr spectra were obtained with JEOL GSX-270 and GX-400 (270 and 400 MHz spectrometer using tetramethylsilane as an internal standard. EI-ms, CI-ms and HR-ms were measured with a JEOL JMS-D300 spectrometer. Thin layer chromatography (tlc) was performed on Merck precoated plate (Kieselgel 60F₂₅₄) with AcOEt as a solvent. Column chromatography was carried out on Kieselgel 60 (70 - 230 mesh). Preparative hplc was performed on an apparatus consisting of a M-6000A pump (Waters Associates Co. Ltd.), U6K injector (Waters Associates Co. Ltd.) and Soma S-310A model II UV detector (operated at 254 nm) using the following columns; A) Chemcosorb 5 ODS L (30 cm x 7.5 mm ϕ), B) μ -Bondapak C₁₈ Semi Prep (30 cm x 7.8 mm ϕ) and C) Chemcosorb 5Si (25 cm x 7.8 mm ϕ) with the following solvent systems; solvent A: CH₃CN/H₂O (4:6,

flow rate 2 ml/min), solvent B: MeOH/H₂O (5:6, flow rate, 2 ml/min), solvent C: CHCl₃/EtOH (9:1, flow rate, 2 ml/min).

Extraction and Isolation — 1

Air dried above-ground material (3 kg) of Gaillardia pulchella (seeds were supplied from Takii Shubyo Co. Ltd.), cultivated at Koganei City near Tokyo in 1985, was chopped small pieces and percolated with methanol (50 l) at ambient temperature for 2 weeks. After concentration of the extract under reduced pressure to 1 l, water (1 l) was added, and the mixture was extracted with benzene (1 l x 3) and AcOEt (1 l x 3). The AcOEt layer was then evaporated in vacuo afforded a brown gum (54 g). The residue was then submitted to silica gel chromatography and the fraction eluted with benzene/AcOEt (1:1) gave a crude mixture of the constituents (13 g).

Recrystallization from AcOEt afforded a mixture of (5) and (6) (9.9 g). Further separation of the mixture (1 g) by hplc (column C, solvent C) gave pulchellin (5) (500 mg, Rt 10.7 min) and neopulchellin (6) (300 mg, Rt 12.2 min). AcOEt eluted a mixture (244 mg) of 4-epipulchellin (1), 4-epineopulchellin (2) and florilenalin 7. Hplc separation (column C, 25 cm x 4.6 mm ϕ , solvent C, flow rate 1 ml/min) of the mixture gave florilenalin (7) (15 mg, Rt 6.6 min), 4-epipulchellin (1) (1 mg, Rt 12.7 min) and 4-epineopulchellin (2) (1 mg, Rt 15.4 min). The ¹H-nmr data of 7 was identical with those reported previously for florilenalin.¹⁴

4-Epipulchellin (1) Colorless oil. Ir (KBr) $\nu_{\max}$ cm⁻¹: 3375 (OH), 1751 (γ -lactone), 1656 (C=C), CI-*ms* *m/z*: 267 (MH⁺, base peak), 249 (MH⁺ - H₂O), 231 (MH⁺ - 2 x H₂O). EI-*ms* *m/z*: 266 (M⁺), 248 (M⁺ - H₂O), 230 (M⁺ - 2 x H₂O). HR-*ms* *m/z*: Calcd. for C₁₅H₂₂O₄: 266.1515. Found: 266.1495. ¹H-Nmr (CDCl₃) δ ppm: 6.17 (1H, ddd, J = 9.5, 9.5, 3.8 Hz, H-8), 4.14 (2H, m, H-2 and H-4), 1.18 (3H, d, J = 6.8 Hz, H-14), 0.90 (3H, s, H-15).

