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ADDITIONAL BIOACTIVE HEPTENES FROM *MELODORUM FRUTICOSUM*

J.H. JUNG, C.-J. CHANG, D.L. SMITH, J.L. MCLAUGHLIN,*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

S. PUMMANGURA, C. CHAICHANTIPYUTH, and C. PATARAPANICH

Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10500, Thailand

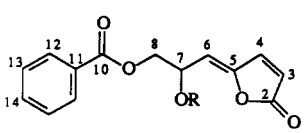
ABSTRACT.—Four additional new bioactive heptenes, melodorinol [**2**], homomelodienone [**4**], 7-hydroxy-6-hydromelodienone [**5**], and homoisomelodienone [**7**] have been isolated from *Melodorum fruticosum*. These compounds were slightly to significantly cytotoxic to human tumor cell lines. Their structures have been determined by comparison of their ^{1}H-nmr, ^{13}C-nmr, and mass spectral data with those of prototype compounds (acetylmelodorinol [**1**], melodienone **3**, and isomelodienone [**6**]).

In our search for natural antitumor compounds, *Melodorum fruticosum* Lour. (Annonaceae) was examined by activity-directed fractionation (1,2). Three novel bioactive compounds have been isolated previously (1). These compounds were cytotoxic to three human tumor cell lines and possessed a novel seven-carbon skeleton, and we named them heptenes. Further phytochemical study on this plant has now yielded four analogous heptene compounds **2**, **4**, **5**, and **7**. In this paper we describe the cytotoxicities to human tumor cell lines and structure elucidation of these four new compounds.

RESULTS AND DISCUSSION

The new heptenes **2**, **4**, **5**, and **7** were weakly to significantly cytotoxic to human tumor cell lines representing lung carcinoma, breast carcinoma, colon adenocarcinoma, and two melanomas (Table 1). Significant cytotoxicity is generally considered when pure compounds show ED_{50} values ≤ 4 μg/ml (3).

The spectra of compound **2** showed very similar patterns to those of acetylmelodorinol [**1**] (1). The major difference in the ir spectrum was the presence of a broad OH absorption. The acetyl protons which were observed in **1** (1) (s, 2.08 ppm) had disappeared from the ^{1}H-nmr spectrum of compound **2**, while an additional OH signal was observed (broad, 2.96 ppm) (Table 2). Moreover, the H-7 signal had shifted significantly upfield (6.12↦5.15 ppm) and the H-8 signal had shifted slightly upfield (4.55, 4.50↦4.45, 4.42 ppm). The methyl carbon (s, 20.85 ppm) and the carbonyl



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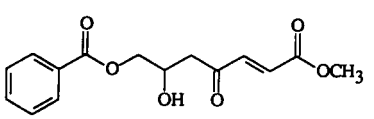
2 R=H



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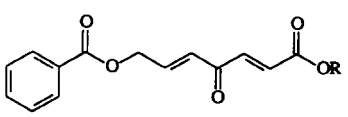
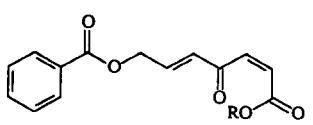


3 R=Me
4 R=Et



6 R=Me
7 R=Et

TABLE 1. Cytotoxicities (ED_{50} values in μg/ml) of Heptenes (compounds **2**, **4**, **5**, and **7**) to Human Tumor Cell Lines.[a,b]

Compound	Cell line				
	A-549	MCF-7	HT-29	SK-MEL-5	Malme-3M
Compound **2**	5.89	1.99	2.87	3.75	3.32
Acetate of compound **2**	2.89	1.96	2.38	4.21	2.74
Compound **5**	3.28	1.92	2.53×10^{-1}	1.04	1.10
Compound **7**	6.70	4.26	36.32	—	—
Compound **4**	36.91	25.61	37.88	—	—

[a]A-549, lung carcinoma (7); MCF-7, breast carcinoma (8); HT-29, colon adenocarcinoma (9); SK-MEL-5, melanoma, metastasis of axillary node (10); Malme-3M, melanoma, metastasis to lung (9).

