ISOLATION AND STRUCTURE OF NEW PSEUDOGUAIANOLIDES, 4-EPIPULCHELLIN AND 4-EPINEOPULCHELLIN FROM GAILLARDIA PULCHELLA[1]

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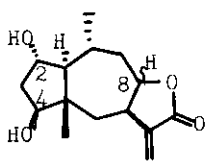
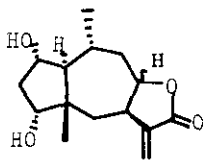
Abstract——The structure of 4-epipulchellin (1a) and 4-epineopulchellin (1b), new pseudoguaianolide sesquiterpene lactones isolated from Gaillardia pulchella, was determined on the basis of spectroscopic data and chemical transformations from pulchellin (2a) and neopulchellin (2b) into 1a and 1b, respectively.

In the course of our search on bioactive sesquiterpenoid constituents of Gaillardia pulchella (Compositae), we have elucidated the absolute structures of several pseudoguaianolide principles, such as pulchellin,[2,3] neopulchellin,[3,4] pulchellidine,[3,5] neopulchellidine,[3,6] pulchellon,[7] pulchelloid A,[8] B[8] and C[9] together with guaianolides such as gaillardin ,[3,10] and neogaillardin ,[3] and eudesmanolides such as pulchellin B, C, D, E and F.[3,11] This communication describes the isolation and structure determination of two new minor pseudoguaianolides possessing 4β-hydroxyl group in this plant, while all the pseudoguaianolides isolated so far from G. pulchella by our and German groups[12] showed, without exception, to hold the α-configuration of both 2- and 4-hydroxyl or alkoxyl groups.

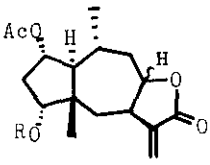
4-Epipulchellin (1a) (0.00003% from dried plant material) and 4-epineopulchellin (1b) (0.00003%) together with a cytotoxic guaianolide, florilenalin[13] (0.0005%) were isolated from the methanol extract of this plant after the separation of pulchellin (2a), and neopulchellin (2b), pulchelloid A and B by repeated silica gel chromatography and subsequent high performance liquid chromatography (column; Chemcosorb 5Si, solvent; $CHCl_3$/EtOH 9:1).



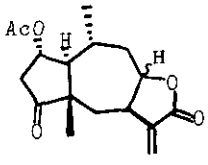
1a,b



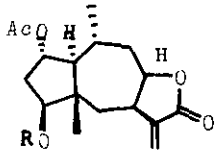
2a,b



3a,b (R=H)
4a,b (R=Ac)



5a,b



6a,b (R=H)
7a,b (R=Ac)

(a: 8βH series, b: 8αH series)

4-Epipulchellin (1a): $C_{15}H_{22}O_4$, colorless oil, ir ν^{KBr} (cm^{-1}) 3375 (OH), 1751 (γ-lactone), 1656 (C=C); cims (isobutane) m/z 267 (MH^+, base peak) 249, 231; eims m/z 266 (M^+), 248, 230; hrms m/z obsd. 266.1495 calcd. 266.1515 for $C_{15}H_{22}O_4$; (^{1}H-nmr, 270 MHz, $CDCl_3$) δ (ppm) 6.17 d (1H, J = 3.3 Hz, H-13'), 5.45 d (1H, J = 2.9 Hz, H-13), 4.22 ddd (1H, J = 9.5, 9.5, 3.8 Hz, H-8), 4.14 m (2H, H-2 and H-4), 1.18 d (3H, J = 6.8 Hz, H-14), 0.90 s (3H, H-15). ^{1}H-Nmr spectrum of 1a was very similar to that of pulchellin (2a) except for the chemical shift and multiplicities of H-4 proton. The partial structure from C-5 to C-10 was assigned by the proton decoupling experiments, but a complete assignment of the whole stereostructure of 1a was hampered by overlapping of H-2 and H-4 signals using most available ^{1}H-nmr spectra. This situation prompted us to synthesize 4-epipulchellin (1a) from 2a which is a major component of this plant.

