*Tetrahedron* Vol. 46, No. 15, pp. 5043-5054, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00


# NEW BIOACTIVE HEPTENES FROM *MELODORUM FRUTICOSUM* (ANNONACEAE)

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(*Received in USA* 18 *September* 1989)

***ABSTRACT***. By guiding fractionation with brine shrimp lethality, three novel compounds, with cytotoxic activities against human tumor cell lines, have been isolated from the bark of *Melodorum fruticosum* Lour.(Annonaceae). These compounds have benzoyl moieties in common with a $C_7$ dienone or lactone terminal which appears to arise from a heptose or the equivalents. They were named melodienone, isomelodienone, and acetylmelodorinol.

## INTRODUCTION

*Melodorum* is a rare genus and is known to contain only three species[1]. Three aporphines (asimilobine, liriodenine, and norushinsunine) have been found previously in *Melodorum punctulatum* Baill.[2] In our search for natural antitumor compounds, the bark of *Melodorum fruticosum* Lour.(Annonaceae) was examined by bioassay-directed procedures. Previously we reported several diverse, bioactive, known natural products.[3] Concurrently, three heptene derivatives, a new type of bioactive compound, were isolated, and, herein we describe the isolation, bioassay results, structure elucidation, and discussion of biogenesis of these novel compounds.

## RESULTS AND DISCUSSION

Screening of the 95% ethanol extract (F001) of the bark by the brine shrimp larvae lethality bioassay[4] detected noteworthy activity ($LC_{50}$ 203 ppm), and antitumor activity was indicated by the inhibition of crown gall tumors (50 % inhibition) on potato discs[5]. Further partition of the EtOH extract (F001) between water and $CH_2Cl_2$ yielded the water solubles (F002), $CH_2Cl_2$ solubles (F003), and the interface (F004). The residue of the $CH_2Cl_2$ solubles (F003) was subsequently partitioned between hexane (F006) and MeOH/$H_2O$ (9:1) (F005). All the fractions were tested for brine shrimp lethality, and the results indicated that the activities concentrated in the aqueous MeOH fraction (F005, $LC_{50}$ 67 ppm). The potato disc assay also indicated that the antitumor activity resided in F005 (80% crown gall tumor inhibition). Thus, F005 was subjected to chromatography on gravity column, medium pressure column, and centrifugal radial thin-layer (chromatotron) to yield the major active components. The activities of all the fractions and compounds were monitored by the brine shrimp assay at each step. Finally three compounds (A-C) toxic to brine shrimp were isolated along with other known bioactive

compounds[3]. These three new compounds were tested for cytotoxicities in human tumor cells, and showed moderate activities in the three cell lines with selective activity against breast cancer and colon cancer (Table 1).

Table 1. Bioassay results of three compounds.

| | Brine shrimp $LC_{50}$ in ppm *a* | cytotoxicities[b] | | |
|---|---|---|---|---|
| | | A-549 | MCF-7 | HT-29 |
| acetylmelodorinol (comp A) | 246 (163/383) | 2.74 | $4.08 \times 10^{-1}$ | $3.04 \times 10^{-1}$ |
| isomelodienone (comp B) | 10 (3/19) | 1.69 | $1.71 \times 10^{-1}$ | $5.14 \times 10^{-1}$ |
| melodienone (comp C) | 24 (15/37) | 8.92 | 4.43 | 3.85 |

*a*: 95 % confidence interval in parentheses.
*b*: Expressed as $ED_{50}$ in μg/ml.
A-549: Human lung carcinoma.
MCF-7: Human breast carcinoma.
HT-29: Human colon adenocarcinoma.