[b]The cytotoxicity of reference adriamycin was in the range of ED_{50} 10^{-2}–10^{-3} μg/ml in these runs and it was nonselective.

carbon (m, 169.71 ppm) signals had disappeared from the proton-decoupled ^{13}C-nmr spectrum of compound **2** (Table 3). There were downfield shifts for C-7, C-6, and C-8. It was difficult to detect an $[MH]^+$ in the isobutane cims; instead, *m/z* 243 was a prominent peak as in the case of **1** (1). The NH_3 adduct ion of *m/z* 278 $[MNH_4]^+$ was detected by NH_3 cims. According to these spectral data it was evident that **2** is a free hydroxyl (deacetylated) form of **1**. The acetate prepared from **2** was identical (tlc, ^{1}H nmr) to **1**, as expected. Thus, a trivial name of melodorinol was given to compound **2**.

Because the ^{1}H-nmr spectral data of the acetate of melodorinal **2** was the same as that of acetylmelodorinol [**1**], the configuration of the 2-butene-4-olide portion of acetylmelodorinol was presumed to be retained in melodorinol.

The absolute configuration at C-7 of melodorinol [**2**] was studied by Horeau's chemical method (partial resolution or kinetic resolution for secondary alcohols) (4) and ^{1}H-nmr spectral analysis. Melodorinol was reacted with an excess of a racemic mixture of 2-phenylbutanoic anhydride in pyridine. The ^{1}H-nmr spectrum of the ester products showed an equimolar concentration of **2** and the 2-phenylbutanoic moiety. Thus, it was speculated that the lactone ring of **2** was intact. The unreacted anhydrides of the reac-

TABLE 2. ^{1}H-nmr Chemical Shift Values (ppm) and Coupling Constants (Hz, in parentheses) of Compounds **2**, **4**, **5**, and **7**.[a]

Proton	Compound			
	2	**4**	**5**	**7**
H-12	8.02 ddd (8.2, 1.3, 0.5)	8.07 d (7.0)	8.03 dd (8.2, 1.3)	8.05 dd (8.0, 1.3)
H-14	7.55 tt (8.2, 1.3)	7.60 t (7.0)	7.57 tt (8.2, 1.3)	7.58 tt (8.0, 1.3)
H-13	7.42 tt (8.2, 1.3)	7.47 t (7.0)	7.44 t (8.2)	7.45 t (8.0)
H-4	7.37 d (5.5)	6.75 d (15.5)[b]	6.71 d (16.1)[b]	6.16 d (12.1)[b]
H-3	6.22 (dd (5.5)	7.35 d (15.5)[b]	7.07 d (16.1)[b]	6.58 d (12.1)[b]
H-7	5.15 ddd (8.1, 6.1, 4.0)	7.08 dt (16.0, 4.0)	4.52 ddddd (7.8, 5.8, 4.4, 4.2, 4.2)	6.86 dt (16.2, 4.4)
H-6	5.39 d (8.1)	6.61 dt (16.0, 2.0)	2.96 dd (17.7, 7.8), 2.91 dd (17.7, 4.2)	6.49 dt (16.2, 1.9)
H-8α	4.45 dd (11.4, 4.0)	5.06 dd (4.0, 2.0)	4.39 dd (11.5, 4.4)[b]	5.02 dd (4.4, 1.9)
H-8β	4.42 dd (11.4, 6.1)	5.06 dd (4.0, 2.0)	4.36 dd (11.5, 5.8)[b]	5.02 dd (4.4, 1.9)
7-OH	2.96 br	—	3.00 d (4.2)	—
CH_2	—	4.25 q (7.5)	—	4.16 q (7.2)
Me	—	1.31 t (7.5)	3.80 s	1.23 t (7.2)

[a]Spectra were recorded at 500 MHz in $CDCl_3$.

[b]Assignments may be reversed.

TABLE 3. ^{13}C-nmr Chemical Shift Values (ppm) of Compounds **2**, **5**, and **7**.[a]

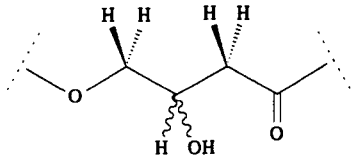
Carbon	Compound		
	2	**5**	**7**
C-2	168.80	166.61[b]	164.78
C-10	166.66	165.69[b]	165.72
C-5	150.01	198.65	193.04
C-4	143.58	139.17	141.99
C-14	133.30	133.37	133.39
C-12	129.71	129.75	129.68
C-11	129.48	129.62	129.31
C-13	128.45	128.52	128.48
C-3	121.00	131.50	126.48
C-6	113.06	44.41	130.01
C-7	67.48	67.50	139.94
C-8	65.88	66.06	62.95
CH_2	—	—	61.27
Me	—	52.48	14.00

[a]The spectrum of compound **2** was recorded at 50.2 MHz, compound **5** and **7** at 125.5 MHz in $CDCl_3$.

