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A New Helenanolide, 11βH-Dihydro-4-epineopulchellin, Isolated from *Gaillardia grandiflora*[1]

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A new pseudoguaianolide named 11βH-dihydro-4-epineopulchellin (1a) was isolated from the methanol extract of *Gaillardia grandiflora*. The stereostructure was determined on the basis of spectral evidence, especially proton nuclear magnetic resonance (including the ^{1}H–^{1}H correlation spectroscopy technique), and was confirmed by the transformation from neopulchellin (2a).

Keywords *Gaillardia grandiflora*; 11βH-dihydro-4-epineopulchellin; 11αH-dihydro-4-epineopulchellin, pseudoguaianolide; ^{1}H–^{1}H COSY; Compositae

In our continuing investigation of bioactive plant principles, several antitumor pseudoguaianolides such as pulchellin,[2] neopulchellin,[3] pulchellidine,[2] neopulchellidine,[3] and pulchellon,[4] as well as congeneric active guaianolides such as gaillardin,[5] and neogaillardin,[6] have been isolated from *Gaillardia* (*G.*) *pulchella* (Compositae). These sesquiterpenolide constituents have a *trans-* or *cis*-fused α-methylene-γ-butyrolactone moiety and carry at least two α-oriented hydroxy or acyloxy groups on the five/seven-membered fused ring system. Recently, we have been searching for highly oxygenated or β-oriented *O*-functionalized congeners in closely related species, especially in *G. grandiflora*. Pseudoguaianolides such as spathulin,[7,8] 9-*O*-desacetylspathulin-2-*O*-isovalerate[9] and 9-*O*-desacetylspathulin-2-*O*-angelate[9] have been reported in this plant. A further investigation of more polar fractions obtained by liquid chromatography of this species yielded a new C(4)-β-hydroxy helenanolide named 11βH-dihydro-4-epineopulchellin (**1a**). In this paper we wish to report the isolation and structure elucidation of this compound, which has not been found in *G. pulchella*, on the basis of spectroscopic data and chemical evidence.

Results and Discussion

The methanol extract of the aerial part of *G. grandiflora* was partitioned between benzene and water. The water layer was extracted with ethyl acetate to afford a brownish gum. Repeated silica gel chromatography and high-performance liquid chromatography (HPLC) gave an oily compound, which was characterized as follows.

11βH-Dihydro-4-epineopulchellin (**1a**), $[\alpha]_D^{27}$ +78.4°, colorless oil, was found to possess the molecular formula $C_{15}H_{24}O_4$, from the high-resolution mass spectrum (HR-MS). The infrared (IR) spectrum revealed hydroxyl (3405 cm^{-1}) and γ-lactone (1757 cm^{-1}) absorptions. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed a tertially methyl signal at δ 0.86 (3H, s) and two secondary methyl signals at δ 1.20 (3H, d, J=7.0 Hz) and 1.34 (3H, d, J=7.3 Hz). In the lower field, three methine protons were observed at δ 4.78 (1H, ddd, J=3.7, 7.6, 11.9 Hz), at δ 4.03 (1H, ddd, J=3.3, 9.2, 9.2 Hz) and at δ 3.95 (1H, dd, J=9.2, 9.2 Hz). Acetylation of **1a** with acetic anhydride–pyridine afforded a diacetate (**1b**), whose molecular formula was found to be $C_{19}H_{28}O_6$ by HR-MS analysis. An assignment of the stereostructure of **1a** was made with the aid of the two dimensional nuclear magnetic resonance (2D-NMR) technique and ^{1}H-NMR decoupling experiments. Figure 1 shows the contour plot of **1a**.

The methine proton signals at δ 3.95 (H_e) and δ 4.03 (H_b) correlated to that at δ 1.14 (H_a) were correlated to the methylene protons at δ 1.96 (H_c) and 2.05 (H_d). Likewise, H_i at δ 4.78 was correlated to H_h at δ 2.26, H_j at δ 1.79 and

