Chemical Components of the Whole Herb of *Mosla dianthera*

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Extracts of the whole herb of *Mosla dianthera* were found to contain three new compounds, 4,5-dimethoxy-2,3-methylenedioxy-1-propenylbenzene, 4,5-dimethoxy-2,3-methylenedioxycinnamaldehyde, and 4,5-dimethoxy-2,3-methylenedioxybenzaldehyde together with eleven known compounds, fatty alcohols, 2,4,5-trimethoxybenzaldehyde, mixture of β-sitosterol and stigmasterol, betulinic acid, oleanolic acid, ursolic acid, arjunolic acid, β-sitosteryl glucopyranoside, palmitic acid, myo-inositol, luteolin and rosmarinic acid.

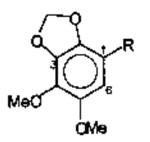
Key words *Mosla dianthera*; Labiatae; 4,5-dimethoxy-2,3-methylenedioxy-1-propenylbenzene; 4,5-dimethoxy-2,3-methylenedioxycinnamaldehyde; 4,5-dimethoxy-2,3-methylenedioxybenzaldehyde

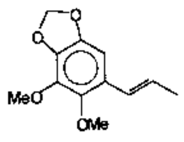
There are five species of *Mosla* genus (Labiatae) indigenous to Taiwan and all of them are herb plants; however, only *M. chinensis* has had its chemical components investigated.[1] Probably because the species of *Mosla* genus are scarce, only one other species *M. soochowensis*[2] has been studied. The chemical components included flavones and their glycosides. One of the flavones, mosloflavone,[1] showed analgesic and antiinflammation effects. This led us to study the chemical constituents of *M. dianthera* Buch.-Ham. Maxim.

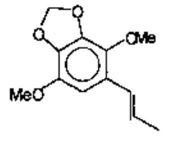
The whole herbs of *M. dianthera* were extracted with methanol, and the extracts were suspended in water and then partitioned with chloroform and *n*-butanol, successively. The chloroform soluble part was subjected to repeated chromatography on silica gel. Fourteen components were isolated, including three new dimethoxymethylenedioxyphenyl derivatives, 4,5-dimethoxy-2,3-methlenedioxy-1-propenylbenzene (**1**), 4,5-dimethoxy-2,3-methylenedioxycinnamaldehyde (**2**), and 4,5-dimethoxy-2,3-methylenedioxybenzaldehyde (**3**), and eleven known compounds, fatty alcohols [mixture of $CH_3(CH_2)_nOH$, $n=29$, 31, 33, 35], 2,4,5-trimethoxybenzaldehyde (asarylaldehyde),[3] steroidal mixtures (β-sitosterol and stigmasterol),[4] betulinic acid,[5] oleanolic acid,[6] ursolic acid,[6] arjunolic acid,[7] β-sitosteryl glucopyranoside,[8] myo-inositol,[9] luteolin,[4] and rosmarinic acid.[10] The known compounds were identified through comparison of their IR, MS, and NMR spectra with anthentic samples or with value taken from the literature. In this paper, we report the structure of three new compounds.