[b]Assignments may be reversed.

tion were hydrolyzed by adding H_2O, and the acid antipodes were isolated from the reaction mixture. The optical rotation of the isolated 2-phenylbutanoic acids mixture was levorotatory [(−)*R*; $[\alpha]^{23}D = -0.25°$, in C_6H_6; optical yield 3.4%], indicating that melodorinol had reacted selectively with (+)-*S*-2-phenylbutanoic acid. However, the optical yield of the isolated 2-phenylbutanoic acids mixture was quite low, so other evidence was sought to support these results. Melodorinol was reacted with each pure enantiomeric 2-phenylbutanoic acid, and each reaction mixture was analyzed by ^{1}H-nmr spectroscopy. Both reaction products showed isomeric peaks [H-12 (7.98, 7.83 ppm), H-3 (6.28, 6.25 ppm), H-6 (5.27, 5.16 ppm), H-8 (4.53, 4.46 ppm)] of unequal intensities in the ^{1}H-nmr spectra. Based on the ^{1}H-nmr spectral analyses, it was concluded that compound **2** is a partial racemic mixture with a higher concentration of the *S* isomer. The stereochemistry of acetylmelodorinol [**1**] was also concluded to be *S* since the cd spectral pattern of **1** was identical with that of melodorinol monoacetate.

^{1}H-nmr spectral data (Table 2) of compound **5** were quite similar to those of melodienone [**3**] (1). However, the signals for H-3 (7.37↦7.07 ppm), H-4 (6.75↦6.71 ppm), H-7 (7.07↦4.52 ppm), and H-8 (5.06↦4.39, 4.36 ppm) had shifted upfield. One hydroxyl proton appeared at 3.00 ppm (d, 4.2 Hz) and disappeared after D_2O exchange. Two alkyl protons next to the carbonyl were also observed at 2.96 ppm (dd) and 2.91 ppm (dd). The proton signals at 4.52, 4.39, 4.36, 3.00, 2.96, and 2.91 ppm constituted a typical six-spin system. The signal at 4.52 ppm (H-7) was coupled to five magnetically non-equivalent protons, and after D_2O exchange this signal collapsed to a simpler splitting pattern which showed coupling to four magnetically non-equivalent protons. From the analyses of this spin system, it was possible to deduce the partial structure **8** for compound **5**. Thus, it was presumed that the double bond at C-6, C-7 of melodienone was saturated, and C-7 was hydroxylated to give structure **5**. ^{13}C-nmr data (Table 3) supported this assumption; the signals for C-7 and C-6 had shifted upfield to 67.50 ppm and 44.41 ppm, respectively. The NH_3 cims detected the adduct ion $[MNH_4]^+$ at *m/z* 310 as expected for structure **5**. The fragment of *m/z* 105 was the base peak in the eims, and the molecular ion was very unstable. Com-



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pound **5** was given the trivial name 7-hydroxy-6-hydromelodienone after melodienone [**3**].

From the ^{1}H-nmr spectral analysis (Table 2), it was clear that the structure of compound **7** is very similar to that of isomelodienone **6** (1). The methyl group (3.69 ppm) of **6** was simply replaced with an ethyl group (4.16 ppm, 1.23 ppm) in compound **7**. ^{13}C-nmr spectral data (Table 3) also supported structure **7**. The major difference from the spectrum of isomelodienone [**6**] was the presence of ethyl carbon signals (61.27 ppm, 14.00 ppm). The expected mol wt of compound **7** was confirmed by the presence of an NH_3 adduct ion (*m/z* 306) in the NH_3 cims. In eims, the molecular ion of compound **7** was very unstable. However, the expected fragments were observed at low intensities. The elemental composition of compound **7** was indirectly confirmed by measuring the exact mass of the major fragment at *m/z* 167 $[M-OCOC_6H_5]^+$ in hreims. The measured mass (167.0703) was in accordance with the calculated mass ($C_9H_{11}O_3$, 167.0708). Compound **7** was given the trivial name homoisomelodienone.

