

Melitric Acids A and B, New Trimeric Caffeic Acid Derivatives from *Melissa officinalis*

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Two new polyphenolic compounds, melitric acids A (2) and B (5), were isolated from the aboveground part of *Melissa officinalis* (Labiatae) and their structures, consisting of three caffeic acid units, were determined from chemical reactions and spectral data. Rosmarinic acid (1) was also isolated.

Keywords *Melissa officinalis*; caffeic acid trimer; melitric acid A; melitric acid B; rosmarinic acid; Labiatae

Rosmarinic acid (1),¹⁾ consisting of two caffeic acid units, is widely distributed in the family Labiatae, and caffeic acid trimers²⁾ and tetramers³⁾ of related structures have also been found in some labiate plants. *Melissa officinalis* L. is a labiate plant used as a folkmedicine⁴⁾ in Europe, under the name of balm or lemon balm, for the treatment of chronic bronchial catarrh, feverish cold, headaches and tension and it also contains 1.^{1b)} We have isolated two new rosmarinic acid derivatives, each consisting of three caffeic acid units, from the aboveground part of *M. officinalis*, and named them melitric acids A (2) and B (5).

Results and Discussion

The EtOAc extract obtained from the aqueous acetone homogenate of *M. officinalis* was subjected to column chromatography using aqueous EtOH as eluant to give rosmarinic acid (1) and melitric acid A (2), along with compound 4.

Structure of Melitric Acid A Melitric acid A (2) was obtained as a light-brown powder. The fast-atom bombardment mass spectrum (FAB-MS) of 2 showed the $[M + Na]^+$ ion peak at m/z 561, which corresponds to the molecular formula $C_{27}H_{22}O_{12}$. The 1H -NMR spectrum of 2 showed three sets of ABX signals due to aromatic rings, two doublets due to an *E*-olefine structure, a singlet signal

due to olefinic proton and three aliphatic protons due to a $-CH_2-CH(OH)-$ structure, suggesting that this compound consists of 3-(3,4-dihydroxyphenyl)lactic acid [segment A in formula 2; δ 2.98 (1H, dd, $J=8, 14$ Hz, H-7), 3.09 (1H, dd, $J=4, 14$ Hz, H-7), 5.18 (1H, dd, $J=4, 8$ Hz, H-8), 6.62 (1H, dd, $J=2, 8$ Hz, H-6), 6.72 (1H, d, $J=8$ Hz, H-5), 6.83 (1H, d, $J=2$ Hz, H-2)], 4'- (or 3'-) *O*-substituted caffeic acid [segment B; δ 6.36 (1H, d, $J=16$ Hz, H-8'), 6.77 (1H, d, $J=8$ Hz, H-5'), 7.01 (1H, dd, $J=2, 8$ Hz, H-6'), 7.25 (1H, d, $J=2$ Hz, H-2'), 7.56 (1H, d, $J=16$ Hz, H-7')] and 8- (or 7-) substituted caffeic acid segment C; δ 6.80 (1H, d, $J=8$ Hz, H-5''), 7.12 (1H, dd, $J=2, 8$ Hz, H-6''), 7.28 [1H, s, H-7'' (or H-8'')], 7.31 (1H, d, $J=2$ Hz, H-2'')]. The ^{13}C -NMR spectrum of 2 supports the presence of these units (Table I).

Methylation of 2 with $(CH_3)_2SO_4$ and K_2CO_3 afforded a heptamethyl derivative 2a [electron-impact mass spectrum

TABLE I. ^{13}C -NMR Data for Melitric Acids A (2) and B (5)

Carbon		2	5
Segment A	C-1	129.0	129.0
	2	117.2	117.0
	3	145.6 ^{a)}	145.7 ^{b)}
	4	144.7 ^{a)}	144.8 ^{b)}
	5	115.9	116.0
	6	121.4	121.3
	7	37.4	37.4
	8	74.1	74.1
	9	171.7	171.2
Segment B	C-1'	130.2	131.2
	2'	116.4	118.2
	3'	148.0 ^{a)}	145.9 ^{b)}
	4'	147.9 ^{a)}	142.2 ^{b)}
	5'	115.4	116.3
	6'	121.8	125.3
	7'	146.0	144.4
	8'	116.4	118.4
	9'	166.9	166.4
Segment C	C-1''	125.3	125.5
	2''	117.9	117.3
	3''	145.9 ^{a)}	140.7 ^{b)}
	4''	148.1 ^{a)}	148.3 ^{b)}
	5''	116.2	117.4
	6''	124.2	126.5
	7''	128.2	120.5
	8''	139.1	134.7
	9''	165.5	156.6

Chemical shifts are given in δ values [100 MHz, in $(CD_3)_2CO + D_2O$]. a, b) Values with the same superscript may be interchanged.