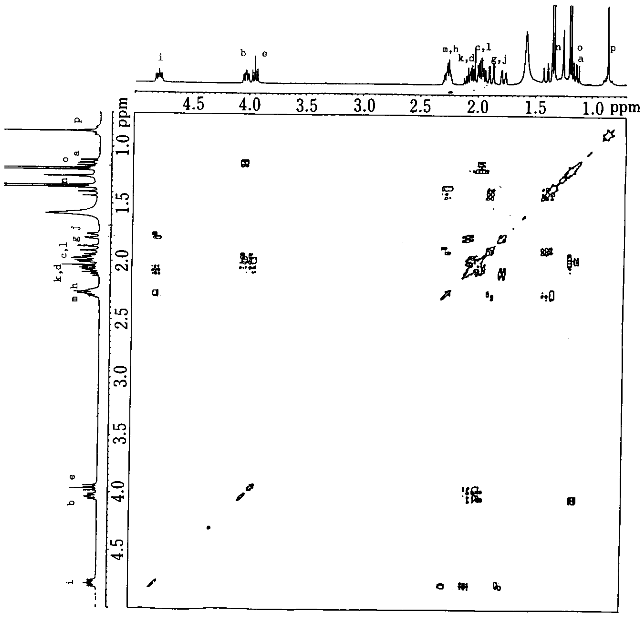
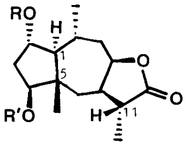
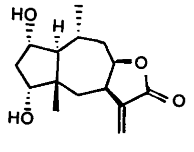


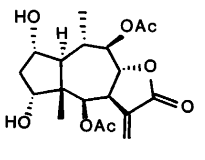
Fig. 1. Contour Plot of ^{1}H–^{1}H Shift-Correlated Spectrum of **1a** in $CDCl_3$



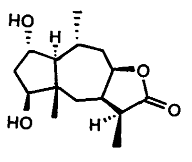
1a : R = R' = H
1b : R = R' = Ac



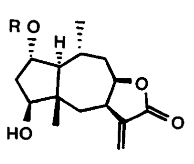
2a



spathulin



1c



2b : R = Ac
2c : R = H

H_k at δ 2.10. H_a at δ 1.14 was further correlated to H_l at δ 1.96 and H_b at δ 4.03. H_l also correlated to H_o at δ 1.20, H_j at δ 1.79, and H_k at δ 2.10. H_h at δ 2.26 was correlated to H_m at δ 2.27 and H_i at δ 4.18 as well as H_f at δ 1.41 and H_g at δ 1.89. Finally, a correlation between H_m at δ 2.27 and H_n at δ 1.34 was observed in this spectrum. On the basis of these data and a consideration of the foregoing observed proton coupling constants, **1a** was presumed to be a helenanolide (a pseudoguaianolide with a C(10)-α-methyl configuration) with C(2)-α- and C(4)-β-hydroxyl groups and a C(7)–C(8) *cis* lactone moiety (Chart 1). Irradiation at the C(11)-methyl doublet signal at δ 1.34 (H_h) changed the multiple methine signal at δ 2.27 (H_m) into a broad doublet ($J=2.9$ Hz). Manipulation of the Dreiding model and the result of this proton decoupling experiment suggested the *cis* relationship between C(7)-H and C(11)-H. In order to confirm this stereostructure and to determine its absolute configuration, we undertook the derivation of **1a** and its C(11)-CH_3 epimer (**1c**) from neopulchellin (**2a**),[3] which was isolated from *G. pulchella*.

2-Acetyl-4-epineopulchellin (**2b**),[10] which was derived from **2a** in three steps as reported previously, was hydrolyzed with 10% KOH in dioxane at room temperature to yield 4-epineopulchellin (**2c**).[10] Compound **2c** was then treated with sodium borohydride ($NaBH_4$) at room temperature to afford an epimeric mixture of 11,13-dihydro-4-epineopulchellin. Silica gel chromatography of the mixture using a chloroform and methanol solvent system, afforded compounds **1a** and **1c** in the ratio of about 1 : 2. The minor component (**1a**) was identical with the aforementioned naturally occurring product (**1a**) in terms of the physicochemical data such as thin layer chromatography (TLC), IR, ^{1}H-NMR, chemical ionization mass spectrum (CI-MS) and $[\alpha]_D$.