Compound A crystallized from the initial column fractions and was recrystallized from hexane/acetone as colorless crystals (m.p. 75-76°). Compounds B and C were obtained as light yellow liquids after several chromatographic purifications. Compound C was recrystallized from hexane/acetone to give yellow crystals (m.p. 69-70°). These three compounds showed very similar spectral characteristics in $^1$H-nmr and $^{13}$C-nmr (Tables 2 and 3). The most significant features of the spectral data of the three compounds were the presence of

| | acetylmelodorinol(comp A) | isomelodienone(comp B) | melodienone(comp C) |
|---|---|---|---|
| H-12 | 8.01 ddd (8.2, 1.3, 0.9) | 8.05 dd (8.2, 1.3) | 8.07 dd (8.4, 1.3) |
| H-14 | 7.56 tt (8.2, 1.3) | 7.58 tt (8.2, 1.3) | 7.58 tt (8.4, 1.3) |
| H-13 | 7.43 tt (8.2, 1.3) | 7.45 tt (8.2, 1.3) | 7.46 tt (8.4, 1.3) |
| H-4 | 7.36 d (5.5) | * 6.17 d (12) | * 6.75 d (15.8) |
| H-3 | 6.26 dd (5.5, 0.5) | * 6.60 d (12) | * 7.37 d (15.8) |
| H-7 | 6.12 ddd (8.1, 6.1, 4.1) | 6.85 dt (16, 4.5) | 7.07 dt (15.9, 4.4) |
| H-6 | 5.31 dd (8.1, 0.5) | 6.49 dt (16, 1.9) | 6.60 dt (15.9, 1.8) |
| H-8α | 4.55 dd (11.7, 4.1) | 5.00 dd (4.5, 1.9) | 5.06 dd (4.4, 1.8) |
| H-8β | 4.50 dd (11.7, 6.1) | 5.00 dd (4.5, 1.9) | 5.06 dd (4.4, 1.8) |
| $CH_3$ | 2.08 s | 3.69 s | 3.80 s |

Table 2. $^1$H-Nmr chemical shift values and coupling constants (in parentheses). Spectra of compounds A and C were recorded at 500 MHz, and compound B was recorded at 200 MHz in $CDCl_3$.
*, * Assignments may be reversed.

a benzoyl moiety, one isolated olefinic spin system, one or two ester linkages, and ABMX or $A^2MX$ proton spin systems.

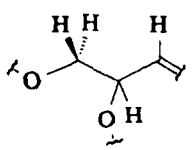
The $MH^+$ of compound A was very weak in the isobutane CIMS. The expected molecular weight was confirmed by ammonia CIMS which gave an ammonium adduct ion ($MNH_4^+$) at m/z 320 as a base peak, indicating the m/z 302 to be $M^+$. $^{13}$C-Nmr and $^1$H-nmr showed 16 carbon signals at 14 different frequencies, and 14 protons, suggesting the molecular formula of $C_{16}H_{14}O_6$. Elemental analysis data matched the proposed formula (calcd. for $C_{16}H_{14}O_6$: C 63.58, H 4.67, O 31.78 ; found: C 63.66, H 4.64, O 31.70). IR spectra showed three carbonyl absorptions at 1790, 1750, and 1735 $cm^{-1}$. All these carbonyl carbons showed signals in the ester

| | acetylmelodorinol(comp A) *a* | | isomelodienone(comp B) | melodienone(comp C) |
|---|---|---|---|---|
| C-16 | 169.71 m | 170.05 | — | — |
| C-2 | 168.43 dd (11, 7.3) | 169.27 | 165.17 dt (12, 2.5) | 165.74 |
| C-10 | 165.94 t (3) | 166.26 | 165.60 t (2.5) | 165.81 |
| C-5 | 150.64 m | 151.95 | 192.72 t (7.5) | 187.74 |
| C-4 | 143.30 t (2) | 145.20 | 141.99 td (5.2, 1.4) | 142.35 td (5.0, 1.5) |
| C-14 | 133.23 t (7.3) | 134.03 | 133.26 t (7.5) | 133.43 t (7.2) |
| C-12 | 129.64 dd (5.4, 5) | 130.57 | 129.56 td (7.6, 1.7) | 129.69 td (6.4, 1.6) |
| C-11 | 129.48 t (7.3) | 130.19 | 129.28 t (7.5) | 129.33 t |
| C-13 | 128.43 d (6.8) | 129.33 | 128.37 d (7) | 128.58 d (7.5) |
| C-3 | 121.41 d (2) | 121.84 | 126.04 d (1.7) | 128.53 |
| C-6 | 108.79 t (2) | 109.24 | 129.77 td (5.2, 2) | 131.40 d (3.1) |
| C-7 | 67.20 s | 67.62 | 140.00 dd (1.4) | 137.79 t (1.4) |
| C-8 | 64.56 t (2) | 65.31 | 62.79 t (6.2) | 63.53 t (6.5) |
| $CH_3$ | 20.85 s | 20.75 | 51.89 s | 52.33 s |

