STRUCTURE AND CONFORMATION OF A NEW HELENANOLIDE, PULCHELLOID C

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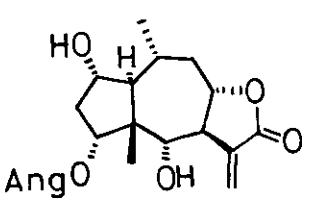
Abstract——The stereostructure, conformation and absolute configuration of *pulchelloid C, a new minor component, helenanolide* isolated from the methanol extract of Gaillardia pulchella, were deduced by combination of chemical and spectroscopic methods as well as X-ray crystallography.

In the course of our studies on biologically active constituents of Gaillardia pulchella (Compositae), several pseudoguaianolides were isolated from the chloroform extraction of a dried material of this plant.[1,2] Recently, we have isolated highly oxygenated pseudoguaianolides, pulchelloid A and B, from the methanol extraction of the fresh whole plant.[3] In this communication we describe the structural determination of pulchelloid C named for a new helenanolide isolated as a minor constituent from the similar material.

Pulchelloid C (1) was found to be contained together with known congeners of



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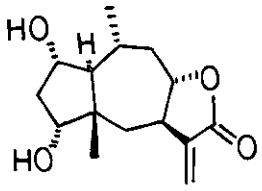
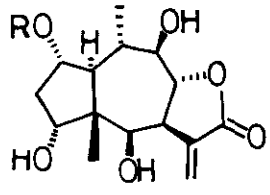


2, 3 (8αH)



4 (R = Ang), 5 (R = i-Val)

Table 1 400 MHz ^{1}H-nmr chemical shifts (multiplicities) and coupling constants of pulchelloid C (1) ($CDCl_3$/TMS)

H	δ (ppm)		J(Hz)	H	δ (ppm)		J(Hz)
1	2.21	dd	9.4, 8.3	10	1.86	m	
2	4.17	m		14	1.25	d	6.7
3α	1.71	ddd	14.4, 8.3, 4.0	15	1.04	s	
3β	2.59	ddd	14.4, 8.3, 5.6	13a	6.25	dd	3.5, 0.8
4	5.05	dd	5.6, 4.0	13b	6.10	dd	2.9, 0.8
6	3.75	dd	9.6, 9.4	3'	6.15	qq	7.2, 1.3
7	3.10	dddd	9.6, 9.4, 3.5, 2.7	4'	1.92	dq	1.3, 1.3
8	4.15	ddd	12.9, 9.4, 3.5	5'	2.03	dq	7.2, 1.3
9α	1.49	ddd	12.9, 12.9, 12.9	2-OH	2.86	d	7.2
9β	2.34	ddd	12.9, 3.5, 3.5	6-OH	3.76	d	9.4

pseudoguaianolides, that is, pulchellin (2),[1] neopulchellin (3),[2] pulchelloid A (4)[3] and pulchelloid B (5)[3] by their clean separation using repeated silica gel chromatography with a gradient mixture of chloroform and ethyl acetate followed by highly efficient isolation of 1 from 4 and 5. Pulchelloid C (1), $C_{20}H_{28}O_6$, CI-ms (isobutane), m/z 365 (MH^+, base peak), mp 98.5-100°C, $[\alpha]_D^{25°}$ -130° (c 0.1, MeOH) contained two hydroxyl groups [ir (KBr) 3540, 3430 cm^{-1}], an α-methylene-γ-butyrolactone moiety [ir ν(KBr) 1748, 1640 cm^{-1}; uv λ(EtOH) 210 nm (logε 4.24); ^{1}H-nmr δ (ppm) 6.10 (1H, dd, J = 2.9, 0.8 Hz), 6.25 (1H, dd, J = 3.5, 0.8 Hz)], an angeloyl ester group [ir ν(KBr) 1688 cm^{-1}; ^{1}H-nmr δ (ppm) 1.92 (3H, dq, J = 1.3, 1.3 Hz), 2.03 (3H, dq, J = 7.2, 1.3 Hz); ^{13}C-nmr δ (ppm) (acetone-d_6/TMS) 15.8 q, 20.8 q, 137.1 d, 129.6 s, 167.1 s], a tertiary methyl [^{1}H-nmr δ (ppm) 1.04 (3H, s)] and a secondary methyl group [^{1}H-nmr δ (ppm) 1.25 (3H, d, J = 6.7 Hz)]. These data suggested that pulchelloid C had a pseudoguaiane skeleton including an α-methylene-γ-butyrolactonic moiety and an angeloyl group. The full structure and relative stereochemistry of 1 were deduced mainly on the basis of 400 MHz ^{1}H-nmr spectrum as described below. As shown in Table 1, the 400 MHz