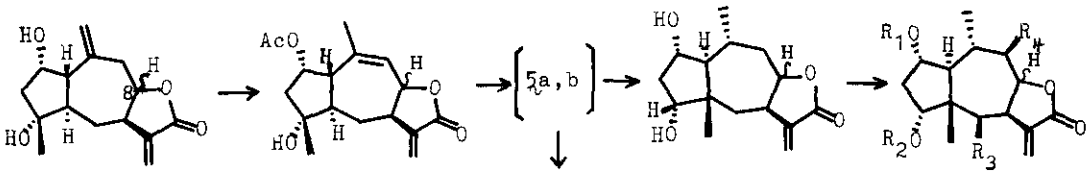
2-Acetyldehydropulchellin (5a)[4] prepared by oxidation of 2-acetylpulchellin (3a)[4] was subjected to reduction with sodium borohydride ($NaBH_4$, 0.5 eq. mol) in MeOH (r.t., 30 min.) yielding in almost quantitative yield, stereospecifically 2-acetyl-4-epipulchellin (6a): $C_{17}H_{24}O_5$, mp 188-191°C, ir ν^{KBr} (cm^{-1}) 3500 (OH), 1756 (γ-lactone), 1731 ($OCOCH_3$); cims (isobutane) m/z 309 (MH^+); eims m/z 248, 230; hrms m/z obsd. 309.1702, calcd. 309.1702 for $C_{17}H_{25}O_5$; ^{1}H-nmr (400 MHz, $CDCl_3$) δ 6.16 d (1H, J = 3.6 Hz, H-13'), 5.47 d (1H, J = 3.1 Hz, H-13), 4.93 ddd (1H, J = 3.4, 9.5, 12.2 Hz, H-8), 4.04 dd (1H, J = 8.6, 10.7 Hz, H-4), 2.84 m (1H, H-7), 2.04 s (3H, $OCOCH_3$), 1.95 m (1H, H-10), 0.97 d (3H, J = 6.7 Hz, H-14), 0.93 s (3H, H-15). Compound 6a was then treated with Ac_2O/pyridine to afford diacetyl-4-epipulchellin (7a): $C_{19}H_{26}O_6$, mp 133-134°C, ir ν^{KBr} (cm^{-1}) 1763 (γ-lactone), 1729 ($OCOCH_3$); cims (isobutane) m/z 351 (MH^+, base peak); eims m/z 290, 230; hrms m/z obsd. 350.1829, calcd. 350.1819 for $C_{19}H_{26}O_6$; ^{1}H-nmr (400 MHz, $CDCl_3$) δ 6.18 d (1H, J = 3.4 Hz, H-13'), 5.44 d (1H, J = 3.4 Hz, H-13), 4.21 ddd (1H, J = 3.4, 9.5,

12.2 Hz, H-8), 2.84 m (1H, H-7), 2.37 ddd (1H, J = 3.4, 3.4, 14.1 Hz, H-9), 2.25 dd (1H, J = 5.8, 14.7 Hz, H-6), 2.10 s (3H, $OCOCH_3$), 2.04 s ($OCOCH_3$), 1.98 m (1H, H-10), 1.39 dd (1H, J = 12.6, 14.4 Hz), 1.35 dd (1H, J = 11.9, 14.4 Hz), 1.00 s (3H, H-15), 0.99 d (3H, J = 7.9 Hz, H-14).

The stereostructure of 6a and 7a were unequivocally determined by the comparison of their ^{1}H-nmr data with those of 4-epipulchellin-2-acetate and 4-epidiacetylpulchellin,[14] which were isolated from Geigeria burkey. Finally treatment of 6a with 10% KOH/dioxane afforded in 81.1% yield, 4-epipulchellin (1a), whose ir and ^{1}H-nmr spectral data accorded in every respects with those of the naturally occurring 4-epipulchellin (1a) mentioned above.