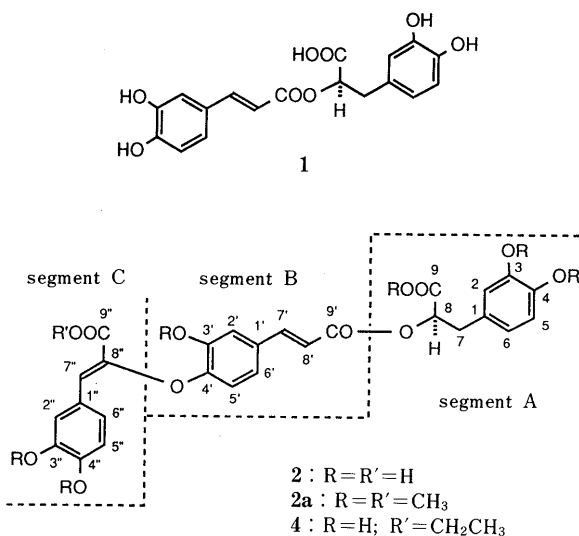
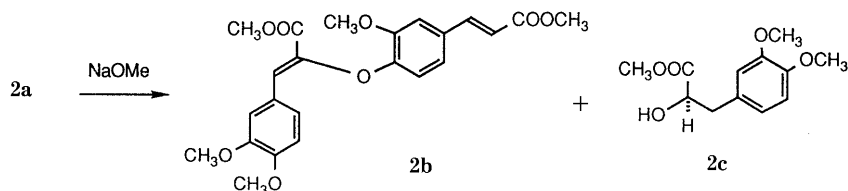
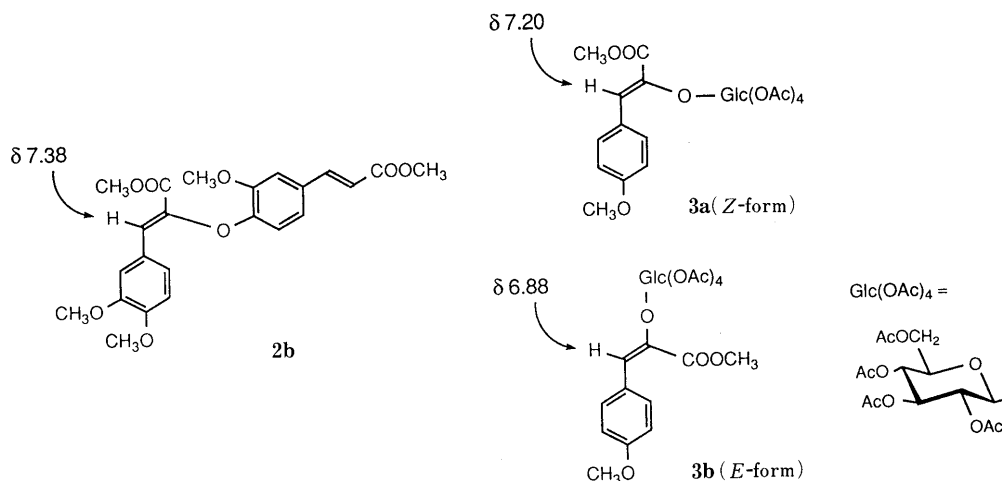


Chart 1. Structures of Rosmarinic Acid (1), Melitric Acid A (2), Dimethyl Penta-O-methylmelitrate (2a) and Ethyl Melitrate A (4)

Chart 2. Methanolysis of Compound **2a**Chart 3. Comparison of the ^1H Chemical Shifts of Olefinic Protons of Compounds **2b**, **3a** and **3b**