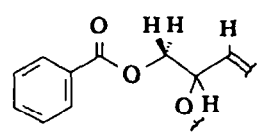
Table 3. $^{13}$C-Nmr chemical shift values, $^{13}$C-$^{1}$H long range coupling pattern, and constants (in parentheses). Spectra of compound A were recorded at 50.2 MHz, compound B at 125.5 MHz in acetone-$d_6$, and compound C at 125.5 MHz in $CDCl_3$.

*a*: acetylmelodorinol in acetone-$d_6$

carbonyl regions in the $^{13}$C-nmr spectra (169.71, 168.43, and 165.94 ppm), so the presence of one unsaturated lactone ring (1790 $cm^{-1}$) and two ester linkages (1750 and 1735 $cm^{-1}$) was assumed. The presence of a benzoyl moiety was assured by comparison of $^{13}$C-nmr and $^{1}$H-nmr spectral data with those of benzylbenzoate. This was further confirmed by hydrolysis of compound A, which gave benzoic acid as a hydrolysis product. Benzoic acid was obtained as colorless crystals, but other hydrolysis fragments could not be identified. An ABMX proton spin system, comprised of signals at 6.12, 5.31, 4.55, and 4.50 ppm, was recognized in the $^{1}$H-nmr spectra (these protons were attached to carbons with signals at 67.20, 108.79, and 64.56 ppm, respectively, in $^{13}$C-nmr spectra), which were attributed to fragment (1). Protons with signals at 4.55 and 4.50 ppm were attached to the same



(1)



(2)

carbon (64.56 ppm) and were magnetically non-equivalent indicating the presence of bulky neighboring functional groups, thus inhibiting free rotation along the single bond. Also, in the COLOC experiment (Figure 1)[6], these geminal protons showed long range coupling to the carbonyl carbon of the benzoyl moiety (this carbon, at 165.94 ppm in the proton coupled $^{13}$C-nmr spectra, was resolved to a triplet due to major three bond coupling with H12). Thus, the fragment (1) was extended to fragment (2). Two olefinic protons (7.36 and 6.26 ppm) of an isolated olefinic spin system, were coupled to each other by unusually small coupling (5.5 Hz), indicating that the double bond is part of a strained ring system. IR absorption at 1790 $cm^{-1}$ also indicated an unsaturated lactone ring. Since three ester carbonyl carbon signals were recognized in the $^{13}$C-nmr spectra, all six oxygens in the molecule were believed to participate in three ester linkages, and one of the three ester linkages was then suspected to constitute a lactone ring. A quaternary carbon at 150.64 ppm might be a typical

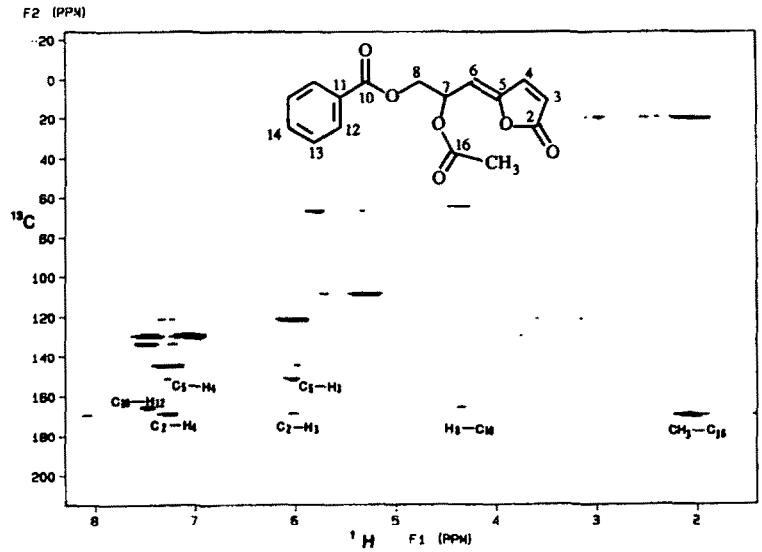
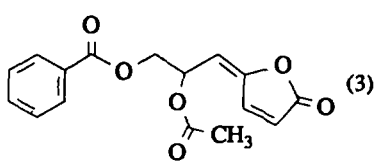
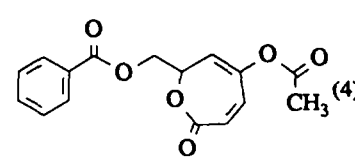