4-Epineopulchellin (2) Colorless oil. Ir (KBr) $\nu_{\max}$ cm⁻¹: 3380 (OH), 1764 (γ -lactone). CI-*ms* (isobutane) *m/z*: 267 (MH⁺). EI-*ms* *m/z*: Calcd. for C₁₅H₂₂O₄: 266.1515. Found: 266.1504. ¹H-Nmr (acetone-d₆) δ ppm: 6.08 (1H, d, J = 2.5 Hz, H-13'), 5.62 (1H, d, J = 2.2 Hz, H-13), 4.81 (1H, ddd, J = 3.2, 7.8, 11.2 Hz, 4-OH), 3.67 (1H, brs, 2-OH), 3.29 (1H, m, H-7), 2.13 (1H, ddd, J = 6.4, 12.9, 12.9 Hz, H-9 β), 1.94 (1H, m, H-10), 1.66 (1H, ddd, J = 1.5, 3.4, 12.9 Hz, H-9 α), 1.41 (1H, dd, J = 13.7, 13.7 Hz, H-6), 1.20 (3H, d, J = 6.8 Hz, H-14), 0.84 (3H, s, H-15).

Extraction and Isolation — 2

Dried above-ground material (6 kg) of G. pulchella (seeds supplied by Daiichi Engei

Co. Ltd.), cultivated at Koganei City near Tokyo in 1985, were cut into small pieces and extracted with MeOH (45 l) at ambient temperature for 3 weeks. After concentration of the extract under reduced pressure to a small volume (ca 1 l), water (1 l) was added. Chlorophyll and wax were then removed with benzene (1 l x 3). The aqueous layer was extracted with AcOEt (1 l x 2). After evaporation of the solvent under reduced pressure, the residue (49 g) was subjected to neutral alumina column chromatography using AcOEt as eluent. Treatment of the eluate as usual afforded a brown gum (30 g), which was then submitted to silica gel chromatography. Elution with a benzene, AcOEt and MeOH solvent system afforded six fractions: Fr. 1, 10 mg (Rf. 0.73); Fr. 2, 320 mg (Rf. 0.67); Fr. 3, 2.3 g (Rf. 0.60); Fr. 4, 5.6 g (Rf. 0.45); Fr. 5, 122 mg (Rf. 0.33); Fr. 6, 237 mg (Rf. 0.26). A solid obtained from Fr. 1 was recrystallized from acetone to give colorless prisms (mp 212–213°C), which were identified with a (3:2) mixture of 9-O-desacetylspathulin-2-O-angelate (or 6-acetylpulchelloid A) (9) and 9-O-desacetylspathulin-2-O-isovalerate (or 6-acetylpulchelloid B) (10) by ¹H-nmr. Recrystallization of the residue obtained from Fr. 5 from AcOEt afforded colorless prisms (mp 261–263°C), which was identical with spathulin (11) in all respects. Separation of the residue 30 mg of Fr. 2 by hplc (column A, solvent B) gave four fractions, Fr. 2-1 (Rt. 18.0 min), Fr. 2-2 (Rt. 19.2 min), Fr. 2-3 (Rt. 21.4 min) and Fr. 2-4 (Rt. 22.0 min). Each fraction afforded 9 mg of 4-angeroyl-6α-hydroxyneopulchellin (12), 3 mg of pulchelloid C (13), 2 mg of 4-acetylpulchelloid A (14) and 4 mg of 6-acetylpulchelloid A (9), respectively. The residue of Fr. 3 (100 mg) was separated by hplc (column A, solvent B) into two fractions of Fr. 3-1 (Rt. 43.0 min) and Fr. 3-2 (Rt. 45.8 min). Fr. 3-1 gave 7 mg of compound (15). Fr. 3-2 was deduced to be a mixture of compounds (16) and (17). Fr. 4 (7 mg) was separated by hplc (column B, solvent B) into two fractions of Fr. 4-1 (Rt. 41.0 min) and Fr. 4-2 (Rt. 43.5 min), which gave 2 mg of pulchelloid A (15) and pulchelloid B (16), respectively. 10 mg of Fr. 6 were separated by hplc (column A, solvent B) into two fractions of Fr. 6-1 (Rt. 9.6 min) and Fr. 6-2 (Rt. 17.2 min), which gave 40 mg of crude compound (17) and 10 mg of mixture of pulchellin (5) and (17), respectively. Fr. 6-1 was further purified by hplc (column C, solvent C) to afford 15 mg of 6α-hydroxy-neopulchellin (17).