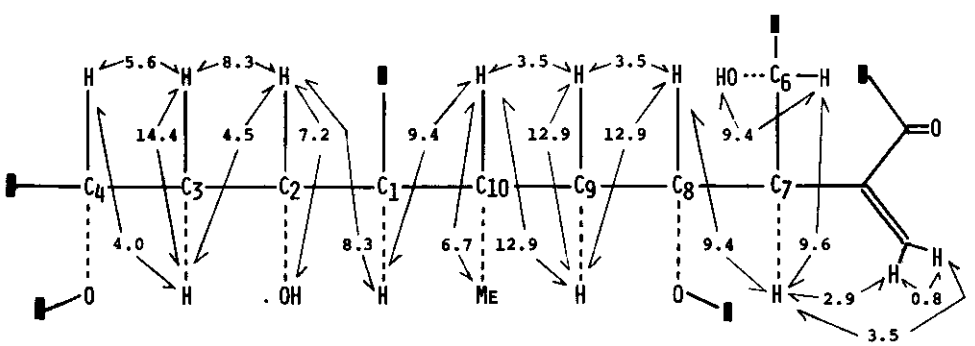


Fig. 1 Partial structure A of pulchelloid C (1) including ^{1}H-nmr J-values (Hz)

^{1}H-nmr spectrum ($CDCl_3$)[4] of 1 displayed signals due to a secondary methyl group at 1.25 ppm (d, J = 6.7 Hz), two vinylic methyl groups at 1.92 and 2.03 ppm coupled with a vinyl proton at 6.15 ppm (due to angelate group), oxygen bearing methine protons at 5.05, 4.17, 4.15 and 3.75 ppm, two terminal olefinic protons at 6.25 and 6.10 ppm, two hydroxyl protons at 3.76 and 2.86 ppm. In addition, extensive ^{1}H-NMDR studies revealed the presence of the partial structure A, in which exo methylene protons showed significant long range coupling $J_{7,13a}$ = 3.5 Hz and $J_{7,13b}$ = 2.9 Hz. These nmr data for 1 indicated a close relationship with pulchellin (2), one of the congeners isolated from the same plant. The significant differences in the nmr spectra of 1 were the low field shift of H-4, lack of H-6α which existed in 2 and appearance of angelate group. On irradiation of a hydroxyl proton at 5.56 ppm (d, J = 6.8 Hz) in $CDCl_3$/pyridine-d_5 (30%), the doublet of doublet signal for H-6 with J = 6.8, 9.7 Hz was changed into a doublet with J = 9.4 Hz. This fact indicated the position of the new hydroxyl group in 1 must be at C(6). The angelate group in 1 was located at C(4) on the basis of the lower field shift of H-4 compared with that of 2. Its configuration was assigned to α from the down field shifted hydroxyl group at C(2) due to the effect of hydrogen bond between this hydroxyl group and carbonyl group of the angelate. Consequently, the structure of pulchelloid C is represented as 1.

The structure and conformation of 1 was finally confirmed by the following X-ray diffraction study of pulchelloid C monohydrate by the direct method.

Pulchelloid C (1) was recrystallized from ethyl acetate as colourless prisms. A single crystal of approximate dimensions 0.4 x 0.4 x 0.2 mm obtained as monohydrate afforded the crystal data as follows: $C_{20}H_{28}O_6 \cdot H_2O$, FW = 382.4 orthorhombic, space group $P2_12_12_1$, Z = 4, D_c = 1.278 gcm^{-3}. $\underline{a}$ = 13.876 (7), $\underline{b}$ = 15.199 (8), $\underline{c}$ = 9.423 (5) Å, U = 1,987.3 $Å^3$. Of the total of 2,024 reflections measured within 2θ angle of 25° on a Philips PW1100 diffractometer using MoKα radiation monochromated by a graphite plate, 1,605 reflections were used for the structure determination as above the 2σ (I) level.

18 Atoms were located on an E-map calculated by MULTAN program[5] and the subsequent structure factor and Fourier calculations revealed the whole structure. Refinement was carried out by the block-diagonal least-squares method including all the 28 hydrogen atoms of the molecule and 2 of those of the

crystallization water. The R factor was reduced to 0.063. The molecular structure is illustrated in Fig. 2 drawn by ORTEP program.[6] Table 2 shows the selected torsion angles within each ring.

