

A NEW SESQUITERPENE FROM *Dendranthema grandiflora* FLOWERS

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Chemical investigation of the dichloromethane extracts of the flowers of Dendranthema grandiflora cv. Yoko Ono afforded a new sesquiterpene lactone, grandiflorolide (1), 1,2-dilinoleoyl-3-linolenoylglycerol (2), a mixture of pseudotaraxasterol (3a), taraxasterol (3b), β -amyrin (3c), α -amyrin (3d), and lupeol (3e) in about 2:1:1:0.5:0.5 ratio, and another mixture of β -sitosterol (4a) and stigmasterol (4b) in about 2:1 ratio. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy, while those of 2–4b were identified by comparison of their NMR data with literature data.

Keywords: *Dendranthema grandiflora* cv. Yoko Ono, Astereaceae, grandiflorolide, sesquiterpene, triterpenes, sterols.

Dendranthema grandiflora Tzvelev (syn. *Chrysanthemum morifolium* Ramat.) consists of about 7000 cultivars [1] and is one of the most popular commercial flowers in the world. *Dendranthema grandiflora* cv. Yoko Ono originated from a cross of *D. grandiflora* (K.O.96.925.1) as the female or seed parent with *D. grandiflora* (K.O.96.768.1) as the male or pollen parent. It is a herbaceous pompon-type cut *Chrysanthemum* [2].

D. grandiflora flowers have previously yielded flavonoids [3–12], anthocyanins, and carotenoids [13–17]. A recent study reported that 21 compounds were isolated and identified as octacosyl alcohol, β -sitosterol, lupeol, α -amyrin, daucosterol, ineupatorolide B, syringin, chlorogenic acid, petasiphenol, physcion, acacetin, eupatilin, quercetin, diosmetin, luteolin, apigenin, apigenin-7-*O*- β -D-glucopyranoside, quercetin-3-*O*- β -D-glucopyranoside, luteolin-7-*O*- β -D-glucopyranoside, apigenin-7-*O*- β -D-neosperoside, and acacetin-7-*O*- β -D-glucoside [18].

We earlier reported the isolation of fatty acid esters of maniladiol, heliantriol C, faradiol, and arnidiol from *C. morifolium* [19]. We report herein the isolation of a new sesquiterpene lactone, grandiflorolide (**1**), together with the known compounds 1,2-dilinoleoyl-3-linolenoylglycerol (**2**), a mixture of pseudotaraxasterol (**3a**), taraxasterol (**3b**), β -amyrin (**3c**), α -amyrin (**3d**), and lupeol (**3e**), and a mixture of β -sitosterol (**4a**) and stigmasterol (**4b**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2–4b** were identified by comparison of their NMR data with reported data. To the best of our knowledge, this is the first report on the isolation of **1–3e** from *D. grandiflora*.

Silica gel chromatography of the dichloromethane extract of *D. grandiflora* flowers afforded a new sesquiterpene lactone, grandiflorolide (**1**). The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ¹H NMR spectrum of **1** indicated resonances for exocyclic methylene protons at δ 5.48 and 6.18, olefinic protons at δ 6.22 and 6.32, oxymethine protons at δ 5.10 (lactone) and 3.76 (hydroxyl), an allylic methyl at δ 1.71, a methyl singlet at δ 1.36, an acetoxy methyl at δ 2.15, allylic methines at δ 3.74 and 2.74, and methylene protons at δ 2.10.

The ¹³C NMR spectrum gave resonances for six olefinic carbons at δ 93.6, 99.2, 121.8, 133.5, 137.3, and 137.5; a lactone carbonyl at δ 169.0; an acetate carbonyl at δ 170.5; two oxygenated methine carbons at δ 71.6 and 75.8 and a nonprotonated oxygenated carbon at δ 70.8; two methine carbons at δ 46.4 and 69.1; a methylene carbon at δ 41.5; and three methyl carbons at δ 13.7, 21.3, and 27.5.