Extraction and Isolation — 3

The chipped and dried whole plant of G. pulchella (ca 1 kg), cultivated at Shiki near Tokyo in 1970, was percolated with methanol (10 l) at room temperature. After concentration in vacuo to 500 ml followed by addition of water (500 ml), the water

layer was extracted with AcOEt (500 ml x 2). The organic extract was dried over anhydrous sodium sulfate. Evaporation in vacuo successively afforded 10 g of a brownish gum, which were subjected to silica gel chromatography using CH_2Cl_2 and AcOEt mixtures as eluents. Eluates with $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ (7:3), (1:1), (3:7) and (0:10) afforded Fr. 1 (2.4 g), Fr. 2 (2.23 g), Fr. 3 (1.89 g) and Fr. 4 (1.1 g), respectively. Recrystallization of Fr. 3 from AcOEt gave colorless prisms (880 mg) of a mixture of compounds (1) and (2). Fr. 1 was further submitted to silica gel chromatography and eluted with $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ (7:3) to afford four fractions: Fr. 1-1 (557 mg), Fr. 1-2 (586 mg), Fr. 1-3 (650 mg) and Fr. 1-4 (164 mg). Fr. 1-1 was further subjected to silica gel chromatography and eluted with a benzene-AcOEt solvent system. The benzene/AcOEt (1:5) fraction afforded Fr. 1-1-1 (95 mg), which was then separated by hplc (column B, solvent $\text{H}_2\text{O}/\text{MeCN}$ 24:19, flow rate 2 ml/min) to afford three fractions: Fr. 1-1-1-1 = compound (3) (Rt 24.0 min, 12 mg); Fr. 1-1-1-2 (Rt. 25.4 min, 2 mg); Fr. 1-1-1-3 (Rt. 29.3 min, 23 mg). Fr. 2 was subjected to silica gel chromatography (benzene/AcOEt) giving two fractions: Fr. 2-1 (50 mg) and Fr. 2-2 (80 mg). Recrystallization of Fr. 2-1 from AcOEt gave colorless prisms (mp 195-198°C), which were identified as gaillardin (8) by ir and ^1H -nmr. Fr. 2-2 was further submitted to hplc (column B, solvent $\text{MeCN}/\text{H}_2\text{O}$ 4:6, flow rate 1 ml/min) to separate into four fractions: Fr. 2-2-1 (Rt. 20.0 min, 13 mg), Fr. 2-2-2 (Rt. 22.0 min, 29 mg), Fr. 2-2-3 (Rt. 4.2 min, 10 mg) and Fr. 2-2-4 (Rt. 37.0 min, 1 mg). Recrystallization of Fr. 2-2-2 from AcOEt/ether gave compound (4) as colorless prisms (mp 139-140°C, 15 mg).