The molecular formula of **1** was determined as $C_{12}H_{14}O_4$ by HR-EI-MS. Analysis of the IR spectrum of **1** suggested it contained an aromatic group (3045, 1601, 1490 cm^{-1}), *trans*-conjugated double bond (1628, 958 cm^{-1}) and a methylenedioxy group (2837, 1230, 1064, 927 cm^{-1}). The ^1H-NMR spectrum exhibited signals for one vinyl methyl group at δ 1.86 (dd, $J=6.6$, 1.7 Hz), two singlet phenolic methyl groups at δ 3.84 and 3.85, a singlet methylenedioxy group at δ 5.93 (2H), two *trans*-olefinic protons at δ 6.09 (dq, 1H, $J=15.7$, 6.6 Hz) and 6.57 (dq, 1H, $J=15.7$, 1.7 Hz), and a singlet phenyl proton at δ 6.54. The UV absorption at λ_{max} 246 and 288 nm suggested compound **1** has an double bond conjugated with aromatic group. Six aromatic carbon signals exhibited at δ 104.4, 124.6, 135.6, 135.7, 138.9, and 139.5. The signal at δ 104.4 (CH) is assigned as a substituted free aromatic carbon and *ortho* to oxygenated carbon. And the signal at δ 124.6 (C) was considered to be linking to the 1-propenyl group and vicinal to oxygenated carbon. The other four phenyl carbons are higher field than δ 140; this result indicates that the four oxygenated phenyl carbons are contiguous. Therefore three possible structures (**1**, **4**, and **5**) for compound **1** were proposed. The correct structure of **1** was confirmed by chemical transformation and spectral evidence. Hydrogenation of compound **1** under catalytic hydrogenation conditions with Pd–C as catalyst in methanol solution gave compound **6** [liquid; δ 0.91 (t, 3H, $J=7.2$ Hz), 1.54 (sex, 2H, $J=7.2$ Hz), 2.48 (t, 2H, $J=7.2$ Hz), 3.82, 3.85 (s, each 3H), 5.90 (s, 2H), and 6.27 (s, 1H)]. The relative position was elucidated by NOE evidence (see structure **6**). Irradiation of the phenyl proton at δ 6.27 afforded an nuclear Overhauser effect (NOE) at the benzyl proton (δ 2.48) (5.7% enhancement) and one of the methoxy groups (δ 3.82) (10.7% enhancement). And irradiation of the signal at δ 2.48 gave only an NOE to the phenyl proton (δ 6.27, 5.2% enhancement). The above evidence determined the structure of **1** as 4,5-dimethoxy-2,3-methylenedioxy-1-propenylbenzene. This is the first time that compound **1** has been isolated from nature though the same compound had been prepared from compound **7** by basic isomerization.[11]

Compound **2** was isolated as a needle crystal (mp 140—



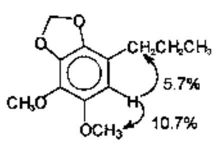




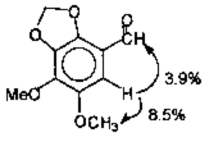
1 R = $\overset{E}{C}H=CHCH_3$
2 R = $\overset{E}{C}H=CHCHO$
3 R = CHO
7 R = $CH_2CH=CH_2$
9 R = $\overset{7}{C}H_2OH$

4

5



6 NOE (→)



8 NOE (→)

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142 °C), and showed the molecular formula $C_{12}H_{12}O_5$, based on HR-EI-MS. The IR spectrum of **2** showed the presence of an aromatic group and a conjugated carbonyl system. The ^{1}H-NMR spectrum of **2** is very similar to that of **1** indicating the presence of a methylenedioxy group [δ 6.02 (s, 2H)], two phenolic methyl groups (δ 3.86, 3.97), and a phenyl proton [δ 6.71 (s, 1H)]. It also contained two *trans*-olefinic protons [δ 6.63 (dd, 1H, J=15.9, 7.7 Hz), 7.69 (d, 1H, J=15.9 Hz)], and one aldehyde proton [δ 9.62 (d, J=7.7 Hz)]. The above spectrum indicated **2** has an aldehyde group conjugated with an olefinic group instead of a methyl group in **1**. The longer wave length absorptions of UV spectrum at $\lambda_{\max}$ 234, 249, and 335 nm further proved the assignment. The phenyl proton (δ 6.71) showed an NOE with a methoxy group (δ 3.86) and an olefinic proton (δ 7.69); this decided the structure of compound **2** as 4,5-dimethoxy-2,3-methylenedioxycinnamaldehyde. The ^{13}C-NMR data also agreed to the assigned structure. Compound **1** was oxidized with selenium dioxide in dioxane under reflux to yield a product, which was identified as compound **2**.