The major component (**1c**) obtained as a colorless oil was found to have the molecular formula $C_{15}H_{24}O_4$ by HR-MS. The IR spectrum showed hydroxyl groups (3400 cm^{-1}) and γ-lactone ring (1757 cm^{-1}) absorptions. The ^{1}H-NMR signals were assigned on the basis of decoupling experiments (Table I). The C(11) quartet of doublets due to a methine proton at δ 2.92 in **1c** was changed to a doublet ($J=9.5$ Hz) by irradiation at the C(11)-methyl doublet at δ 1.15. The chemical shift of the C(11)-β-methyl signal in **1c** was at higher field (Δ 0.19 ppm) than that of its C(11)-α-methyl epimer (**1a**), while the C(11)-αH signal in **1c** was at much lower field (Δ0.65 ppm) than that of **1a**. This result could be rationalized by the consideration that catalytic hydrogenation or $NaBH_4$ reduction of helenanolides having a *cis* fused α-methylene-γ-butyrolactone moiety should yield mainly the C(11)-β-CH_3 dihydro derivative, since the thermodynamically more stable 11β-methyl lactone should be produced by proton capture by the lactone enolate intermediate. Thus, the absolute structure of the naturally occurring product **1a** was established to be as shown in Chart 1.

TABLE I. ^{1}H-NMR Chemical Shifts and Coupling Constants of 11βH-Dihydro- (**1a**) and 11αH-Dihydro-4-epineopulchellin (**1c**)

Proton	No.	**1a**[a]	**1c**[a]
1	H_a	1.14 dd $J=7.7$, 11.7 Hz	1.28 dd $J=8.1$, 10.6 Hz
2	H_b	4.03 ddd $J=3.3$, 8.1, 9.2 Hz	4.04 ddd $J=3.3$, 8.1, 8.8 Hz
3α	H_c	1.96 m	1.9—2.1 m
3β	H_d	2.05 m	1.9—2.1 m
4	H_e	3.95 dd $J=9.2$, 9.2 Hz	3.88 dd $J=9.2$, 9.2 Hz
6α	H_f	1.41 dd $J=13.2$, 14.7 Hz	1.08 dd $J=14.7$, 14.7 Hz
6β	H_g	1.89 dd $J=3.3$, 15.0 Hz	1.78 dd $J=2.6$, 14.7 Hz
7	H_h	2.26 m	2.55 m
8	H_i	4.78 ddd $J=3.7$, 7.6, 11.9 Hz	4.73 ddd $J=4.8$, 6.6, 11.0 Hz
9α	H_j	1.79 ddd $J=1.1$, 3.8, 13.6 Hz	1.9—2.1 m
9β	H_k	2.10 m	1.9—2.1 m
10	H_l	1.96 m	1.90 m
11	H_m	2.27 m	2.92 qd $J=7.7$, 9.5 Hz
13	H_n	1.34 d $J=7.3$ Hz	1.15 d $J=7.7$ Hz
14	H_o	1.20 d $J=7.0$ Hz	1.20 d $J=7.3$ Hz
15	H_p	0.86 s	0.89 s

a) 400 MHz ^{1}H-NMR (in $CDCl_3$ solution, TMS as internal standard).

The conformation in solution of the seven-membered ring of **1a** was deduced from the ^{1}H-NMR data as follows. Since its observed coupling constants, that is, $J_{6\alpha,7}$ (3.3 Hz), $J_{6\beta,7}$ (13.2 Hz), $J_{7,8}$ (7.6 Hz), $J_{8,9\alpha}$ (3.8 Hz) and $J_{8,9\beta}$ (11.9 Hz) are comparable with those of neopulchellin [$J_{6\alpha,7}$ (4.1 Hz), $J_{6\beta,7}$ (13.2 Hz), $J_{7,8}$ (8.0 Hz), $J_{8,9\alpha}$ (3.6 Hz), and $J_{8,9\beta}$ (12.3 Hz)], **1a** clearly adopts a boat conformation with appreciable similarity to the boat form of **2a**.[3b] Reports have appeared on the isolation of helenanolides possessing 2α,4β-dihydroxy or diacetoxy groups such as 4-epipulchellin and 4-epineopulchellin from *G. pulchella*,[10] as well as 4-epipulchellin-2-acetate, 4-epipulchellin diacetate and 4-epineopulchellin diacetate from *Geigeria burkey*.[11] It is interesting to note that the existence of the thermodynamically less stable 11α-methyl-7β,8β-*cis*-helenanolide (usually in 11β-methyl configuration), including 2α- and 4β-hydroxyl groups in the molecule, is unprecedented; all the above helenanolides have an α-methylene-γ-butyrolactone moiety.