Pulchelloid D (3) Colorless oil obtained from Fr. 1-1-1. Ir (KBr) $\nu_{\max}$ cm^{-1} : 3440 (OH), 1772 (γ -lactone), 1716 (ester carbonyl). CI-*ms* *m/z*: 463 (MH^+). ^1H -nmr (CDCl_3) δ ppm: 6.43 (1H, d, $J = 3.5$ Hz, H-13), 6.16 (1H, qq, $J = 1.5, 7.1$ Hz, H-3' or H-3"), 6.08 (1H, qq, 1.5, 7.1 Hz, H-3' or H-3"), 5.55 (1H, d, $J = 3.1$ Hz, H-13'), 5.16 (1H, ddd, $J = 1.9, 6.7, 9.2$ Hz, H-2), 5.02 (1H, d, $J = 4.6$ Hz, H-4), 4.62 (1H, dd, $J = 9.4, 9.4$ Hz, H-8), 4.43 (1H, dd, $J = 3.5, 3.5$ Hz, H-6), 3.35 (1H, dd, $J = 9.5, 9.4$ Hz, H-9), 3.00 (1H, m, H-7), 2.76 (1H, ddd, $J = 4.6, 9.2, 14.1$ Hz, H-3), 2.30 (1H, dd, $J = 6.7, 11.2$ Hz, H-1), 2.02 (3H, dq, $J = 1.5, 7.3$ Hz, H-4' or H-4"), 1.98 (3H, dq, $J = 1.5, 7.3$ Hz, H-4' or H-4"), 1.93 (1H, m, H-10), 1.89 (3H, qq, $J = 1.5, 1.5$, H-5' or H-5"), 1.84 (3H, qq, $J = 1.5, 1.5$ Hz, H-5' or H-5"), 1.70 (1H, dd, $J = 1.9, 14.1$ Hz, H-3 α), 1.17 (3H, d, $J = 6.6$ Hz, H-14), 1.06 (3H, s, H-15).

2-Acetylflorilenalin (4) Colorless prisms. mp 139-140°C. $[\alpha]_D^{20} +98.9^\circ$ ($c = 0.15$, CHCl_3), ir (KBr) $\nu_{\max}$ cm^{-1} : 3530 (OH), 1760 (γ -lactone), 1723 (acetyl

carbonyl), EI-ms m/z : 306 (M^+), 246 ($M^+ - CH_3COOH$), 228 (246 - H_2O), 204 (246 - 2 x H_2O), CI-ms (isobutane) m/z : 307 (MH^+), 289 ($MH^+ - H_2O$), 247 ($MH^+ - CH_3COOH$), 229 (247 - H_2O), HR-ms m/z : Calcd. for $C_{15}H_{22}O_5$: 306.1468, Found: 306.1494. 1H -Nmr ($CDCl_3$) δ ppm: 6.31 (1H, d, $J = 2.8$ Hz, H-13'), 5.68 (1H, d, $J = 2.4$ Hz, H-13), 5.31 (1H, dd, $J = 5.5, 2.8$ Hz, H-2), 5.08 (1H, brs, H-14'), 4.89 (1H, brs, H-14), 4.63 (1H, ddd, $J = 11.3, 7.9, 3.7$ Hz, H-8), 3.24 (1H, m, H-7), 2.32 (1H, dd, $J = 12.8, 11.3$ Hz, H-9 α), 2.73 (1H, dd, $J = 12.8, 3.7$ Hz, H-9 β), 2.21 (1H, dd, $J = 15.9, 5.5$ Hz, H-3 β), 2.07 (3H, s, acetyl CH_3), 1.93 (1H, d, $J = 15.9$ Hz, H-3 α), 1.22 (3H, s, H-15). 2-Acetyl-4-epipulchellin (19) To a solution of 2-acetyl-4-dehydropulchellin (18)^{3c} (120 mg), prepared from pulchellin (1), in MeOH (8 ml) was added $NaBH_4$ (8 mg) and the mixture was stirred at room temperature for 30 min. After addition of water, the reaction mixture was extracted with AcOEt and the organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave a crystalline mass in almost quantitatively, which was then recrystallized from $CHCl_3$ /ether to give 2-acetyl-4-epipulchellin (19) as colorless prisms, mp 188-191°C, ir (KBr) ν_{max} cm^{-1} : 3500 (OH), 1756 (γ -lactone), 1731 (acetyl carbonyl), EI-ms m/z : 248 ($M^+ - CH_3COOH$), 230 ($M^+ - CH_3COOH - H_2O$), CI-ms (isobutane) m/z : 309 (M^+), HR-ms m/z : Calcd. for $C_{17}H_{24}O_5$: 308.1624, Found: 308.1623. 1H -Nmr ($CDCl_3$) δ ppm: (1H, d, $J = 3.6$ Hz, H-13'), 5.47 (1H, d, $J = 3.1$ Hz, H-13), 4.93 (1H, ddd, $J = 2.1, 8.3, 9.5$ Hz, H-2), 4.23 (1H, ddd, $J = 3.4, 9.5, 12.2$ Hz, H-8), 4.04 (1H, dd, $J = 8.6, 10.7$ Hz, H-4), 2.84 (1H, m, H-7), 2.04 (3H, s, acetyl CH_3), 1.95 (1H, m, H-10), 0.97 (3H, d, $J = 6.7$ Hz, H-14), 0.93 (3H, s, H-15).