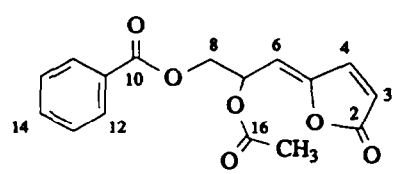

FIGURE 1. COLOC spectrum showing $^{13}C$-$^{1}H$ long range coupling of acetylmelodorinol(comp A). Crucial couplings are marked. The spectrum was recorded at 200 MHz for proton and 50 MHz for carbon.

$sp^2$ carbon attached to oxygen. Thus, fragment(2) would possibly be extended to structure (3) or (4). The nmr


(3)


(4)

spectral data of (3) and (4) might be almost the same, and we could not confirm the long range coupling between the methyl group and any other carbon. Finally, X-ray diffraction analysis gave structure (5) for compound A


(5)

(Figure 2). Atomic coordinates and their estimated standard deviations, bond lengths, and bond angles are given in Tables 4 and 5.

This represents a new class of heptene natural product, and it was given a trivial name acetylmelodorinol. The assignments of $^{13}C$-nmr and $^{1}H$-nmr spectra were straightforward except for H3, H4, C3, C4, C2, and C16. Carbons C3, C4, C2, and C16 were assigned on the basis of their $^{13}C$-$^{1}H$ long range coupling patterns (Table 3). The carbonyl carbon signal at 169.71 ppm was a multiplet and the one at 168.43 ppm was a doublet of doublets

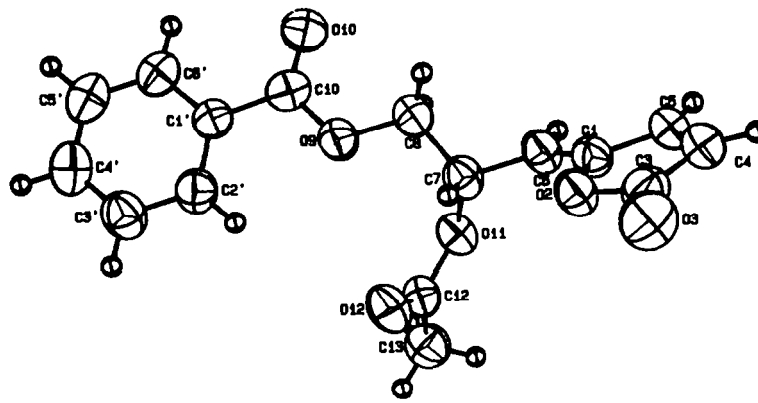



FIGURE 2. ORTEP drawing of acetylmelodorinol(comp A).

Table 4. Atomic coordinates ($x10^4$) and ESD for acetylmelodorinol(comp A).