Compound **1a** and its C(11)-epimer (**1c**) showed no antitumor activity against KB cells, like related 11,13-dihydrohelenanolides such as dihydropulchellin,[2b] and dihydroneopulchellin.[3a] These results support the hypothesis that the exocyclic methylene group of α-methylene-γ-butyrolactones[12a] and the equivalent moiety in sesquiterpenoids[12b] are essential for the expression of cytotoxicity including antitumor activity.[2b,13] The biological data of the above mentioned helenanolides will be reported in detail from the viewpoint of the structure–activity relationship.

Experimental

Optical rotations were measured with a JASCO DIP360 digital polarimeter and IR spectra were recorded on a Hitachi EPI-G3 spectrometer. Electron impact (EI)-, CI- and HR-MS were all taken on a JEOL JMS-D300 spectrometer. The 400 and 270 MHz ^{1}H-NMR spectra were obtained using JEOL GX-400 and GSX-270 spectrometers, respectively. Chromatographic purification was carried out on Kieselgel 60 (70—270 mesh) and TLC was performed on precoated silica gel plates (Merck, Kieselgel 60 F_{254}). Preparative HPLC was performed on an apparatus consisting of an M-6000A pump (Waters Associates Co., Ltd.), a U6K injector (Waters Associates Co., Ltd.) and a Soma S-310A model II UV detector (set at 254 nm) and using a Chemcosorb 5Si column (300 × 3.9 mm i.d.) with the solvent system CH_2Cl_2–MeOH (9 : 1).

Extraction and Fractionation The methanol extract (700 g) of the aerial part of *G. grandiflora* was dissolved in methanol–water (1 : 2) (1.5 l). The aqueous methanol solution was treated with benzene (1.5 l) to remove chlorophyll and wax. The water layer was then extracted with ethyl acetate (1 l × 3), and the organic layer, after drying over Na_2SO_4 and evaporation *in vacuo*, yielded 14.9 g of a brownish gum. Silica gel (100 g) chromatography with a gradient mixture of benzene and ethyl acetate gave fractions 1 (1.95 g), 2 (4.18 g) and 3 (2.11 g). Fraction 2 was chromatographed on silica gel with CH_2Cl_2–MeOH to give four fractions 2—1 through 2—4.

Isolation of 11*β*H-Dihydro-4-epineopulchellin (1a) Fraction 2—3 (178 mg) was submitted to HPLC (column, Chemcosorb 5Si; solvent, CH_2Cl_2–MeOH (9 : 1); flow rate, 1.5 ml/min. Among four fractions, the last fraction (t_R 11.0 min) gave the 11*β*H-dihydro derivative (**1a**) (4 mg) as a colorless oil, $[\alpha]_D^{27}$ +78.4° (c=0.102, $CHCl_3$). EI-MS m/z 268: (M^+), 250, 232, 123 (base peak). HR-MS m/z Calcd for $C_{15}H_{24}O_4$: 268.1674. Found: 268.1694. IR ν_{max}^{KBr} cm^{-1}: 3378, 1752.

Acetylation of 1a **1a** (1 mg) was dissolved in pyridine (1 ml) and Ac_2O (0.1 ml) as usual and the mixture was stirred for 2.5 h at ambient temperature. After addition of a catalytic amount of *N*,*N*-dimethylaminopyridine, the reaction was continued with stirring for 2 h at 80 °C. The reaction mixture was diluted with 0.4 ml of water and then extracted with 0.5 ml of ethyl acetate, and the organic layer was dried over anhydrous sodium sulfate (Na_2SO_4). Evaporation of the solvent afforded 1 mg of the diacetate (**1b**). Colorless oil. EI-MS m/z: 292 (M^+ − AcOH), 250, 232, 44 (base peak). CI-MS (isobutane) m/z 353 (MH^+, base peak). IR ν_{max}^{KBr} cm^{-1}: 1768, 1750, 1730, 1245.

Hydrolysis of 2-Acetyl-4-epineopulchellin (2b) A solution of **2b** (5 mg) in 1 ml of dioxane was allowed to stand with 10% KOH (0.75 ml) at room temperature overnight (17 h). The reaction mixture was then acidified and extracted with ethyl acetate, and the organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* afforded 4 mg of 4-epineopulchellin (**2c**), which was identical with an authentic sample of **2c**[10] in terms of the IR, ^{1}H-NMR, and MS data and optical rotation.