Table 2 Ring torsion angles of pulchelloid C (1)

Lactone ring		Cyclopetane ring	
C(7)-C(8)-O(1)-C(12)	-21.8°	C(1)-C(2)-C(3)-C(4)	4.1
C(8)-O(1)-C(12)-C(11)	8.3	C(2)-C(3)-C(4)-C(5)	23.4
O(1)-C(12)-C(11)-C(7)	9.0	C(3)-C(4)-C(5)-C(1)	-40.5
C(12)-C(11)-C(7)-C(8)	-21.0	C(4)-C(5)-C(1)-C(2)	44.1
C(11)-C(7)-C(8)-O(1)	25.3	C(5)-C(1)-C(2)-C(3)	-29.9
Cycloheptane ring-1		**Cycloheptane ring-2**	
C(5)-C(6)-C(7)-C(8)	77.6	C(9)-C(10)-C(1)-C(5)	87.7
C(6)-C(7)-C(8)-C(9)	-84.8	C(10)-C(1)-C(5)-C(6)	-59.1
C(7)-C(8)-C(9)-C(10)	61.6	C(1)-C(5)-C(6)-C(7)	-18.0
C(8)-C(9)-C(10)-C(1)	-62.9		

As shown in Fig. 2, the cyclopentane ring was trans-fused to the central cycloheptane ring, and had an envelope form with the flap at C(5). Torsion angles C(1)-C(2)-C(3)-C(4) was close to 0° (+4.1°). This fact showed that C(1), C(2), C(3), and C(4) were closely planar. The cycloheptane ring revealed a deformed chair conformation, and the γ-lactone ring being trans-fused to the cycloheptane ring had a slightly twisted form.

The absolute configuration was supposed to be as shown in Fig. 2 on the basis of the analogy of the structure with pulchellin,[1] along with the considerations on the biosynthesis of the pseudogauianolides.[7] The cd Cotton effect of the n-π* transition of the α-methylene-γ-lactone chromophore in 1 was not observed in the range between 246 and 261 nm,[8] mainly due to the angeloyl ester chromophore present in this molecule.

In order to confirm the absolute configuration, the precise measurement of the diffraction intensities of several Friedel pairs was attempted. The structure factor calculations including anomalous dispersion corrections for carbon and oxygen atoms for CuKα radiation showed that 35 Friedel pairs should have the intensity difference greater than 3%. The intensities of these reflections along with their symmetry related ones were measured with CuKα radiation and the average values of the four symmetry equivalent reflections were compared with those of Friedel reflections. The 2θ angles of the measured reflections were ranged from 40° to 120° and no absorption corrections were applied. The result showed very good agreement with the calculated values: 27 out of

35 pairs have the same relations of $I(hk\ell)/I(\bar{h}\bar{k}\bar{\ell})$ with the calculated values and the remaining 8 pairs have either very weak intensity or very small intensity differences. The proposed absolute configuration shown in Fig. 2 was also supported by the present X-ray study.

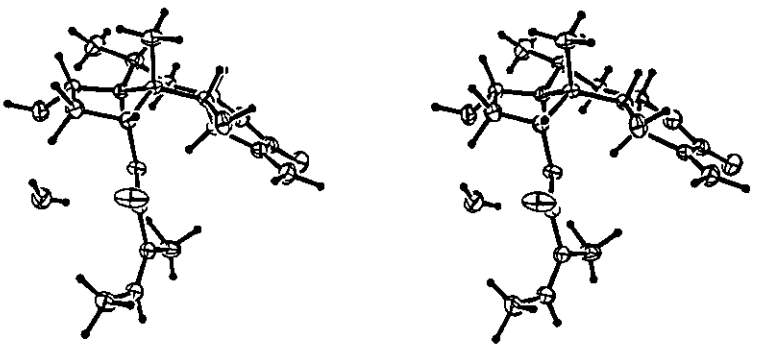


Fig. 2 Stereoview with absolute configuration of pulchelloid C monohydrate

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The fractional atomic coordinate for pulchelloid C monohydrate (1)

