

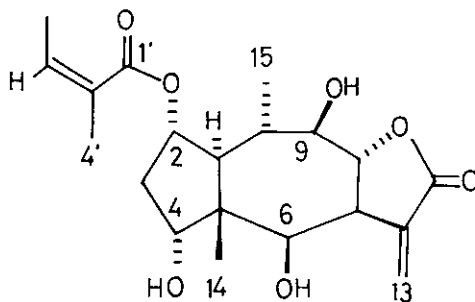
ISOLATION AND STRUCTURES OF PULCHELLOID A AND PULCHELLOID B,
NEW PSEUDOGUAIANOLIDES FROM GAILLARDIA PULCHELLA^{1,2}

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Abstracts

The structures of new pseudoguaianolides named pulchelloid A (1) and pulchelloid B (2) isolated from the methanol extraction of Gaillardia Pulchella together with the known pulchellin (3) and neopulchellin (4) were determined mainly by 400 MHz ¹H nmr associated with decoupling experiments and ¹³C nmr in addition to uv, ir and MS spectral data.

In the course of our studies on biologically active constituents of Gaillardia pulchella (Compositae), several sesquiterpene lactones³ including active pseudoguaianolides pulchellin^{4,5} and neopulchellin⁶ and closely related alkaloids pulchellidine^{4,5} and neopulchellidine⁶ along with a modified pseudoguaianolide pulchellon⁷ have been isolated from the consistent chloroform extraction of the dried whole plant. A further attention was focussed on the possible occurrence of diverse components in addition to less hydrophillic sesquiterpenolides already isolated as mentioned above. In this communication we wish to describe the isolation and structural elucidation of two new highly oxidised pseudoguaianolides named pulchelloid A (1) and pulchelloid B (2) besides the dominant constituents pulchellin (3) and neopulchellin (4) from the methanol extract of the fresh material.



Pulchelloid A (1); B (2) (2-isovalerate)

Table I 400 MHz ^1H nmr Chemical Shifts and Coupling Constants of
Pulchelloid A (1) and Pulchelloid B (2)^a

Pulchelloid A				Pulchelloid B			
H	δ (ppm)		J (Hz)	δ (ppm)		J (Hz)	
1	2.453	dd	6.8(1,2), 11.7(1,10)	2.354	dd	7.1(1,2), 11.5(1,10)	
2	5.074	ddd	1.6(2,3 α), 7.0(2,1) 8.0(2,3 β)	5.608	ddd	1.7(2,3 α), 7.2(2,1) 7.2(2,3 β)	
3 α	1.575	dd	2.0(3 α ,2), 15.6(3 α ,3 β)	1.520	dd	2.0(3 α ,2), 15.4(3 α ,3 β)	
3 β	2.594	ddd	4.6(3 β ,4), 8.8(3 β ,2) 13.2(3 β ,3 α)	2.551	ddd	4.8(3 β ,4), 8.8(3 β ,2) 13.6(3 β ,3 α)	
4	3.851	dd	4.0(4,4-OH), 4.0(4,3 β)	3.822	dd	3.6(4,4-OH), 4.0(4,3 β)	
6	4.992	dd	5.6(6,6-OH), 3.6(6,7)	4.981	dd	5.6(6,6-OH), 4.0(6,7)	
7	3.092	m		3.084	m		
8	4.537	dd	9.5(8,9), 9.3(8,7)	4.519	dd	9.5(8,9), 9.3(8,7)	
9	3.306	ddd	4.8(9,9-OH), 8.8(9,8) 8.8(9,10)	3.281	ddd	4.7(9,9-OH), 9.1(9,8) 9.0(9,10)	
10	1.8-1.9	m		1.83	m		
13a	6.196	d	3.6(13a,7)	6.192	d	3.4(13a,7)	
13b	5.625	d	3.2(13b,7)	5.616	d	3.2(13b,7)	
14	0.952	s		0.936	s		
15	1.155	d	6.6(14,10)	1.137	d	6.6(14,10)	
2'	—			2.138	brd	7.0(2',3')	
3'	6.068	qq	4.0(3',5'), 0.7(3',4')	1.93-1.99	m		
4'	1.857	brs		0.936	d	6.8(4',3')	
5'	1.951	brd	9.0(5',3')	0.936	d	6.8(5',3')	
4-OH	4.193	d	3.4(4-OH,4)	4.190	d	3.7(4-OH,4)	
6-OH	3.994	d	5.9(6-OH,6)	3.976	d	5.9(6-OH,6)	
9-OH	4.421	d	4.6(9-OH,9)	4.402	d	4.9(9-OH,9)	

a: Assignments based on proton decoupling experiments.

Run in $(\text{CD}_3)_2\text{CO/TMS}$.