Reduction of 4-Epineopulchellin (2c) **2c** was dissolved in MeOH (1 ml) and an excess amount of $NaBH_4$ was added. The mixture was stirred overnight (15 h). Water was added to the reaction mixture, which was then extracted with ethyl acetate. The extract was dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 4 mg of a mixture of 11,13-dihydro derivatives. Silica gel chromatography eluted with $CHCl_3$–2—5% MeOH gave 11*α*H-dihydro-4-epineopulchellin (**1c**) (2 mg) and 11*β*H-dihydro-4-epineopulchellin (**1a**) (1 mg) both as pale yellow oils.

1c: EI-MS m/z: 268 (M^+), 44 (base peak). CI-MS (isobutane) m/z: 269 (MH^+, base peak). HR-MS m/z Calcd for $C_{15}H_{24}O_4$: 268.1674. Found: 268.1667. IR ν_{max}^{KBr} cm^{-1}: 3400, 1757.

1a: $[\alpha]_D^{26}$ +82.5° (c=0.0606 $CHCl_3$). EI-MS m/z: 268 (M^+), 44 (base peak). HR-MS m/z Calcd for $C_{15}H_{24}O_4$: 268.1674. Found: 268.1631. IR ν_{max}^{KBr} cm^{-1}: 3405, 1757.

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References and Notes

1) Abstracts of Papers The 32nd Syposium on the Chemistry of Perfume, Terpene and Essential Oil, Miyazaki, Oct., 1988 p. 202.
2) *a*) M. Yanagita, S. Inayama, T. Kawamata, T. Ohkura, and W. Herz, *Tetrahedron Lett.*, 2073, 4170 (1969); *b*) M. Yanagita, S. Inayama, and T. Kawamata, *ibid.*, **131** (1970); *c*) T. Sekita, S. Inayama, and Y. Iitaka, *ibid.*, **135** (1970); *d*) T. Kawamata and S. Inayama, *Chem. Pharm. Bull.*, **19**, 643, (1971); *e*) S. Inayama, K. Harimaya, N. Shimizu, H. Hori, T. Ohkura, and T. Kawamata, *Heterocycles*, **23**, 377 (1985).
3) *a*) M. Yanagita, S. Inayama, and T. Kawamata, *Tetrahedron Lett.*, 3007 (1970); *b*) S. Inayama, K. Harimaya, H. Hori, T. Kawamata, T. Ohkura, H. Nakamura, and Y. Iitaka, *Heterocycles*, **19**, 1801 (1982).
4) *a*) S. Inayama, T. Kawamata, T. Ohkura, A. Itai, and Y. Iitaka, *Chem. Pharm. Bull.*, **23**, 2998 (1975); *b*) S. Inayama, T. Kawamata, and T. Ohkura, *Tetrahedron Lett.*, 1557 (1978).
5) T. A. Dulforce, G. A. Sim, D. N. J. White, J. E. Kelsey, and S. M. Kupchan, *Tetrahedron Lett.*, 973 (1969).
6) S. Inayama, T. Kawamata, and M. Yanagita, *Phytochemistry*, **12**, 1741 (1973).
7) W. Herz and A. Srinivasan, *Phytochemistry*, **19**, 2049 (1980).
8) S. Inayama, T. Ohkura, and Y. Iitaka, *Chem. Pharm. Bull.*, **28**, 282 (1980).
9) S. Gill, W. Dembinska-Migas, E. Sliwinska, W. M. Deniewski, and F. Bohlmann, *Phytochemistry*, **19**, 2049 (1980).
10) K. Harimaya, H. Hori, T. Ohkura, T. Kawamata, J-F. Gao, and S. Inayama, *Heterocycles*, **27**, 83 (1988).
11) F. Bohlmann, C. Zdero, and M. Ahmed, *Phytochemistry*, **21**, 1697 (1982).
12) *a*) S. Inayama, T. Kawamata, and K. Sata, *Chem. Pharm. Bull.*, **28**, 277 (1980); *b*) S. Inayama, T. Kawamata, and N. Shimizu, *ibid.*, **28**, 282 (1980).
13) S. M. Kupchan, M. A. Eavin, and A. M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).