(EI-MS) m/z : 636 (M^+)). The ^{13}C -NMR spectrum of **2a** indicated the presence of five methoxyl groups on the aromatic ring [δ 55.5, 55.9 (3C) and 56.3], and two carboxymethyl groups (δ 52.3 and 52.4). Therefore, one of the six phenolic hydroxyl groups of the three caffeic acid segments [including the 3-(3,4-dihydroxyphenyl)lactic acid moiety] in **2** forms an ether linkage. Methanolysis of **2a** gave **2b** and methyl (*R*)-3-(3,4-dihydroxyphenyl)lactate (**2c**).^{3c)}

The ^1H -NMR spectrum of **2b** showed two sets of ABX signals, two doublets due to an *E*-olefine structure and a singlet signal due to olefinic proton, attributable to two caffeic acid segments [δ 7.11 (d, $J=2$ Hz, H-2'), 6.74 (1H, d, $J=8$ Hz, H-5'), 6.96 (1H, dd, $J=2, 8$ Hz, H-6'), 7.58 (1H, d, $J=16$ Hz, H-7'), 6.29 (1H, d, $J=16$ Hz, H-8') (segment B); δ 7.36 (1H, d, $J=2$ Hz, H-2''), 6.79 (1H, d, $J=8$ Hz, H-5''), 7.18 (1H, dd, $J=2, 8$ Hz, H-6''), 7.38 (1H, s, H-7'') (segment C)], along with five methoxyl signals [δ 3.72, 3.75, 3.77, 3.84, 3.95 (3H each, s)]. These data are in accord with the structure **2b** in which the phenolic hydroxyl group at C-3' or C-4' of segment B is bound to one of the olefinic carbons of segment C. The molecular ion peak at m/z 428 in the EI-MS spectrum of **2b**, corresponding to the molecular formula $\text{C}_{23}\text{H}_{24}\text{O}_8$, also fits this structure.

The upfield shift of one (δ 163.9) of the two ester carbonyl carbons in the ^{13}C -NMR spectrum of **2a** suggested the presence of an electron-donating group at C-8''. The ^1H - ^{13}C long-range shift-correlation coherence spectroscopy (COLOC) of **2b** showed a cross-peak due to the three-bond coupling between H-6'' (δ_{H} 7.18) and a proton-bearing olefinic carbon at δ_{C} 128.0 (C-7''). Therefore, the C-8'' of segment C is bound to the phenolic hydroxyl group at C-3' or C-4' of segment B. In addition, nuclear Overhauser enhancement spectroscopy (NOESY) of **2b** showed a

nuclear Overhauser effect (NOE) between H-2' (δ 7.12) and the methoxyl signal at δ 4.00 (MeO at C-3'), indicating that the ether linkage in segment B is at C-4'.

The configuration of the olefinic bond at C-7''-C-8'' in **2** was assigned to *Z* configuration based on comparison of the chemical shift of the olefinic proton H-7'' (δ 7.38) of **2b** with those of the corresponding protons of compounds **3a** and **3b** (*Z*-form **3a**, δ 7.20; *E*-form **3b**, δ 6.88) which have analogous partial structures (see Chart 3).⁵⁾

Structure of Compound 4 Compound **4** was obtained as a light-brown powder. The ^1H -NMR spectrum of **4** is almost the same as that of **2**, except for the presence of signals due to an ethyl group [δ 1.16 (3H, t, $J=7$ Hz), 4.14 (2H, q, $J=7$ Hz)] in the spectrum of **4**. Methylation of **4** and subsequent methanolysis gave **2b** and **2c**. Therefore, one of the two carboxyl groups in **2** forms the ethyl ester in **4**.

The FAB-MS of **4** showed an ion peak at m/z 369 ($[\text{M}-197]^+$), along with the $[\text{M}+\text{Na}]^+$ ion peak at m/z 589. This fragment peak is attributable to the ion produced by the elimination of the dihydroxyphenyllactic acid moiety from the molecular ion (see Chart 4). In addition, the spectrum of **2** showed the corresponding $[\text{M}-197]^+$ ion peak at m/z 341, which is 28 mass units smaller than the fragment peak of **4**. Based on these data, structure **4**, in which the carboxyl group at C-8'' forms the ethyl ester, was assigned as ethyl melitrate A.