From examination of the ^{1}H-nmr spectral data (Table 2), it was clear that compound **4** is chemically analogous to melodienone [**3**] (1). As in the case of homoisomelodienone [**7**], the methyl group (3.80 ppm) of the prototype molecule (melodienone) was replaced by an ethyl group (4.25 ppm, 1.31 ppm). The expected mol wt of **4** was confirmed by the presence of the NH_3 adduct ion (*m/z* 306) in the NH_3 cims. Fragmentation patterns in the NH_3 cims of **4** were similar to those of **7**. Again, the molecular ion of **4** was very unstable in eims, and the fragmentations were similar to those of homoisomelodienone. The elemental composition of **4** was supported by measuring the mass of the major fragment at *m/z* 167 $[M-OCOC_6H_5]^+$ in hreims. The measured mass (167.0704) was in good agreement with the calculated mass ($C_9H_{11}O_3$, 167.0708). The trivial name homomelodienone was given to compound **4**.

Ethyl esters such as **4** and **7** are relatively rare in nature, and one might speculate that these compounds could be extraction artifacts with their ethyl groups arising from the EtOH used in the original extraction. Similarily, the co-occurrence of the methyl esters, **3** and **6**, suggests that the parent heptene acid might be present and that the methyl groups of **3** and **6** might arise from the MeOH used. Because our work focuses only on bioactive compounds, the presence of such acids was not sought in this study. Likewise, logical mechanisms can be rationalized for the alcohol initiated ring openings of **1** leading to **3** and **4**, and by hydrations leading to **2** and **5**. Partial racemization at the hydroxyl positions of **2** and **5** can also be speculated. Thus, the possibilities exist that these isolated compounds may be extraction artifacts of nonbioactive precursors which would not be detected or isolated during activity-directed fractionation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Mettler FP5 and are uncorrected. Optical rotations were measured at room temperature (23°) by a Perkin-Elmer 241 polarimeter using a 10 ml or a 1 ml optical cell, or by a Rudolph Research Autopol III using a 7 ml optical cell. Ir spectra were obtained by Beckman IR-33 double beam instruments either in KBr discs or as liquids. Cd was measured by a Jasco J 600 spectropolarimeter. ^{1}H-nmr spectra were recorded on a Varian VXR-500s instrument. ^{13}C-nmr spectra were recorded on a Chemagnetics A-200 or the Varian VXR-500s operating

at 50.2 MHz or 125.7 MHz, respectively. Chemical shifts were reported relative to the residual solvent peaks ($CDCl_3$: 7.24 ppm, 77.0 ppm). Low resolution eims and cims were obtained on a Finnigan 4000, and high resolution ms were obtained on a Kratos MS 50 through peak matching.

PLANT MATERIAL.—Stem bark of *M. fruticosum* was collected in Thailand where voucher specimens are maintained at the Herbarium of the Faculty of Pharmaceutical Chemistry, Chulalongkorn University.