| Atom | x | y | z | B(Å$^2$) |
|---|---|---|---|---|
| O-2 | 1726 (4) | 9370 | 7650 (3) | 4.64 (6) |
| O-3 | 20 (5) | 11006 (3) | 6839 (4) | 6.69 (9) |
| O-9 | 4521 (4) | 6489 (3) | 11714 (3) | 4.80 (6) |
| O-10 | 3130 (4) | 4789 (3) | 11811 (3) | 6.19 (8) |
| O-11 | 6295 (3) | 7467 (2) | 9700 (3) | 4.47 (6) |
| O-12 | 6957 (4) | 8766 (3) | 11583 (3) | 6.00 (7) |
| C-1 | 1997 (5) | 8375 (3) | 6947 (4) | 4.07 (8) |
| C-3 | 453 (5) | 10081 (4) | 6517 (4) | 5.1 (1) |
| C-4 | -100 (6) | 9453 (4) | 5070 (5) | 5.3 (1) |
| C-5 | 821 (6) | 8458 (4) | 5333 (4) | 4.8 (1) |
| C-6 | 3135 (6) | 7527 (4) | 7719 (4) | 4.49 (9) |
| C-7 | 4191 (5) | 7497 (4) | 9424 (4) | 4.36 (9) |
| C-8 | 3661 (6) | 6428 (4) | 10067 (4) | 4.9 (1) |
| C-1' | 5202 (5) | 5645 (4) | 14140 (4) | 4.04 (8) |
| C-10 | 4171 (5) | 5576 (4) | 12465 (4) | 4.57 (9) |
| C-12 | 7495 (6) | 8173 (4) | 10772 (4) | 4.69 (9) |
| C-13 | 9539 (6) | 8088 (5) | 10784 (5) | 5.9 (1) |
| C-2' | 6386 (6) | 6573 (4) | 14835 (4) | 4.77 (9) |
| C-3' | 7328 (6) | 6589 (4) | 16420 (5) | 5.7 (1) |
| C-4' | 7088 (6) | 5686 (4) | 17287 (4) | 5.6 (1) |
| C-5' | 5922 (6) | 4765 (4) | 16602 (4) | 5.6 (1) |
| C-6' | 4973 (6) | 4748 (4) | 15026 (5) | 4.92 (9) |

with relatively large $^2J_{CH}$ and $^3J_{CH}$ values (7.3 Hz and 11 Hz, respectively). So, the doublet of doublets was assigned to C2, since C2 might show strong coupling to a transoid H4 and weaker coupling to H3, and the multiplet was assigned to C16. One $sp^2$ carbon at 143.30 ppm was a triplet, and the other at 121.41 ppm was a doublet. The doublet was assigned to C3, and the triplet was assigned to C4 assuming the coupling with H3 and H6. Proton signals of H3 and H4 (7.36 ppm and 6.26 ppm) were assigned by a COLOC experiment. The signal at 7.36 ppm showed stronger coupling with C2 than C5; in contrast, the signal at 6.26 ppm showed stronger coupling to C5 than C2; so the peak at 7.36 ppm was assigned to H4, and the one at 6.26 ppm was assigned to

Table 5. Bond lengths(Å) and angles(°) of acetylmelodorinol(comp A).

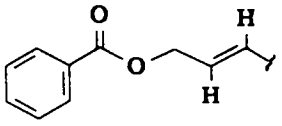
| Bond lengths | | | |
|---|---|---|---|
| O2-C1 | 1.384(5) | C4-C5 | 1.318(6) |
| O2-C3 | 1.401(5) | C6-C7 | 1.500(5) |
| O3-C3 | 1.192(6) | C7-C8 | 1.496(6) |
| O9-C8 | 1.440(5) | C1'-C10 | 1.475(5) |
| O9-C10 | 1.350(5) | C1'-C2' | 1.391(6) |
| O10-C10 | 1.208(5) | C1'-C6' | 1.382(6) |
| O11-C7 | 1.459(5) | C12-C13 | 1.487(6) |
| O11-C12 | 1.348(5) | C2'-C3' | 1.391(6) |
| O12-C12 | 1.192(5) | C3'-C4' | 1.379(7) |
| C1-C5 | 1.445(5) | C4'-C5' | 1.376(7) |
| C1-C6 | 1.325(5) | C5'-C6' | 1.384(6) |
| C3-C4 | 1.463(7) | | |

| Bond angles | | | |
|---|---|---|---|
| C1-O2-C3 | 107.9(3) | O9-C8-C7 | 108.3(3) |
| C8-O9-C10 | 115.2(3) | C10-C1'-C2' | 122.1(4) |
| C7-O11-C12 | 118.3(3) | C10-C1'-C6' | 118.1(4) |
| O2-C1-C5 | 107.9(3) | C2'-C1'-C6' | 119.9(4) |
| O2-C1-C6 | 122.5(3) | O9-C10-O10 | 122.7(4) |
| C5-C1-C6 | 129.6(4) | O9-C10-C1' | 113.0(4) |
| O2-C3-O3 | 120.6(4) | O10-C10-C1' | 124.4(4) |
| O2-C3-C4 | 106.8(4) | O11-C12-O12 | 123.7(4) |
| O3-C3-C4 | 132.7(4) | O11-C12-C13 | 110.1(4) |
| C3-C4-C5 | 108.5(4) | O12-C12-C13 | 126.3(4) |
| C1-C5-C4 | 108.9(4) | C1'-C2'-C3' | 119.6(4) |
| C1-C6-C7 | 125.2(4) | C2'-C3'-C4' | 119.8(5) |
| O11-C7-C6 | 107.0(3) | C3'-C4'-C5' | 120.7(4) |
| O11-C7-C8 | 108.4(3) | C4'-C5'-C6' | 119.6(4) |
| C6-C7-C8 | 109.9(4) | C1'-C6'-C5' | 120.4(4) |