Structure of Melitric Acid B Compound **4** may be an artifact produced upon the treatment of the extract with aqueous ethanol during purification. Therefore, we tried to separate the polyphenolic compounds using solvents other than ethanol and isolated a new compound named melitric acid B (**5**).

Melitric Acid B (**5**) was obtained as a light-brown powder. The ^1H -NMR spectrum of **5** indicated that this compound

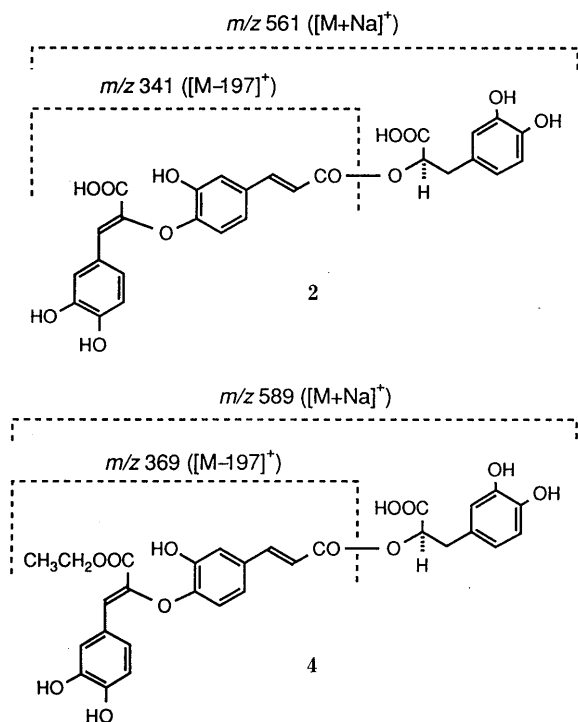


Chart 4. Observed Ions in the FAB-MS Spectra of Compounds 2 and 4

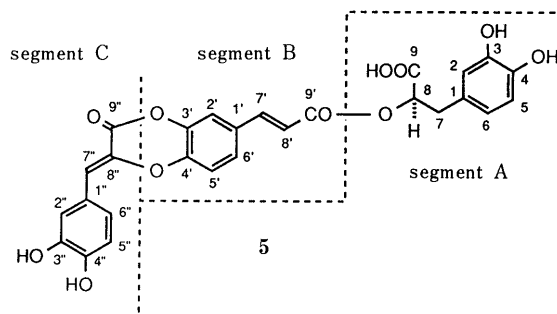


Chart 5. Structure of Melitric Acid B (5)

is composed of 3-(3,4-dihydroxyphenyl)lactic acid [segment A in formula 5; δ 3.00 (1H, dd, $J=8, 14$ Hz, H-7), 3.12 (1H, dd, $J=4, 14$ Hz, H-7), 5.22 (1H, dd, $J=4, 8$ Hz, H-8), 6.65 (1H, dd, $J=2, 8$ Hz, H-6), 6.74 (1H, d, $J=8$ Hz, H-5), 6.84 (1H, d, $J=2$ Hz, H-2)], *O*-substituted caffeic acid [segment B; δ 6.54 (1H, d, $J=16$ Hz, H-8'), 6.90 (1H, d, $J=8$ Hz, H-5'), 7.28 (1H, dd, $J=2, 8$ Hz, H-6'), 7.62 (1H, d, $J=2$ Hz, H-2'), 7.70 (1H, d, $J=16$ Hz, H-7')] and 8-substituted caffeic acid [segment C; δ 6.97 (1H, s, H-7''), 7.36 (1H, d, $J=8$ Hz, H-5''), 7.49–7.50 (2H, m, H-2'', 6'')], suggesting that the structure of 5 is closely related to that of 2. The FAB-MS of 5 shows the $[M+Na]^+$ ion peak at m/z 543, which is 18 mass unit smaller than that of 2. Heating an aqueous solution of 5 afforded 2 and treatment of 5 with aqueous EtOH gave 4. Methylation of 5 with $(CH_3)_2SO_4$ and K_2CO_3 afforded 2a.