Diacetyl-4-epipulchellin (20) Acetylation of 2-acetyl-4-epipulchellin (22) (20 mg) with a mixture of Ac_2O (0.1 ml) and pyridine (1.0 ml) at room temperature overnight gave a crystalline mass (20) (22 mg), whose recrystallization from ether/n-hexane afforded 4-epipulchellin diacetate (20) as colorless prisms, mp 133-134°C (lit. mp 137°C as the natural product).¹⁶

4-Epipulchellin (1) To a solution of 2-acetyl-4-epipulchellin (22) (50 mg) in dioxane (2 ml) was added 5% KOH (1 ml), and the mixture was stirred at room temperature for 18.5 h. After acidifying with 10 % HCl, the reaction mixture was extracted with AcOEt. The dried organic layer was evaporated in vacuo to give 4-epipulchellin (1) (35 mg) as a colorless oil, which was identical with the natural product in all respects.¹²

2-Dehydropulchellin (51) Pulchellin (5) (50 mg) was stirred with PDC (100 mg) in 1 ml of DMF at room temperature for 17 h. Water (20 ml) and EtOH (1 ml) were added

into the reaction mixture, which was then extracted with AcOEt. The dried organic layer was evaporated in vacuo to give a brown oil, which was purified by neutral alumina column chromatography and eluted with AcOEt to afford 2-dehydropulchellin (51) (42 mg) as colorless powder in 84.6 % yield, mp 156-159°C, ir (KBr) $\nu_{\max}$ cm^{-1} : 3450 (OH), 1759 (γ -lactone), 1736 (acetyl C=O), 1633 (C=C), EI-ms m/z: 264 (M^+), 246, 192, 163, 136. $^1\text{H-Nmr}$ (CDCl_3) δ ppm: 1.01 (3H, s, H-15), 1.32 (3H, d, $J = 3.0$ Hz, 13-H), 6.23 (1H, d, $J = 3.0$ Hz, 13'-H).

2-Dehydro-4-acetylpulchellin (52) 2-Dehydropulchellin (51) (310 mg) was mixed with pyridine (6 ml) and Ac_2O (6 ml) and the mixture was stirred at room temperature for 13 h. The reaction mixture was worked up as usual to afford 274 mg (75.8 %) of 2-dehydro-4-acetylpulchellin (52) as a colorless oil, ir (KBr) $\nu_{\max}$ cm^{-1} : 1755 (γ -lactone), 1735 (acetyl C=O), EI-ms: m/z, 246 ($M^+ - \text{AcOH}$), CI-ms (isobutane) m/z: 307 (MH^+), $^1\text{H-nmr}$ (CDCl_3) δ ppm: 1.07 (3H, s, 15-H), 1.42 (3H, d, $J = 6.0$ Hz, 14-H), 4.20 (1H, m, 8-H), 5.03 (1H, d, $J = 4.8$ Hz, 4-H), 5.45 (1H, d, $J = 3.0$ Hz, 13-H), 6.23 (1H, d, $J = 3.0$ Hz, 13'-H).