A mixture of **1** and **2** was first separated from the so-called pulchellin mixture consisting of **3** and **4** by repetition of silica gel chromatography of the crude extract. Pulchelloid A and B were finally isolated pure by submitting this mixture to a reversed phase high performance LC [μ -Bondapak C_{18} Semi Prep column, using $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (2:8) as the solvent and uv detection at 254 nm] at the average ratio of 1:2.

Pulchelloid A (**1**), $\text{C}_{20}\text{H}_{28}\text{O}_7$, mp 165-167°C, exhibited prominent CI-MS base peak at m/e 381 (MH^+). **1** displays characteristic absorptions of α -methylene- γ -lactone grouping, which are ir bands (KBr) at 1747 (γ -lactone) and 1645 cm^{-1} (conjugated double bond) and uv maximum (EtOH) 212.5 nm (ϵ 12551) as well as a pair of low

field doublets ($J = 3.2, 3.6$ Hz) appeared at 5.625 and 6.196 ppm in ^1H nmr spectrum (see Table I). In addition, 1 exhibits an ir band at 1695 cm^{-1} (α, β -unsaturated ester) and prominent MS fragment ion peaks at m/e 55 and 83 as well as ^{13}C nmr signals (see Table II) at 167.9 (carbonyl carbon), 129.2 and 137.3 ppm (olefinic carbons) and ^1H nmr signals at 6.068 (1H, qq, $J = 4.0, 0.7$), 1.857 (3H, br s) and 1.951 ppm (3H, br d, $J = 9.0$ Hz). The above-mentioned data suggest the presence of both α -methylene- γ -butyrolactone and angelate⁸ moiety in the molecule.

Table II ^{13}C nmr Spectra (100 MHz) of Pulchelloid A (1) & Pulchelloid B (2)^e

C	1		2		C	1		2	
1	49.7	d	49.5	d	11	138.3	s	138.3	s
2	80.5	d ^a	80.4	d ^c	12	170.0	s	170.0	s
3	41.4	t	41.3	t	13	121.0	t	121.0	t
4	79.3	d ^a	79.2	d ^c	14	18.3	q ^b	18.3	q ^d
5	53.3	s	53.3	s	15	17.9	q ^b	17.8	q ^d
6	64.8	d	64.8	d	1'	167.9	s ^f	172.7	s
7	47.6	d	47.6	d	2'	129.2	s ^f	44.3	t
8	81.3	d ^a	81.3	d ^c	3'	137.3	d ^f	26.3	d
9	79.5	d ^a	79.4	d ^c	4'	20.7	q ^f	22.7	q
10	38.2	d	38.1	d	5'	15.8	q ^f	22.7	q

a,b,c,d: Assignments interchangeable. e: Assignments based on single-frequency off-resonance. Run in $(\text{CD}_3)_2\text{CO/TMS}$. f: see ref. 8.

The determination of the whole structure and relative stereochemistry of 1 has met with an indifferent solution mainly by the use of 400 MHz ^1H nmr in order to overcome the difficulty due to the complex multiplicity of signals brought about by employing conventional nmr spectrometers and techniques. Since there exists a considerable discrepancy for the interpretation of these ^1H nmr (Table I) with the decoupling studies mentioned below and ^{13}C nmr (Table II) providing that the structure has either a guaiane or eudesmane skeleton, 1 should be regarded to possess a pseudoguaiane skeleton on which the α -methylene- γ -lactone moiety and the angelate group are possibly located at $\text{C}_7\text{-C}_8$ and at C_2 respectively as evidenced below.² An extensive ^1H nmr study through proton decoupling experiments allowed us to assign all the proton peaks except for H_{10} to each chemical shift and coupling constant appeared in the complex spectra as indicated in Table I. Comparison of coupling constants thus observed to those deduced from consideration of Dreiding models led to the confirmation of the stereochemistry given in formula 1. The large values observed in the above decoupling experiments for $J_{7,8}$ (9.0 Hz), $J_{8,9}$ (9.0 Hz), $J_{9,10}$ (8.5 Hz), and $J_{10,1}$ (11.0 Hz), which are acceptable even