These results indicate that melitric acid B is a dehydrated analog of 2, in which the carboxyl group at C-8'' is esterified with the phenolic hydroxyl group at C-3'. The depside linkage formation⁶⁾ of the carboxyl group at C-8'' was also shown by the distinctive upfield shift of the C-9'' in the ^{13}C -NMR spectrum of 5 (see Table I), relative to the

corresponding carbon signal of 2 [δ 165.5 (2) $\rightarrow$ δ 156.6 (5)]. The assignment of C-9'' for the ^{13}C signal at δ 156.6 was substantiated by a cross-peak for the coupling of δ_H 6.97 (H-7'') and δ_C 156.6 in the COLOC spectrum of 5. The participation of the hydroxyl group at C-3' in the depside linkage was supported by downfield shifts of H-2' [δ 7.25 (2) $\rightarrow$ δ 7.62 (5)], H-5' [δ 6.77 (2) $\rightarrow$ δ 6.90 (5)] and H-6' [δ 7.01 (2) $\rightarrow$ δ 7.28 (5)]. Structure 5 is therefore assigned for melitric acid B, based on the assumption that the configuration of the olefinic bond at C-7''–C-8'' is retained during the transformation of 5 into 2.

Experimental

1H - and ^{13}C -NMR spectra were recorded on a JEOL JNM-EX400 instrument (400 MHz for 1H -NMR and 100 MHz for ^{13}C -NMR), and chemical shifts are given in δ values (ppm). High-performance liquid chromatography (HPLC) was performed using a YMC A-312 (ODS) column (6 $\times$ 150 mm; Yamamura Chemical Co.) in an oven at 40 $^\circ$ C, using solvent system A [0.01 M KH_2PO_4 –0.01 M H_3PO_4 –EtOH–EtOAc (50:50:28:7)] or B [0.01 M KH_2PO_4 –0.01 M H_3PO_4 – CH_3CN (9:9:10)]. Flow rate was set at 1.0 ml/min. TLC was conducted on Kieselgel 60 F254 (Merck) plates.

Materials *Malissa officinalis* used in this study was cultivated in the Medicinal Botanic Garden, Higashi Nippon Gakuen University. The aboveground part of the plant was collected in September and air-dried.

Isolation of Polyphenols 1) The dried material (1.1 kg) was pulverized, and the homogenized in 70% acetone. The filtrate of the homogenate was concentrated and extracted successively with Et_2O (300 ml $\times$ 10), EtOAc (300 ml $\times$ 20) and *n*-BuOH (300 ml $\times$ 10). The EtOAc extract (22.5 g) was chromatographed on a column of Sephadex LH-20 (Pharmacia) using 70% EtOH, to give rosmarinic acid (1) (3.15 g) and a mixture of polyphenols. The mixture was further chromatographed on a Sep-Pak C_{18} cartridge (Waters), eluting with increasing concentrations of EtOH in water, to afford 2 (17.5 mg) and 4 (8.9 mg).

2) The dried aboveground part (4.25 kg) was treated in an analogous way and the EtOAc extract (56 g) was chromatographed on a Sephadex LH-20 column with 70% CH_3CN . A mixture containing 5 was further purified by chromatography on a Toyopearl HW-40F (Tosoh) column with 40% CH_3CN to give 5 (17 mg).