Aldehyde **3** melted at mp 106—108 °C with formula $C_{10}H_{10}O_5$ based on HR-MS. Its IR spectrum indicated aromatic (1590, 1497 cm^{-1}) and conjugated carbonyl groups (1655 cm^{-1}). The ^{1}H-NMR signal at δ 10.21 (s) in addition to the UV absorption bands at $\lambda_{\max}$ 230 and 301 nm indicated an oxygenated benzaldehyde functionality. The ^{1}H-NMR signals presented a singlet phenyl proton at δ 7.06, a methylenedioxyl group at δ 6.07 (s, 2H), and two phenolic methyl groups at 3.86 and 4.03. Four contiguous oxygenated aromatic carbons were discernible from the ^{13}C-NMR signals at δ 138.1, 139.8, 142.2, and 143.1. NOE correlation of aldehyde **3** is shown in structure **8**, which confirmed the structure of **3** as 4,5-dimethoxy-2,3-methylenedioxycinnamaldehyde. Reduction of **3** with sodium borohydride in methanol produced alcohol **9** [amorphous; 3305 cm^{-1}; δ 4.57 (s, 2H, $-C\underline{H}_2OH$) and 6.46 (s, 1H, phenyl H)]. Aldehyde **3** could be prepared from **1** by oxidation with osmium tetraoxide and sodium periodate in dioxane. Aldehyde **3** had been synthesized by Dallacher,[12] but this is the first time thus isolated from a natural source.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. ^{1}H- and ^{13}C-NMR spectra were run on a Brucker AM 300 in $CDCl_3$ solution with tetramethylsilane (TMS) as internal standard. Chemical shifts are given in hertz (Hz). EI-MS and UV spectra were taken on a JEOL-JMS-100 and Hitachi RMS-4 spectrometer , respectively.

Extraction and Isolation Air-dry whole herb of *Mosla dianthera* (4.4 kg) was extracted with MeOH (60 l) at room temperature three times (6 d each time). The crude extracted was evaporated *in vacuo* to leave a black syrup (400 g), and then water was added to a total volume of 1.3 l. The aqueous layer was partitioned with $CHCl_3$ (1 l×3) and *n*-BuOH (1 l×3), successively. The weight of the $CHCl_3$ extract was about 230 g, and a portion of this residue (120 g) was subjected to repeated chromatography on silica gel. The eluent solvent system was a combination of hexane and ethyl acetate. The components were eluted in order as **1** (90 mg), **2** (20 mg), and **3** (10 mg) (5% EtOAc in hexane), fatty alcohol [$CH_3(CH_2)_nOH$, n=29, 31, 33, 35; 30 mg], 2,4,5-trimethoxybenzaldehyde (110 mg), and steroidal mixture (β-sitosterol and stimasterol, 800 mg) (10% EtOAc in hexane), betulinic acid (100 mg), oleanolic acid (150 mg), and ursolic acid (120 mg) (20% EtOAc in hexane), arjunolic acid (80 mg) (80% EtOAc in hexane), β-sitosteryl glucopyranoside (300 mg) (EtOAc), luteolin (20 mg) (from 5% MeOH in EtOAc), rosmarinic acid (10 mg) (10% MeOH in EtOAc), and myo-inositol (150 mg) (30% EtOAc in MeOH).

4,5-Dimethoxy-2,3-methylenedioxy-1-propenylbenzene (1) mp 56—57 °C. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 246 (4.36), 288 (4.00). IR (KBr) cm^{-1}: 3045, 2837, 1628, 1601, 1490, 1230, 1064, 958, and 927. ^{13}C-NMR ($CDCl_3$) δ: 18.7, 56.7, 60.3, 101.6, 104.4, 124.6, 124.9, 125.4, 135.6, 135.7, 138.9, 139.5. EI-MS (70 eV) (rel. int.) m/z: 222 (M$^+$, 100), 207 (11), 177 (23), 149 (27). HR-EI-MS m/z: 222.0892 (M$^+$, Calcd for $C_{12}H_{14}O_4$: 222.0895).