4-Epineopulchellin (1b), $C_{15}H_{22}O_4$, was isolated as a colorless oil, ir ν^{KBr} (cm^{-1}) 3380 (OH), 1764 (γ-lactone); cims (isobutane) m/z 267 (MH^+); eims m/z 266 (M^+), 248, 230; hrms m/z obsd. 266.1504, calcd. 266.1515 for $C_{15}H_{22}O_4$, ^{1}H-nmr (400 MHz, acetone-d_6) δ 6.08 d (1H, J = 2.5 Hz, H-13'), 5.62 d (1H, J = 2.2 Hz, H-13), 4.81 ddd (1H, J = 3.2, 7.8, 11.2 Hz, H-8), 4.01 dd (1H, J = 8.8, 8.8 Hz, H-4), 3.93 dd (1H, J = 8.4, 8.4 Hz, H-2), 3.83 brs (1H, 4-OH), 3.67 brs (1H, 2-OH), 3.29 m (1H, H-7), 2.13 ddd (1H, J = 6.4, 12.9, 12.9 Hz, H-9β), 1.94 m (1H, H-10), 1.66 ddd (1H, J = 1.5, 3.4, 13.8 Hz, H-9α), 1.41 dd (1H, J = 13.7, 13.7 Hz, H-6), 1.20 d (3H, J = 6.8 Hz, H-14), 0.84 s (3H, H-15). ^{1}H-Nmr spectrum of 1b showed to be much similar to that of neopulchellin (2b) possessing a cis fused γ-lactone ring.[4] The coupling constants ($J \leqq 3$) for H-13 (2.2 Hz) and H-13' (2.5 Hz) in 1b suggested that 1b has a cis fused γ-lactone group as in the case of 2b.[4b,15] The only striking difference between those of 1b and 2b was observed in their chemical shifts and coupling constants of H-4. This implicates that 1b seems to be 4-epimer of 2b. In order to confirm this estimation conclusively, chemical transformation from 2b into 1b was undertaken.

Acetylation of 2b with Ac_2O/pyridine gave a 1:1 mixture of 2-acetylneopulchellin (3b) and diacetylneopulchellin (4b). The acetate (3b) was then oxidized with Jones' reagent to afford ketoacetate (5b) (93.8%). Subsequent reduction with $NaBH_4$ (1.3 eq. mol) yielded 4-epineopulchellin-2-acetate (6b) in a stereospecific manner almost quantitatively. 6b: $C_{17}H_{24}O_5$, mp 128-130°C, ir ν^{KBr} (cm^{-1}) 3430 (OH), 1757 (γ-lactone), 1707 ($OCOCH_3$); cims (isobutane) m/z 309 (MH^+, base peak), 249; hrms m/z obsd. 308.1633, calcd. 308.1623 for $C_{17}H_{24}O_5$. Treatment of 6b with 10% KOH/dioxane gave rise to 4-epineopulchellin (1b) in 94.2% yield, which was



8a,b 9a,b 5a,b 1a,b 2a,b 10a:R_1=R_2=H,R_3=R_4=OAc
11a:R_1=Ang,R_2=H,R_3=R_4=OH
12a:R_1=iVal,R_2=H,R_3=R_4=OH
13a:R_1=H,R_2=Ang,R_3=αOH,R_4=H

(a: 8βH series, b: 8αH series)

Scheme 1 *Possible* Biogenetic Scheme of Sesquiterpenoids in G. pulchella

acetylated as usual to afford diacetyl-4-epineopulchellin (7b): Colorless oil, ir ν^{KBr} (cm^{-1}) 1769 (γ-lactone), 1735 ($OCOCH_3$); cims (isobutane) m/z 351 (MH^+, base peak); *eims* m/z 350 (M^+), 290, 248, 230; hrms m/z obsd. 350.1705, calcd. 350.1726 for $C_{19}H_{26}O_6$; ^{1}H-nmr (400 MHz, $CDCl_3$) δ 6.27 d (1H, J = 2.5 Hz, H-13'), 5.66 d (1H, J = 2.2 Hz, H-13), 4.95 dd (1H, J = 9.0, 9.0 Hz, H-4), 4.87 ddd (1H, J = 2.9, 9.3 9.3 Hz, H-2), 4.78 ddd (1H, J = 3.4, 8.6, 11.2 Hz, H-8), 3.22 m (1H, H-7), 2.10 s (3H, $OCOCH_3$), 2.03 s (3H, $OCOCH_3$), 1.04 d (3H, J = 6.8 Hz, H-14), 0.95 s (3H, H-15). These spectroscopic data are in good agreement with those of naturally occurring 4-epineopulchellindiacetate.[14] The physical properties of synthetic 4-epineopulchellin (1b) also accoreded with those of the natural 4-epineopulchellin (1b) in all respects.