2-Dehydro-4-anhydropulchellin (49) 2-Dehydro-4-acetylpulchellin (52) (200 mg) was dissolved in 10 ml of pyridine and heated at 110°C for 12 h. After usual workup the product was purified by neutral alumina column chromatography by eluting with AcOEt to afford compound (49), which gave a pure sample of 2-dehydro-4-anhydropulchellin (49) 140 mg (87.1 %) on recrystallization from ether/AcOEt as colorless prisms, mp 228-231°C, ir (KBr) $\nu_{\max}$ cm^{-1} : 1752 (γ -lactone), 1701 (cyclopentenone), 1669 (C=C), EI-ms m/z: 246 (M^+), 228, 213, HR-ms Calcd. for $\text{C}_{15}\text{H}_{18}\text{O}_3$: 246.1256, Found: 246.1248, $^1\text{H-nmr}$ (CDCl_3) δ ppm: 1.33 (3H, s, H-15), 1.45 (3H, d, $J = 7$ Hz, H-14), 3.80 (1H, ddd, $J = 5, 11, 11$ Hz, H-8), 5.38 (1H, d, $J = 3$ Hz, H-13), 6.16 (1H, d, $J = 3.2$ Hz, H-13'), 7.17 (1H, d, $J = 6.0$ Hz, H-3).

2-Dehydrodihydropulchellin (54) To the solution of dihydropulchellin (32) (120 mg) in DMF (2.0 ml), PDC (200 mg) was added. The mixture was then stirred at room temperature for 26 h. An additional solution of PDC (100 mg) in DMF (2.0 ml) was added, and the mixture was then stirred for 4 days. After water and a small amount of EtOH had been added, the mixture was then extracted with AcOEt. The dried organic layer was worked up as usual and then purified by neutral alumina column chromatography by eluting with AcOEt. After evaporation of the solvent, a crystalline residue was then recrystallized from ether/AcOEt to give 100 mg (83.3 %) of 2-dehydrodihydropulchellin (55) as colorless prisms, mp 182-185°C, ir (KBr) $\nu_{\max}$ cm^{-1} : 3450 (OH), 1746 (γ -lactone), 1734 (cyclopentenone C=O). EI-ms m/z: 248 ($M^+ - \text{H}_2\text{O}$),

CI-ms (isobutane) m/z : 267 (MH^+), 249. 1H -Nmr ($CDCl_3$) δ ppm: 0.99 (3H, s, H-15), 1.23 (3H, d, $J = 6$ Hz, H-13), 1.39 (3H, d, $J = 6$ Hz, H-14), 3.92 (1H, d, $J = 3.6$ Hz, H-4), 4.2 (1H, m, 8-H).

2-Dehydro-4-acetyldihdropulchellin (56) A solution of 2-dehydrodihdropulchellin (55) (60 mg) in pyridine (3 ml) and Ac_2O (1 ml) was stirred at room temperature for 2 h. After water had been added, the reaction mixture was extracted with CH_2Cl_2 . The dried organic layer was evaporated to dryness to give 59 mg (84.4 %) of 2-dehydro-4-acetyldihdropulchellin (56) as colorless powder. Recrystallization from petroleum ether afforded colorless prisms. mp 135-137°C. Ir (KBr) ν_{max} cm^{-1} : 1763 (γ -lactone), 1736 (acetyl C=O). EI-ms m/z : 308 (M^+), 248, CI-ms (isobutane) m/z : 309 (MH^+). 1H -Nmr ($CDCl_3$) δ ppm: 1.05 (3H, s, H-15), 1.19 (3H, d, $J = 6$ Hz, H-13 or H-14), 1.43 (3H, d, $J = 6$ Hz, H-13 or H-14), 2.07 (3H, s, acetyl CH_3), 4.09 (1H, m, H-8), 4.98 (1H, d, $J = 3.6$ Hz, H-4).