EXTRACTION AND ISOLATION.—The extraction and fractionation procedures were the same as those described previously (1,2). A portion (4.9 g) of F005 was dissolved in Me_2CO, and the Me_2CO solubles (4.5 g) were chromatographed on Si gel column (60–200 mesh, 250 g) with the gradient of hexane-CH_2Cl_2 (1:1)→CH_2Cl_2-Me_2CO (4:6). A total of 28 fractions were collected. The fractions were analyzed by tlc (hexane-CH_2Cl_2-Me_2CO (10:2:1), Si gel), and one spot with the same color reaction (0.3% potassium dichromate in H_2SO_4) but lower R_f value than acetylmelodorinol [**1**] was observed in fraction 15. This fraction (391 mg) was chromatographed on the Chromatotron (Si gel, 2 mm) with a gradient of CH_2Cl_2→CH_2Cl_2-Me_2CO (20:1) to give 25 fractions. Fractions 3–14 (195 mg) were chromatographed again on the Chromatotron with a gradient of hexane-CH_2Cl_2-Me_2CO (10:2:0.5)→hexane-CH_2Cl_2-Me_2CO (10:2:1.5) to give 24 fractions. A colorless liquid of **2** was obtained from fractions 13–22 (37.5 mg). Fractions 2–5 (10 mg) were combined and rechromatographed on a microcolumn of Si gel with a gradient of hexane-CH_2Cl_2-Me_2CO (10:2:0.5)→hexane-CH_2Cl_2-Me_2CO (10:2:1) to give 13 fractions. Yellow crystals of **5** were obtained from fractions 10–12 (2.5 mg, mp 96–97°). The fractions from various chromatographic analyses that were abundant in acetylmelodorinol [**1**] were combined to make a single pool (2.2 g). Two seemingly novel heptenes were detected in trace quantities in the ^{1}H-nmr spectra of this pool. A portion of this pool (pool 33–35, 1.3 g) was chromatographed on the Chromatotron (4 mm, Si gel rotor) with a gradient of hexane-CH_2Cl_2-Me_2CO (10:2:0.2)→(10:2:0.5) to give 27 fractions. Fractions 16–23 (205 mg) from the Chromatotron separation were further resolved on a 1 mm Si gel rotor with a solvent system of hexane-CH_2Cl_2-Me_2CO (75:15:1) to give 4 fractions. A pure liquid of **7** (1.5 mg) was obtained from fraction 3. Compound **7** appeared just above isomelodienone [**6**] and below melodienone [**3**] on Si gel tlc developed with hexane-CH_2Cl_2-Me_2CO (10:2:1). The rest (890 mg) of pool 33–35 and fraction 4 (200 mg) from chromatographic resolution on the 1-mm Chromatotron rotor were combined and further resolved by the Chromatotron (4 mm, Si gel rotor) with a gradient of hexane-CH_2Cl_2-Me_2CO (150:30:1→20:6:1) to give 20 fractions. Fractions 3–9 (250 mg) were resolved again by Chromatotron on 1-mm Si gel rotor with a gradient of hexane-C_6H_6-CH_2Cl_2-Me_2CO (20:5:3:0→100:25:20:1) to give 25 fractions. Compound **4** was obtained from fraction 21 as a liquid (1.5 mg).

BIOLOGICAL EVALUATIONS.—The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae. The brine shrimp lethality bioassay (5) was performed in our laboratory. In vitro antitumor activities on human tumor cell lines were determined in the Purdue Cell Culture Laboratory, Purdue Cancer Center, following the protocols established by the National Cancer Institute (2, 6–10).

MELODORINOL [**2**].—Colorless liquid: $[\alpha]_D$ −37° (c = 10 mg/ml, $CHCl_3$); ir ν max (neat) cm^{-1} 3500 (OH stretch), 3100 (aromatic CH), 2950 (aliphatic CH), 1790 (lactone C=O), 1730 (benzoyl C=O), 1280, 1120; hrcims (NH_3) *m/z* 243.0658 (calcd for $C_{14}H_{11}O_5$, 243.0657); cims (isobutane) *m/z* (%) $[MH]^+$ 261 (2), $[MH-H_2O]^+$ 243 (100), 195 (4); cims (NH_3) *m/z* (%) $[MNH_4]^+$ 278 (43), $[MNH_4-H_2O]^+$•260 (0.4), 243 (100); ^{1}H nmr see Table 2; ^{13}C nmr see Table 3.

ACETYLATION OF MELODORINOL [**2**].—Melodorinol [**2**] (12.5 mg) was mixed with 3 drops of pyridine and 20 drops of Ac_2O and stirred at room temperature for 6 h. The reaction mixture was purified through a microcolumn to yield 9 mg of the acetate. ^{1}H-nmr data were the same as for acetylmelodorinol [**1**]: cd (c = 0.05 mg/ml, EtOH) $[\theta]^{23}$ (nm) 104 (489.4), 4046 (270.6), 3539 (265.8), 2136 (255.2), 8999 (229.4), 10241 (223.8), 6879 (209), 7896 (201.8).

PREPARATION OF RACEMIC 2-PHENYLBUTANOIC ANHYDRIDE.—Racemic 2-phenylbutyric acid (10 g) was mixed with 10 g of Ac_2O and refluxed (ca. 144°) for 1 h. The HOAc produced in the reaction was distilled away under vacuum. Another 10 g of Ac_2O was added to the reaction mixture and refluxed (ca. 155°) for 1 h. Again the HOAc was distilled away under vacuum. A third portion of the Ac_2O was added to the reaction mixture and refluxed (ca. 165°) for 1 h, and the HOAc and Ac_2O were distilled away under vacuum. A yellow liquid was obtained. Examination of the ^{1}H-nmr spectrum showed that the product consisted of essentially equal amounts of meso and threo diastereomeric 2-phenylbutanoic anhydrides.