Melitric Acid A (2) A light-brown powder, mp 135–138 $^\circ$ C. $[\alpha]_D^{25} +45^\circ$ ($c=0.23$, MeOH). HPLC: retention time (t_R) 19.7 min (solvent A), 5.2 min (solvent B). *Anal.* Calcd for $C_{27}H_{22}O_{12} \cdot 1/2H_2O$: C, 59.24; H, 4.23. Found: C, 59.18; H, 4.30. FAB-MS m/z : 561 ($[M+Na]^+$). UV λ_{max}^{MeOH} nm (log ϵ): 286 (4.42), 321 (4.42). IR ν_{max}^{KBr} cm^{-1} : 1720–1690 (CO), 1610, 1518–1510 (aromatic). 1H -NMR [$(CD_3)_2CO+D_2O$] δ : 2.98 (1H, dd, $J=8, 14$ Hz, H-7), 3.09 (1H, dd, $J=4, 14$ Hz, H-7), 5.18 (1H, dd, $J=4, 8$ Hz, H-8), 6.36 (1H, d, $J=16$ Hz, H-8'), 6.62 (1H, dd, $J=2, 8$ Hz, H-6), 6.72 (1H, d, $J=8$ Hz, H-5), 6.77 (1H, d, $J=8$ Hz, H-5'), 6.80 (1H, d, $J=8$ Hz, H-5''), 6.83 (1H, d, $J=2$ Hz, H-2), 7.01 (1H, dd, $J=2, 8$ Hz, H-6'), 7.12 (1H, dd, $J=2, 8$ Hz, H-6''), 7.25 (1H, d, $J=2$ Hz, H-2'), 7.28 (1H, s, H-7''), 7.31 (1H, d, $J=2$ Hz, H-2''), 7.56 (1H, d, $J=16$ Hz, H-7'). ^{13}C -NMR: See Table I.

Methylation of Melitric Acid A (2) A mixture of 2 (10 mg), $(CH_3)_2SO_4$ (0.1 ml) and anhydrous K_2CO_3 (0.3 g) in dry acetone (3 ml) was stirred for 10 min at room temperature and refluxed for 3 h. The insoluble material was then filtered off, and the filtrate evaporated to dryness. The residue was purified by preparative TLC (benzene–acetone, 19:1), to give dimethyl penta-*O*-methylmelitrate A (2a) (6 mg) as a pale-yellow powder, mp 52–55 $^\circ$ C. $[\alpha]_D^{26} +24.7^\circ$ ($c=1.0$, $CHCl_3$). EI-MS m/z : 636 (M^+). UV λ_{max}^{MeOH} nm (log ϵ): 233 (4.43), 288 (4.40), 328 (4.50). IR ν_{max}^{KBr} cm^{-1} : 1720 (CO), 1598, 1514 (aromatic). 1H -NMR ($CDCl_3$) δ : 3.07 (1H, dd, $J=8, 14$ Hz, H-7), 3.15 (1H, dd, $J=5, 14$ Hz, H-7), 3.72 (6H, s), 3.75, 3.83, 3.84, 3.85, 3.96 (3H, each, s) ($7 \times CH_3O$), 5.35 (1H, dd, $J=5, 8$ Hz, H-8), 6.31 (1H, d, $J=16$ Hz, H-8'), 6.74 (1H, d, $J=8$ Hz, H-5'), 6.77–6.78 (3H, H-2, 5, 6), 6.80 (1H, d, $J=8$ Hz, H-5''), 6.95 (1H, d, $J=2, 8$ Hz, H-6'), 7.10 (1H, d, $J=2$ Hz, H-2'), 7.18 (1H, dd, $J=2, 8$ Hz, H-6''), 7.36 (1H, s, H-7''), 7.36 (1H, d, $J=2$ Hz, H-2''), 7.61 (1H, d, $J=16$ Hz, H-7'). ^{13}C -NMR ($CDCl_3$) δ : 37.2 (C-7), 52.3, 52.4, 55.5, 55.9 (3C), 56.3 ($7 \times CH_3O$), 73.1 (C-8), 111.0, 111.5 (2C), 112.7, 112.8, 114.0 (C-2, 2', 2'', 5, 5', 5''), 115.7 (C-8'), 121.5, 122.4 (C-6, 6'), 124.9, 125.2, 128.1, 128.5, 129.2 (C-1, 1', 1'', 6'', 7''), 138.6 (C-8''), 145.6 (C-7'), 148.0, 148.3, 149.0 (2C), 149.2, 150.8 (C-3, 3', 3'', 4, 4', 4''), 163.9, 166.1, 170.3 (C-9, 9', 9'').