2-Dehydro-4-anhydrodihdropulchellin (50) 2-Dehydro-4-acetyldihdropulchellin (56) (45 mg) was dissolved in pyridine (2 ml) and the solution was heated at 110°C for 15 h. After evaporation in vacuo, the residue was submitted to neutral alumina column chromatography ($AcOEt$), which yielded 33 mg (91.1 %) of 2-dehydro-4-anhydrodihdropulchellin (50). Recrystallization from ether/ $AcOEt$ gave colorless prisms. mp 160-163°C. Ir (KBr) ν_{max} cm^{-1} : 1774 (γ -lactone), 1694 (cyclopentenone C=O). EI-ms m/z : 248 (M^+). CI-ms (isobutane) m/z : 249 (MH^+). 1H -Nmr ($CDCl_3$) δ ppm: 1.03 (3H, d, $J = 7.2$ Hz, H-13), 1.24 (3H, s, H-15), 1.35 (3H, d, $J = 7.0$ Hz, H-14), 3.90 (1H, m, H-8), 6.18 (1H, d, $J = 5.4$ Hz, H-3), 7.16 (1H, d, $J = 5.4$ Hz, H-4).

The partition coefficients ($P = C_{octanol}/C_{water}$) were determined essentially according to Hansch-Fujita method using a Hitachi 220A spectrometer. For example, a sample (0.2 mg) was dissolved in 10 ml of water saturated with octanol. Water-saturated octanol (5 ml) was added into 5 ml of the above solution, moderately and continuously shaken at 25°C for 3 h. After this mixture had been centrifuged at 300 rpm for 5 min, absorbance of the water phase was measured at 210 - 220 nm, the absorption maximum of the α -methylene- γ -lactone chromophore. The biological activity $\log 1/C_{50}$ was correlated with lipophilicity ($\log P$) by a least-squares analysis.

ACKNOWLEDGEMENTS

One of the authors (S. I.) acknowledges a grant in aid from the Ministry of Education, Science and Culture of Japan and a financial support by our University authority. Our thanks are due to Mr. M. Nakamura and Miss S. Takei, Joint Laboratory

in this School for ms and ir spectral measurements, respectively. Antitumor screening tests were executed by Dr. S. Tsukagoshi and T. Tashiro of the Chemotherapeutic Center of Cancer Institute to whom we are indebted.

REFERENCES AND NOTES

- + This paper was used in part for a work of Doctor thesis (K. H.), Tokyo University, 1989.
- * All correspondence should be addressed to S. I.
- 1) This article is dedicated to the late Professor Tetsuji Kametani in the special issue of HETEROCYCLES to the memories of himself.
 - 2) a) W. Herz, K. Ueda, and S. Inayama, Tetrahedron, 1963, 19, 483; b) M. Yanagita, S. Inayama, and T. Kawamata, Tetrahedron Lett., 1970, 131; c) T. Sekita, S. Inayama, and Y. Iitaka, ibid., 1970, 135; d) Idem, Acta Crystallogr., 1971, B27, 877; e) K. Aota, C. N. Caughlan, M. T. Emerson, W. Herz, S. Inayama, and M. ul-Haque, J. Org. Chem., 1970, 35, 627.
 - 3) a) M. Yanagita, S. Inayama, T. Kawamata, T. Ohkura, and W. Herz, Tetrahedron Lett., 1969, 2073, 4170; b) T. Kawamata and S. Inayama, Chem. Pharm. Bull., 1971, 19, 643; c) S. Inayama, K. Harimaya, N. Shimizu, H. Hori, T. Ohkura, T. Kawamata, and Y. Iitaka, Heterocycles, 1985, 23, 377.
 - 4) a) M. Yanagita, S. Inayama, and T. Kawamata, Tetrahedron Lett., 1970, 3007; b) S. Inayama, K. Harimaya, H. Hori, T. Kawamata, T. Ohkura, H. Nakamura, and Y. Iitaka, Heterocycles, 1982, 19, 1801.
 - 5) S. Inayama, K. Harimaya, T. Ohkura, and T. Kawamata, Heterocycles, 1982, 17, 219.
 - 6) S. Inayama, K. Harimaya, H. Hori, T. Kawamata, I. Miura, and Y. Iitaka, Heterocycles, 1983, 20, 1505.
 - 7) a) S. Inayama, T. Kawamata, and T. Ohkura, Tetrahedron Lett., 1978, 1557; b) S. Inayama, T. Kawamata, T. Ohkura, A. Itai, and Y. Iitaka, Chem. Pharm. Bull., 1975, 23, 2998.
 - 8) S. Inayama, T. Kawamata, and M. Yanagita, Phytochemistry, 1973, 12, 1741.
 - 9) F. Bohlmann, C. Zdero, R. M. King, and H. Robinson, Phytochemistry, 1984, 23, 1979.
 - 10) a) S. M. Kupchan, J. M. Cassady, J. Bailey, and J. R. Knot, J. Pharm. Soc., 1964, 54, 1703; b) S. M. Kupchan, J. M. Cassady, J. E. Kelsey H. K. Schnoes, D. H. Smith, and A. L. Burlingame, J. Am. Chem. Soc., 1966, 88, 5292;