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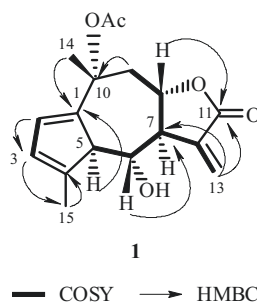


Fig. 1. ^1H - ^1H COSY and ^1H - ^{13}C long-range correlations for **1**.

Correlation spectroscopy (COSY) analysis (Fig. 1) indicated two isolated spin systems as follows: coupled olefinic protons (H-2 and H-3); and a methine proton (H-5) coupled to an oxymethine proton (H-6), which was coupled to another methine proton (H-7), which was in turn coupled to exocyclic methylene protons (H₂-13) and a lactonic proton (H-8), which was finally coupled to methylene protons (H₂-9).

Protons attached to carbons were assigned from heteronuclear single quantum coherence (HSQC) 2D NMR data, and the structure of **1** was elucidated by analysis of heteronuclear multiple bond coherence (HMBC) 2D NMR data: key HMBC correlations are shown in Fig. 1. The hydroxyl was located at C-6 on the basis of long-range correlations between the oxymethine proton (H-6) and the methine carbons (C-5 and C-7). The exocyclic methylene protons were attached to C-13 due to long-range correlations between these protons (H₂-13) and C-7, C-11, and C-12. The allylic methyl was assigned to C-15 since long-range correlations were observed between this methyl (H₃-15) and the olefinic carbons (C-3 and C-4) and the methine carbon (C-5). The methyl singlet was attributed to C-14 based on long-range correlations between this methyl (H₃-14) and C-1, C-9, and C-10. All long-range correlations observed are consistent with the structure of **1**.

The relative configuration of **1** was deduced from nuclear Overhauser effect spectroscopy (NOESY) analysis. The methyl singlet (H₃-14) was close in space to the olefinic proton (H-2), while the lactonic proton (H-8) was close to the oxymethine proton (H-6). On the opposite face of **1**, the methine protons (H-5 and H-7) were close to each other. All NOESY correlations were consistent with the relative configuration of **1**.

The structure of **1** was confirmed by HR-ESI-MS analysis, which revealed a molecular ion of m/z 304.1312 [M]⁺, corresponding to a molecular formula of C₁₇H₂₀O₅. Literature search revealed that **1** is a new compound. The trivial name grandiflorolide is proposed for **1**.

In addition to the above sesquiterpene lactone isolated from *Dendranthema grandiflora* flowers, several other constituents (**2–4d**) were isolated from this plant. The NMR data of **2** are in accordance with data reported in the literature for 1,2-dilinoleoyl-3-linolenoylglycerol [20]; **3a** for pseudotaraxasterol [21, 22]; **3b** for taraxasterol [22–24]; **3c** for β -amyirin [25–27]; **3d** for α -amyirin [26–28]; **3e** for lupeol [28–30]; **4a** for β -sitosterol [31, 32]; and **4b** for stigmasterol [33, 34].

The presence of α -linolenic acid in the triacylglycerol (**2**) was deduced from the methyl triplet at δ 0.96 (t, J = 7.8 Hz), the double allylic methylenes at δ 2.78, and the olefinic protons at δ 5.34 (m) [33]. The presence of linoleic acid was deduced from the methyl triplet at δ 0.86 (t, J = 6.6 Hz), the double allylic methylene at δ 2.80, and the olefinic protons at δ 5.34 (m) [33]. Based on integrations of the triacylglycerol methyls at δ 0.96 (t, J = 7.8 Hz) and 0.86 (t, J = 6.6 Hz), the ratio of linolenic acid and linoleic acid in the triglycerides is about 1:2 [33].

The ratio of about 2:1:1:0.5:0.5 for the mixture of **3a**, **3b**, **3c**, **3d**, and **3e** was deduced from integrations and relative intensities of the ^1H NMR resonances for the olefinic protons of **3a** at δ 5.24 (br.d, J = 7.2 Hz), **3b** at δ 4.60 (d, J = 2.4 Hz) and 4.58 (d, J = 2.4 Hz), **3c** at δ 5.16 (t, J = 3.6 Hz), **3d** at δ 5.10 (t, J = 3.6 Hz), and **3e** at δ 4.55 (d, J = 2.4 Hz) and 4.66 (d, J = 2.4 Hz).