Biogenetic relationship between guaianolides and pseudoguaianolides isolated from the title plant is thus shown in Scheme 1. Gaillardin (9a) or neogaillardin (9b) derived from florilenalin (8b)[16] could be transformed to 5a or 5b by way of trans antiparallel consecutive 1,2-shifts of two hydrides and one methyl anion and deprotonation.[3] Subsequent hydrolysis and reduction of 5a and 5b would then result in the formation of 2a and 2b accompanying 1a and 1b, respectively. Biological oxygenation and esterification of 2a conceivably lead to spathulin (10a),[17] pulchelloid A (11a), B (12a) and C (13a). Antitumor activities of 4-epipulchellin (1a) and 4-epineopulchellin (1b) together with the above-mentioned other sesquiterpenoid constituents, which were all isolated from this plant and were ornamented by α-methylene-γ-butyrolactone moiety, will soon be reported elsewhere.

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REFERENCES AND NOTES

1. Presented at the 30th Symposium of the Chemistry of Terpenes, Essential Oils and Aromatics. Abstract p. 245, Hiroshima, Oct. 2-5, 1986.
2. a) M. Yanagita, S. Inayama, and T. Kawamata, Tetrahedron Lett., 1970, 131; b) T. Sekita, S. Inayama, and Y. Iitaka, Acta Crystallogr., 1971, B27, 877; cf. Idem. Tetrahedron Lett., 1970, 135; References loc. cit.
3. S. Inayama, T. Kawamata, and M. Yanagita, Phytochemistry, 1973, 12, 1743.
4. a) M. Yanagita, S. Inayama, and T. Kawamata, Tetrahedron Lett., 1970, 135; b) S. Inayama, K. Harimaya, H. Hori, T. Kawamata, T. Ohkura, H. Nakamura, and Y. Iitaka, Heterocycles, 1982, 19, 1801.
5. a) M. Yanagita, S. Inayama, T. Kawamata, and T. Ohkura, Tetrahedron Lett., 1969, 2073; b) S. Inayama, K. Harimaya, N. Shimizu, H. Hori, T. Ohkura, Y. Iitaka, and T. Kawamata, Heterocycles, 1985, 23, 377.
6. M. Yanagita, S. Inayama, and T. Kawamata, Tetrahedron Lett., 1970, 3007.
7. a) S. Inayama, T. Kawamata, T. Ohkura, A. Itai, and Y. Iitaka, Chem. Pharm. Bull., 1975, 23, 2998; b) S. Inayama, T. Kawamata, and T. Ohkura, Tetrahedron Lett., 1978, 1557.
8. S. Inayama, K. Harimaya, T. Ohkura, and T. Kawamata, Heterocycles, 1982, 17, 219.
9. S. Inayama, K. Harimaya, H. Hori, T. Kawamata, T. Ohkura, I. Miura, and Y. Iitaka, Heterocycles, 1983, 20, 1501.
10. T. A. Dullforce, G. A. Sim, D. N. White, J. E. Kelsey, and S. M. Kupchan, J. Org. Chem., 1975, 35, 617.
11. a) W. Herz and S. Inayama, Tetrahedron, 1964, 20, 341 ; b) T. A. Mabry, N. Dennis, and W. Herz, J. Org. Chem., 1975, 35, 627.
12. F. Bohlmann, C. Zdero, R. M. King, and H. Robinson, Phytochemistry, 1984, 23, 1979.
13. Acetylation of florilenalin isolated from G. pulchella with Ac_2O/pyridine/DMAP at 80°C afforded florilenalin diacetate, mp 127-128°C, whose ^{1}H-nmr data coincided with those of diacetylflorilenalin (mp 128-129°C) reported by K. H. Lee, T. Ibuka, and M. Kozuka, Tetrahedron Lett., 1970, 3007.
14. F. Bohlmann, C. Zdero, and M. Ahmed, Phytochemistry, 1982, 21, 1697.
15. Z. Samek, Tetrahedron Lett., 1970, 671.
16. 8-Epiflorilenalin (8a), which is presumably a possible precursor of gaillardin (9a), has not been isolated so far in this plant.
17. S. Inayama, T. Ohkura, and Y. Iitaka, Chem. Pharm. Bull., 1977, 25, 1928.

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