somewhat different from the simply observed values shown in Table I, implicate the trans diaxial relationship with respect to each neighboring hydrogen atom attached at the serial carbons, i.e. C₇, C₈, C₉, C₁₀ and C₁, which are embodied in the chair form of the cycloheptane ring of the pseudoguaiane skeleton. The trans-fused α -methylene- γ -lactone is further verified according to the generalization of $J_{7,13}(\text{trans}) \leq 3 \text{ Hz} \leq J_{7,13}(\text{cis})$.⁹ The magnitude of $J_{6,7}$ (3.5 Hz) and $J_{1,2}$ (6.5 Hz) requires β and α -orientation¹⁰ of the hydroxyl group at C₆ and C₂, respectively, and the small values of $J_{3\alpha,4}$ (~0 Hz) and $J_{3\beta,4}$ (4.5 Hz) suggest α configuration of the hydroxyl group at C₄. When deuterium oxide was added, a pair of doublet of doublets at 3.851 (H₄, J = 4.0, 4.0 Hz) and 4.992 (H₆, J = 5.6, 4.0 Hz) collapsed to doublets (H₄, J = 4.5; H₆, J = 3.5 Hz), and a doublet of double doublets at 3.306 ppm (H₉, J = 4.8, 8.8, 8.8 Hz) also changed to a doublet of doublets (J = 9.5, 9.5 Hz). In addition, disappeared three signals of doublet at 3.994 (J = 5.9 Hz), 4.193 (J = 3.4 Hz), and 4.421 ppm (J = 4.6 Hz). These data indicate that the three hydroxyl groups were located at C₄, C₆ and C₉, respectively. The ir stretching vibration (3425 cm⁻¹) due to these hydroxyl groups in 1 was exchanged by newly born acetoxy-carbonyl bands (1735, 1743 cm⁻¹) of the corresponding triacetate, C₂₆H₃₄O₁₀, mp 168-171°C, in which the signals attributed to methine protons on the carbons bearing the hydroxyls in 1 shifted downward by approximately 1 ppm as expected. Hence the stereostructure of pulchelloid A has been defined.

Pulchelloid B (2), C₂₀H₃₀O₇, mp 167-169°C, CI-MS (isobutane) m/e 383 (MH⁺), has an α -methylene- γ -lactone group [ir (KBr) ν 1762, 1662 cm⁻¹; uv (EtOH) λ 212 nm (ϵ 10667); ¹H nmr δ (ppm) 6.19, d, J = 3.4 Hz, 5.62, d, J = 3.2 Hz]. The formation of triacetate, C₂₆H₃₆O₁₀, EI-MS m/e 508 (M⁺), mp 65-67°C, suggests the presence of three hydroxyl groups in 2. 2 exhibited ir absorption at 1734 cm⁻¹ (saturated ester) and prominent MS fragment ion peaks at m/e 57 and 85. The specific signals for 2 were observed at 22.7 (2x -CH₃), 26.3 ($\geq$ CH), 44.3 ($>$ CH₂) and 172.7 ppm (-O-COR) in the ¹³C nmr spectrum as well as at 0.936 (6H, d, J = 6.8 Hz, 2x -CH₃), 1.93-1.99 (1H, m, $\geq$ CH) and 2.138 ppm (2H, d, J = 7.0 Hz, $>$ CH₂) in the ¹H nmr spectrum. These data evidently suggest the existence of an isovalerate group in 2. The conclusive data of ¹H nmr and ¹³C nmr spectra shown in Table I and II were well coincident with those of 1 except for the ester moiety. The structure and relative stereochemistry of 2 were likewise confirmed by proton decoupling experiments as in the case of 1. The stereostructure of pulchelloid B has thus been deduced as shown in 2. Albeit the absolute configurations of these highly

substituted pseudoguaianolides may preliminarily be assigned as in the present structures, in harmony with all the spectral data described above and the preceding biogenetic point of view,³ further studies of the establishment are in progress.

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References and Notes

1. This paper is dedicated for Professor Kyōsuke Tsuda, Emeritus Professor of Tokyo University on the occasion of his 75th birthday.
2. Presented to the 25th Symposium of Perfume, Terpene and Essential Oil, Yamaguchi, 1981, Oct. 2-4, wherein pulchelloid A and B were tentatively referred to pulangelin A and B. Paper abstracts pp. 107-109.
3. S. Inayama, T. Kawamata, and M. Yanagita, Phytochemistry, 1973, 12, 1741.
4. M. Yanagita, S. Inayama, and T. Kawamata, Tetrahedron Lett., 1970, 131; T. Sekita, S. Inayama, and Y. Iitaka, ibid., 1970, 135.
5. M. Yanagita, S. Inayama, and T. Kawamata, ibid., 1970, 3007.
6. M. Yanagita, S. Inayama, T. Kawamata, T. Ohkura, and W. Herz, ibid., 1969, 2073, 4170.
7. S. Inayama, T. Kawamata, and T. Ohkura, ibid., 1978, 1557; S. Inayama, T. Kawamata, T. Ohkura, A. Itai, and Y. Iitaka, Chem. Pharm. Bull., 1975, 23, 2998.
8. G. R. Pettit, C. L. Herald, D. Gust, D. L. Herald, and L. D. Vanell, J. Org. Chem., 1978, 43, 1092.
9. Z. Samek, Tetrahedron Lett., 1970, 671.
10. The prefix of α or β for the configuration of substituents on this pseudoguaianolide was described as reference to the angular methyl group, which should commonly be β-oriented from the biogenetic point of view.

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