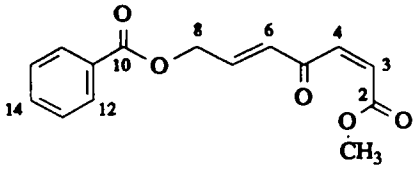
H3, assuming the transoid configuration of H3-C5 and H4-C2. The measured dihedral angle from X-ray diffraction analysis (the dihedral angle of H3-C5 and H4-C2 were 179.42°) supported the analysis of the COLOC experimental data. Other $^{13}C$-$^{1}H$ long range couplings of H8-C10 and C10-H12 were also detected by the COLOC experiment. In $^{1}H$-nmr spectra, H3 showed small extra long range coupling (0.5 Hz), while H4 did not. Presumably, this long range $^{1}H$-$^{1}H$ coupling was due to 'W' type coupling with H6, and it was confirmed by a decoupling experiment. When H3 was decoupled, the intensity of the H6 signal significantly increased, and when H6 was decoupled, the signal of H3 increased. The carbon signal at 165.94 ppm showed long range coupling with H12 and H8 in the COLOC experiment, so this carbon signal was assigned to C10.

$^{13}C$-nmr and $^{1}H$-nmr spectra of compound B showed 15 carbon signals, at 13 different frequencies, and 14 protons. EIMS showed $M^+$ at m/z 274 and isobutane CIMS showed $MH^+$ at m/z 275. Exact mass gave the expected composition of $C_{15}H_{14}O_5$ (found: 274.0839, calcd.: 274.0841). $^{13}C$-Nmr and $^{1}H$-nmr spectra were similar to acetylmelodorinol, and again the presence of a benzoyl moiety was clear. An $A^2MX$ proton spin system, comprised of signals at 5.0, 6.49, and 6.85 ppm (these protons were attached to carbons of 62.79, 129.77, and 140.00 ppm, respectively), was recognized as being connected to the benzoyl moiety suggesting the fragment (6). One isolated olefinic proton spin system was indicated by proton signals at 6.60 ppm and 6.17 ppm with a cis-configuration ($^{3}J_{HH}$=12 Hz). No long range $^{1}H$-$^{1}H$ coupling of those protons with other protons was detected in regular 1-D and COSY experiments. Proton homo decoupling experiments showed long range coupling between two separate olefinic systems. Extra keto and ester carbonyl carbons were recognized in $^{13}C$-nmr (192.72 ppm and 165.17 ppm, respectively). Thus, the possible structure for compound B was proposed as (7).

Low resolution EIMS gave the expected fragment ions for the proposed structure (8). Exact masses were measured for the fragment ions of m/z 215, 161, and 113, to give the expected compositions of $C_{13}H_{11}O_3$ (found: 215.0710, calcd.: 215.0708), $C_{10}H_9O_2$ (found: 161.0605, calcd.: 161.0602), and $C_5H_5O_3$ (found: 113.0242 calcd.:113.0238). The oxime derivative of compound B was prepared and directly analyzed by low resolution CIMS. The $MH^+$ and expected major fragment of a mono-oxime derivative were recognized at m/z 304 and m/z 182 ($M^+$-$C_6H_6COO$) and helped to confirm structure (7).