CONFIGURATION OF MELODORINOL [**2**].—Melodorinol [**2**] (11 mg) was added to a solution of 2-phenylbutanoic anhydride (83 mg) in anhydrous pyridine (0.5 ml), and the resulting mixture was allowed to stand at room temperature for 6 h. H_2O was then added to effect hydrolysis, and the mixture was left to

stand for 30 min. The hydrolysis mixture was titrated with 0.1 N NaOH solution in the presence of C_6H_6 (10 ml) and a drop of phenolphthalein solution as an indicator. The mixture was transferred to a separatory funnel and separated, and the pink aqueous basic phase was washed with $CHCl_3$ to remove traces of esters. The aqueous phase was acidified with 1 N HCl, and the free 2-phenylbutanoic acids thus formed were extracted with C_6H_6. The C_6H_6 extract was dried and weighed before measuring the optical rotation.

The mixture of the diastereomeric 2-phenylbutanoic esters of melodorinol [**2**] was separated, and its ^{1}H-nmr spectrum was recorded. Proton signals of isomeric pairs (*R-R, S-S,* and *R-S, S-R*) were observed: ^{1}H-nmr (500 MHz, $CDCl_3$) δ 7.98 and 7.83 (H-12), 6.28 and 6.25 (H-3), 6.12 (H-7), 5.27 and 5.16 (H-6), 4.53 and 4.46 (H-8), 3.49 (CH of 2-phenylbutanoyl moiety), 2.10 (CH_2 of 2-phenylbutanoyl moiety), 1.80 (CH_2 of 2-phenylbutanoyl moiety), 0.86 (Me of 2-phenylbutanoyl moiety).

7-HYDROXY-6-HYDROMELODIENONE [**5**].—Yellow crystals: mp 96–97°; hr cims (NH_3) *m/z* 294.1097 (calcd for $C_{15}H_{16}O_6$, 294.1103); eims *m/z* (%) $[M-Me]^+$ 277 (>1), $[M-COOMe]^+$ 233 (>1), $[M-77]^+$ 215 (0.3), 170 (11), $[M-179]^+$ 113 (19), $[M-OCOC_6H_5]^+$ 105 (100), $[C_5H_6]^+$ 77 (41); cims (NH_3) *m/z* (%) $[MNH_4]^+$ 310 (100), 294 (28), $[MNH_4-H_2O]^+$ 292 (9), 280 (6), 190 (6), 182 (14), 180 (13), 174 (31), 165 (25), 157 (15), 148 (18), 74 (20); ^{1}H nmr see Table 2; ^{13}C nmr see Table 3.

HOMOISOMELODIENONE [**7**].—Liquid: hreims 167.0703 (calcd for major fragment $C_9H_{11}O_3$, 167.0708); cims (NH_3) *m/z* (%) $[MNH_4]^+$ 306 (16.7), $[MH]^+$ 289 (15.7); eims *m/z* (%) $[M]^+$ 288 (0.1), $[M-C_2H_5]^+$ 259 (0.4), $[M-OC_2H_5]^+$ 243 (0.4), $[M-COOC_2H_5]^+$ 215 (0.8), $[M-OCOC_6H_5]^+$ 167 (6.0), 127 (2.5), $[C_6H_5COO]^+$ 121 (4.5), $[C_6H_5CO]^+$ 105 (100); ^{1}H nmr see Table 2; ^{13}C nmr see Table 3.

HOMOMELODIENONE [**4**].—Liquid: hreims 167.0704 (calcd for major fragment $C_9H_{11}O_3$, 167.0708); cims (NH_3) *m/z* (%) $[MNH_4]^+$ 306 (32.4), $[MH]^+$ 289 (26.4); eims *m/z* (%) $[M-COOC_2H_5]^+$ 215 (1.0), $[M-OCOC_6H_5]^+$ 167 (5.8), 149 (8.0), $[C_6H_5COO]^+$ 121 (5.5), $[C_6H_5CO]^+$ 105 (100); ^{1}H nmr see Table 2.

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