NO	ATOM	X 10**4	Y 10**4	Z 10**4	BEQ A**2
1	C1	633(4)	1306(4)	4845(6)	2.96(.08)
2	C2	1538(4)	1355(4)	5710(7)	3.37(.09)
3	C3	2167(5)	573(5)	5159(8)	4.52(.11)
4	C4	1614(4)	160(4)	3924(7)	3.18(.09)
5	C5	952(4)	907(3)	3398(6)	2.66(.08)
6	C6	144(4)	602(4)	2383(6)	2.77(.08)
7	C7	-835(4)	469(4)	3100(6)	2.65(.08)
8	C8	-1325(4)	1363(4)	3356(7)	3.21(.09)
9	C9	-1090(4)	1880(4)	4652(7)	3.53(.09)
10	C10	-32(4)	2149(4)	4747(7)	3.28(.09)
11	C11	-1610(4)	-18(4)	2307(6)	3.22(.08)
12	C12	-2534(4)	422(4)	2623(7)	3.74(.09)
13	C13	-1577(5)	-733(5)	1499(8)	4.60(.11)
14	C14	94(6)	2789(5)	5983(8)	4.92(.12)
15	C15	1615(5)	1548(4)	2565(8)	3.97(.10)
16	C16	1292(4)	-1367(4)	4369(6)	3.23(.09)
17	C17	583(5)	-2005(4)	4982(7)	3.18(.09)
18	C18	870(5)	-2788(4)	5434(7)	3.78(.10)
19	C19	1858(5)	-3176(4)	5525(8)	4.66(.12)
20	C20	-444(5)	-1709(4)	5123(9)	4.49(.11)
21	O1	-2366(3)	1174(3)	3328(5)	4.05(.07)
22	O2	1329(3)	1281(3)	7193(5)	4.65(.07)
23	O3	973(3)	-539(2)	4477(4)	3.00(.06)
24	O4	483(3)	-171(3)	1656(5)	4.18(.07)
25	O5	-3344(3)	174(3)	2359(6)	5.58(.09)
26	O6	2060(4)	-1562(3)	3855(7)	6.27(.10)
27	OW	216(4)	93(4)	8788(5)	5.52(.09)

NO	ATOM	X 10**3	Y 10**3	Z 10**3	BEQ A**2
28	H(C1)	18(4)	88(4)	543(6)	4.(1.)
29	H(C2)	195(5)	188(4)	559(7)	6.(2.)
30	H(C3)	292(4)	79(3)	496(6)	3.(1.)
31	H'(C3)	226(5)	10(5)	603(8)	8.(2.)
32	H(C4)	212(3)	-10(3)	317(6)	3.(1.)
33	H(C6)	21(4)	103(4)	165(6)	4.(1.)
34	H(C7)	-83(4)	24(4)	412(6)	4.(1.)
35	H(C8)	-124(3)	173(3)	265(5)	2.(1.)
36	H(C9)	-113(4)	161(4)	539(6)	4.(1.)
37	H'(C9)	-155(4)	243(4)	461(7)	6.(2.)
38	H(C10)	5(4)	242(3)	372(5)	3.(1.)
39	H(C13)	-232(4)	-94(4)	101(6)	4.(2.)
40	H'(C13)	-100(5)	-103(4)	151(7)	6.(2.)
41	H(C14)	-3(4)	258(4)	686(6)	5.(1.)
42	H'(C14)	-53(5)	331(4)	601(7)	6.(2.)
43	H"(C14)	89(6)	287(5)	596(9)	9.(2.)
44	H(C15)	176(5)	117(5)	155(8)	8.(2.)
45	H'(C15)	222(4)	169(4)	307(7)	5.(2.)
46	H"(C15)	128(5)	212(5)	227(8)	7.(2.)
47	H(C18)	20(5)	-308(4)	595(8)	6.(2.)
48	H(C19)	236(5)	-277(5)	577(9)	9.(2.)
49	H'(C19)	205(5)	-349(5)	456(9)	9.(2.)
50	H"(C19)	189(5)	-364(5)	637(8)	8.(2.)
51	H(C20)	-77(4)	-212(4)	548(6)	4.(1.)
52	H'(C20)	-64(5)	-147(4)	420(7)	6.(2.)
53	H"(C20)	-43(5)	-99(5)	569(8)	8.(2.)
54	H(O2)	175(6)	152(6)	792(10)	11.(3.)
55	H(O4)	41(4)	-11(4)	68(7)	5.(2.)
56	H(OW)	-29(5)	3(4)	843(8)	6.(2.)
57	H'(OW)	78(7)	19(6)	845(10)	12.(3.)

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