Methanolysis of Dimethyl Penta-*O*-methylmelitate A (2a) Dimethyl penta-*O*-methylmelitate A (2a) (6 mg) was treated with 0.5% NaOMe in MeOH (2 ml) at room temperature for 3.5 h. Acetic acid (0.1 ml) and water (0.1 ml) were added to the solution and then the solvent was evaporated. The residue was extracted with EtOAc and the EtOAc-soluble portion of the residue was subjected to preparative TLC (benzene-acetone, 93:7), to give dimethyl tri-*O*-methylmelidate A (2b) (2 mg) and methyl 3-(3,4-dimethoxyphenyl)lactate (2c) (1 mg).

Dimethyl Tri-*O*-methylmelidate A (2b) A pale-yellow powder, mp 49–52°C. EI-MS m/z : 428 (M^+). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 234 (4.32), 294 (4.41), 325 (4.50). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1724 (CO), 1598, 1514 (aromatic). $^1\text{H-NMR}$ (CDCl_3) δ : 3.72, 3.75, 3.77, 3.84, 3.95 (3H each, s), (5 $\times$ CH_3O), 6.29 (1H, d, $J=16$ Hz, H-8'), 6.74 (1H, d, $J=8$ Hz, H-5'), 6.79 (1H, d, $J=8$ Hz, H-5''), 6.96 (1H, dd, $J=2$, 8 Hz, H-6'), 7.12 (1H, d, $J=2$ Hz, H-2'), 7.18 (1H, dd, $J=2$, 8 Hz, H-6''), 7.36 (1H, d, $J=2$ Hz, H-2''), 7.38 (1H, s, H-7''), 7.58 (1H, d, $J=16$ Hz, H-7'). $^{13}\text{C-NMR}$ (CDCl_3) δ : 51.6, 52.4, 55.5, 55.9, 56.3 (5 $\times$ CH_3O), 111.0, 111.5, 112.7, 114.0 (C-2', 2'', 5', 5''), 116.5 (C-8'), 122.1, 124.9, 125.4 (C-6', 6'', 1''), 128.0 (C-7''), 129.4 (C-1'), 137.7 (C-8''), 144.3 (C-7'), 147.8, 148.9, 149.2, 150.7 (C-3', 3'', 4', 4''), 163.9, 167.4 (C-9', 9'').

Methyl (R)-3-(3,4-Dimethoxyphenyl)lactate (2c)^{3a)} A white powder, $[\alpha]_{\text{D}}^{25} -2^\circ$ ($c=0.25$, MeOH). $^1\text{H-NMR}$ (CDCl_3) δ : 2.90 (1H, dd, $J=7$, 14 Hz, H-7), 3.06 (1H, dd, $J=4$, 14 Hz, H-7), 3.76, 3.84, 3.85 (3H each, s, 3 $\times$ CH_3O), 4.42 (1H, m, H-8), 6.73–6.77 (3H, H-2', 5', 6').

Ethyl Melitrate A (4) A light-brown powder, mp 118–121°C. $[\alpha]_{\text{D}}^{24} +33.7^\circ$ ($c=0.27$, MeOH). HPLC: t_{R} 71.2 min (solvent A), 13.3 min (solvent B). FAB-MS m/z : 589 ($[M+Na]^+$). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 292 (4.40), 330 (4.46). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1702–1695 (CO), 1615–1608, 1515–1508 (aromatic). $^1\text{H-NMR}$ [$(\text{CD}_3)_2\text{CO}+\text{D}_2\text{O}$] δ : 1.16 (3H, t, $J=7$ Hz, CH_3), 2.99 (1H, dd, $J=9$, 14 Hz, H-7), 3.12 (1H, dd, $J=4$, 14 Hz, H-7), 4.14 (2H, q, $J=7$ Hz, CH_2), 5.20 (1H, dd, $J=4$, 9 Hz, H-8), 6.38 (1H, d, $J=16$ Hz, H-8'), 6.64 (1H, dd, $J=2$, 8 Hz, H-6), 6.73 (1H, d, $J=8$ Hz, H-5), 6.76 (1H, d, $J=8$ Hz, H-5'), 6.82 (1H, d, $J=8$ Hz, H-5''), 7.02 (1H, dd, $J=2$, 8 Hz, H-6'), 7.13 (1H, dd, $J=2$, 8 Hz, H-6''), 7.27 (1H, s, H-7''), 7.33 (1H, d, $J=2$ Hz, H-2''), 7.57 (1H, d, $J=16$ Hz, H-7'). Signals at δ 6.83 (H-2) and 7.27 (H-2') are in part overlapped by other signals. $^{13}\text{C-NMR}$ [$(\text{CD}_3)_2\text{CO}+\text{D}_2\text{O}$] δ : 14.3 (CH_3), 37.4 (C-7), 61.7 (CH_2O), 73.9 (C-8), 115.4 (C-5'), 115.8 (C-5), 116.1 (C-5''), 116.3 (C-2'), 116.6 (C-8'), 117.2 (C-2), 117.7 (C-2''), 121.4 (C-6), 121.7 (C-6'), 124.6 (C-6''), 125.2 (C-1''), 128.3 (C-7''), 129.0 (C-1'), 130.4 (C-1), 138.5 (C-8''), 144.7 (C-3), 145.6 (C-3'), 145.9 (2C) (C-4, 7'), 147.6 (C-4'), 148.1 (C-3''), 148.3 (C-4''), 163.8 (C-9''), 166.7 (C-9'), 171.3 (C-9).