- c) T. A. Dulforce, G. A. Sim, D. N. J. White, J. E. Kelsey and S. M. Kupchan, Tetrahedron Lett., 1969, 973.
- 11) a) W. Herz and S. Inayama, Tetrahedron, 1964, 20, 341; b) H. Yoshioka, T. J. Mabry, N. Dennis, and W. Herz, J. Org. Chem., 1970, 35, 627
- 12) For a preliminary account for Δ -epimers of (5) and (6).
K. Harimaya, H. Hori, T. Ohkura, T. Kawamata, Ji-Fu Gao, and S. Inayama, Heterocycles, 1988, 27, 38
- 13) W. Herz and V. Sosa, Phytochemistry, 1988, 27, 155.
- 14) Isolated first from Helenium autumnale (K. H. Lee, T. Ibuka, M. Kozuka, A. T. Mcphail, and K. D. Onan, Tetrahedron Lett., 1974, 2287; cf. T. Mcphail, K. D. Onan, and D. M. Gross, J. Chem. Soc., Perkin Trans. II, 1975, 5, 492).
- 15) Isolated from a cultivated species of G. grandiflora, which is closely related to G. pulchella [S. Inayama, T. Ohkura, and Y. Iitaka, Chem. Pharm. Bull., 1977, 25, 1928] and found first in a collection of G. spathulata [W. Herz, S. Rajapa, M. V. Lahshmikantham, D. Raulais, and J. J. Schmid, J. Org. Chem., 1967, 32, 1042].
- 16) F. Bohlmann, C. Christa, and M. Ahmed, Phytochemistry, 1982, 21, 1679.
- 17) S. M. Kupchan, M. A. Eakin, and A. M. Thomas, J. Med. Chem., 1971, 14, 1147.
- 18) I. H. Hall, W. L. Williams Jr, A. A. Grippo, K. H. Lee, D. J. Holbrook, and S. G. Chaney, Anticancer Research, 1988, 8, 33; References loc. cit.
- 19) K. Harimaya, N. Arai, and S. Inayama, Chem. Pharm. Bull., 1989, 37, 2525.
- 20) K. Harimaya, Y. Iitaka, and S. Inayama, unpublished data; cited in K. Harimaya, Doctor thesis, Tokyo University in 1989.
- 21) T. Fujita, J. Iwasa, and C. Hansch, J. Am. Chem. Soc., 1964, 86, 5175; a limitation due to low solubility and other complicating factors seem to be unfavorable for a rigorous Hansch analysis [J. L. Hartwell and B. J. Aobott, Advan. Pharmacol. Chemothera., 1969, 7, 117].

Received, 26th September, 1989