4,5-Dimethoxy-2,3-methylenedioxycinnamaldehyde (2) mp 140—142 °C, UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 234 (4.15), 249 (4.18), 335 (4.27). IR (KBr) cm^{-1}: 3035, 1683, 1615, 1600, 1500, 1352, 1242, 1188, 1116, 1064. ^{13}C-NMR ($CDCl_3$) δ: 56.8, 60.3, 102.4, 106.9, 120.1, 127.8, 138.2, 138.6, 139.6, 140.1, 147.5, 194.1. EI-MS (70 eV) (rel. int.) m/z: 236 (44), 205 (89), 182 (66), 140 (100), 139 (54), 127 (18), 110 (54). HR-EI-MS m/z: 236.2243. (M$^+$, Calcd for $C_{12}H_{12}O_5$: 236.2245).

4,5-Dimethoxy-2,3-methylenedioxybenzaldehyde (3) mp 106—108 °C; UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 230 (4.15), 301 (3.90). IR (KBr) cm^{-1}: 3041, 1655, 1590, 1497, 1237, 1045. ^{13}C-NMR ($CDCl_3$) δ: 55.5, 60.6, 102.7, 106.0, 122.2, 138.1, 139.8, 142.2, 143.1, 187.8. EI-MS (70 eV) (rel. int.): m/z 210 (100), 195 (30), 181 (12), 164 (23); HR-EI-MS m/z: 210.0528 (M$^+$, Calcd for $C_{10}H_{10}O_5$: 210.0531).

Catalytic Hydrogenation of 1 A solution of **1** (20 mg) in 10 ml of MeOH was hydrogenated in the presence of 10% Pd–C (5 mg). After 5 h, the catalyst was removed by filtration and washed several times with MeOH. The combined filtrate and washings gave a product **6** (19 mg): liquid, IR (KBr) cm^{-1}: 3037, 1630, 1610, 1495, 1260, 1150, 1185, 1075, 960.

Selenium Dioxide Oxidized Compound 1 to Produce 2 Selenium dioxide (22.5 mg) was added to a solution of **1** (45 mg) in dioxane (1.2 ml). The reaction mixture was heated under reflux for 4.5 h. The reaction mixture was cooled to ambient temperature, and then 30 ml of H_2O was added. The reaction mixture was extracted with Et_2O (20 ml×3) to yield a solid which was purified to afford compound **2** (20 mg).

Reduction of 3 with $NaBH_4$ in MeOH Excess $NaBH_4$ (10 mg) was added in small portion to a solution of **3** (20 mg) in MeOH (2 ml), and the reaction mixture was stirred for 30 min. The reaction mixture was diluted (15 ml 1 : 1 of EtOAc : hexane) and washed with H_2O (10 ml×3). Evaporation of the organic layer under reduced pressure gave a residue that was chromatographed on SiO_2 to produce **9** [amorphous; IR (KBr) $\nu_{\max}$ cm^{-1}: 3305, 1610, 1500, 1145. ^{1}H-NMR ($CDCl_3$) δ: 3.46 (s, 1H, –OH), 3.84, 3.94 (s, each 3H), 4.57 (s, 2H, H-7), 5.94 (2H, m), 6.46 (s, 1H, H-6)].

Oxidation of 1 with OsO_4 and $NaIO_4$ in Dioxane Compound **1** (20 mg) was dissolved in a mixture of dioxane (1.2 ml) and H_2O (0.4 ml), and then a catalytic amount of OsO_4 (5 mg) was added. After 30 min, the oxidizing agent $NaIO_4$ (60 mg) was added, and then stirred at room temperature for 3 h. The reaction mixture was poured into 50 ml of water, and then the aqueous solution was extracted with ether (30 ml) three times. After purification on silica gel, the product (12 mg) was identified as **3**.

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