The ratio of about 2:1 for the mixture of **4a** and **4b** was deduced from integrations and relative intensities of the ^1H NMR resonances for the olefinic protons of **4a** at δ 5.33 (dd, J = 1.8, 3 Hz, H-5) and **4b** at δ 5.33 (dd, J = 1.8, 3 Hz, H-5), 5.13 (dd, J = 9, 15 Hz, H-22), and 5.01 (dd, J = 8.4, 15 Hz, H-23) [32].

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl₃ at 600 MHz for ¹H NMR and 150 MHz for ¹³C NMR spectra. HR-ESI-MS was obtained on a Thermo Scientific Q exactive focus orbitrap instrument. Column chromatography was performed with silica gel 60 (70–230 mesh). Thin-layer chromatography was performed with plastic backed plates coated with silica gel F₂₅₄, and plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

Plant Material. The flowers were collected from Baguio City, Philippines in September 2016. The sample was identified as *Dendranthema grandiflora* cultivar Yoko Ono by Virgilio Linis of the Biology Department, De La Salle University-Manila, Philippines.

Extraction and Isolation. The *D. grandiflora* cv. Yoko Ono (147.7 g) was freeze-dried (18.5 g), then ground in a blender, soaked in CH₂Cl₂ for 3 days, and filtered. The filtrate was concentrated under vacuum to afford a crude extract (87.05 mg), which was chromatographed by gradient elution with petroleum ether, 5%, 10%, and 15% EtOAc in petroleum ether, CH₂Cl₂, CH₃CN–Et₂O–CH₂Cl₂ (0.5:0.5:9), CH₃CN–Et₂O–CH₂Cl₂ (1:1:8), and CH₃CN–Et₂O–CH₂Cl₂ (1.5:1.5:7). The 10% EtOAc in the petroleum ether fraction was rechromatographed using 2.5% EtOAc in petroleum ether to afford **2** (5 mg). The 15% EtOAc in the petroleum ether fraction was rechromatographed using 15% EtOAc in petroleum ether to yield a mixture of **3a–3e** (2.8 mg) after washing with petroleum ether. The CH₂Cl₂ fraction was rechromatographed using 20% EtOAc in petroleum ether to provide a mixture of **4a** and **4b** (3.7 mg) after washing with petroleum ether. The CH₃CN–Et₂O–CH₂Cl₂ (1.5:1.5:7) fraction was rechromatographed using CH₃CN–Et₂O–CH₂Cl₂ (0.5:0.5:9) to afford **1** (2.4 mg) after washing with petroleum ether.

Grandiflorolide (1), colorless solid; [α]_D²⁵ + 108.3° (*c* 0.14, CH₃OH); mp 102–104°C. ¹H NMR (600 MHz, CDCl₃, δ , ppm, J/Hz): 1.36 (s, H₃-14), 1.71 (s, H₃-15), 2.102 (d, J = 5.4, H-9b), 2.104 (d, J = 3.0, H-9a), 2.15 (s, OAc), 2.74 (d, J = 10.2, H-5), 3.74 (t, J = 10.2, H-6), 3.76 (tt, J = 3.0, 9.6, H-7), 5.10 (ddd, J = 3.0, 5.4, 9.6, H-8), 5.48 (d, J = 3.0, H-13a), 6.18 (d, J = 3.6, H-13b), 6.22 (d, J = 6, H-2), 6.32 (d, J = 6, H-3). ¹³C NMR (150 MHz, CDCl₃, δ , ppm): 99.2 (C-1), 133.5 (C-2), 137.5 (C-3), 93.6 (C-4), 69.1 (C-5), 75.8 (C-6), 46.4 (C-7), 71.6 (C-8), 41.5 (C-9), 70.8 (C-10), 169.0 (C-11), 137.3 (C-12), 121.8 (C-13), 27.5 (C-14), 13.7 (C-15), 21.3, 170.5 (OAc). HR-ESI-MS *m/z* 304.1312 [M]⁺ (calcd for C₁₇H₂₀O₅, 304.1311).

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