Methanolysis after Methylation of 4 Compound 4 (8.5 mg) was treated similarly to 2, and the product (without purification) was reacted with NaOMe in MeOH (2 ml) at room temperature for 3.5 h, to give 2b (1.5 mg) and 2c (1 mg), which were identified by their $^1\text{H-NMR}$ spectra.

Melitric Acid B (5) A light-brown powder, mp 133–135°C. $[\alpha]_{\text{D}}^{24}$

+119.7° ($c=0.33$, MeOH). HPLC: t_{R} 16.5 min (solvent B). *Anal.* Calcd for $\text{C}_{27}\text{H}_{20}\text{O}_{11} \cdot 3/2\text{H}_2\text{O}$: C, 59.24; H, 4.23. Found: C, 59.18; H, 4.30. FAB-MS: m/z 543 ($[M+Na]^+$). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 288 (4.28), 3.38 (4.38). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1714–1690 (CO), 1610, 1512 (aromatic). $^1\text{H-NMR}$ [$(\text{CD}_3)_2\text{CO}+\text{D}_2\text{O}$] δ : 3.00 (1H, dd, $J=8$, 14 Hz, H-7), 3.12 (1H, dd, $J=4$, 14 Hz, H-7), 5.22 (1H, dd, $J=4$, 9 Hz, H-8), 6.54 (1H, d, $J=16$ Hz, H-8'), 6.65 (1H, dd, $J=2$, 8 Hz, H-6), 6.74 (1H, d, $J=8$ Hz, H-5), 6.84 (1H, d, $J=2$ Hz, H-2), 6.90 (1H, d, $J=8$ Hz, H-5'), 6.97 (1H, s, H-7''), 7.28 (1H, dd, $J=2$, 8 Hz, H-6'), 7.36 (1H, d, $J=8$ Hz, H-5''), 7.49 (1H, br s, H-2''), 7.50 (1H, m, H-6''), 7.62 (1H, d, $J=2$ Hz, H-2'), 7.70 (1H, d, $J=16$ Hz, H-7'). $^{13}\text{C-NMR}$: See Table I.

Methylation of Melitric Acid B (5) Melitric acid B (5) (68 mg) was treated with $(\text{CH}_3)_2\text{SO}_4$ (0.3 ml) and K_2CO_3 (0.5 g) in acetone (10 ml), analogously to the methylation of 2, to give 2a (46 mg), which was identified by $^1\text{H-}$ and $^{13}\text{C-NMR}$.

Transformation of Melitric Acid B (5) into Melitric Acid A (2) A solution of melitric acid B (5) (5 mg) in water (5.4 ml) containing CH_3CN (0.4 ml) was heated on a boiling-water bath for 8 h and then the solvent was evaporated to give 2 (5 mg), which was identified by $^1\text{H-NMR}$ and HPLC.

Formation of Ethyl Melitrate A (4) from Melitric Acid B (5) A solution of melitric acid B (5) (5 mg) in 90% EtOH (7 ml) was refluxed for 9 h and then the solvent was evaporated to give 4 (5 mg), which was identified by $^1\text{H-NMR}$ and HPLC.

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