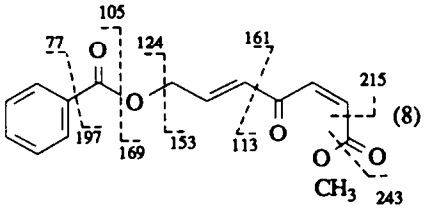



(6)

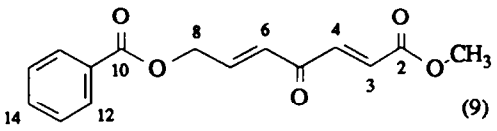



(7)

Compound B (7) also represents the novel heptene class and was named isomelodienone. Spectral assignments of isomelodienone were straightforward except for H3, H4, C3, C4, C6, and C7. The olefinic protons H3 and H4 were assigned by decoupling experiments. Irradiation of H6 (6.49 ppm) caused an increase of the proton signal at 6.60 ppm, while there was no change in the proton signal at 6.17 ppm, and the irradiation of the proton at 6.17 ppm did not affect the intensity of the signal at 6.49 ppm. Thus, as with H3 and H4 of acetylmelodorinol, the signal at 6.60 ppm was assigned to H3, and the signal at 6.17 ppm to H4. The carbon signals of C3, C4, C6, and C7 were assigned on the basis of their $^{13}C$-$^{1}H$ long range coupling patterns. The $sp^2$ carbon signals at 141.99 and 129.77 ppm showed large long range couplings which were attributed to cisoid three bond coupling (5.2 Hz), so one signal at lower field was assigned to C4, and the other to C6, assuming the mesomeric effects of the α,β-unsaturated carbonyl structure. Likewise, of the remaining two olefinic carbons, the doublet of doublets at lower field (140.00 ppm, 1.4 Hz) was assigned to C7, and the other (126.04 ppm, d, 1.7 Hz) was assigned to C3. As in the case of acetylmelodorinol (compound A), the C2 carbon signal showed large




(8)




(9)

$^{13}C$-$^{1}H$ long range coupling (12 Hz).

Spectral data of compound C were very close to those of isomelodienone (compound B)(7), with an identical molecular weight (CIMS: $MH^+$ m/z 275). 15 carbon signals, at 13 different frequencies, and 14 protons were recognized in the $^{13}C$-nmr and $^{1}H$-nmr spectra. The $MH^+$ in the isobutane CIMS was observed at m/z 275, but the $M^+$ was very unstable in EIMS, instead two major fragment ions were prevalent which corresponded to the benzoyl moiety ($C_7H_5O_2$; calcd.: 121.0289, found: 121.0292) and the $C_7$ unit ($C_8H_9O_3$; calcd.: 153.0551, found: 153.0551). Thus, the elemental composition was determined to be $C_{15}H_{14}O_5$, the same as isomelodienone. The only difference from isomelodienone (7) in the nmr spectra was observed in the two olefinic systems. Three olefinic carbon signals were shifted downfield (141.99 → 142.35, 129.77 → 131.40, 126.04 → 128.53 ppm), and the remaining one was shifted upfield (140.00 → 137.79 ppm) compared to that of isomelodienone. The protons of the isolated olefinic spin system were shifted downfield (6.60 → 7.37, 6.17 → 6.75 ppm), and the $^{3}J_{HH}$ was changed to 15.9 Hz from 12 Hz, indicating a change to the trans configuration from the cis configuration. Fragmentation patterns in the EIMS were similar to isomelodienone except that the molecular ion was very unstable. Thus, structure (9) was proposed for compound C which was named melodienone. The spectral assignments of melodienone (7) were straightforward except for H3, H4, C3, C4, C6,

C7, C2, and C10. When H6 (6.60 ppm) was decoupled, the signal at 7.37 ppm was increased, but there was no change to the signal at 6.75 ppm. Thus, the peak at 7.37 ppm was assigned to H3 and the peak at 6.75 ppm was assigned to H4. C2 and C10 were very close in chemical shifts (165.81 and 165.74 ppm) and $^{13}C$-$^{1}H$ long range coupling could not be observed due to the low intensities of these quaternary carbon signals. Consequently, these two carbons were assigned tentatively on the basis of chemical shifts compared to those observed with isomelodienone. Carbon signals of C3, C4, C6, and C7 were assigned on the basis of $^{13}C$-$^{1}H$ long range coupling constants and patterns (Table 3), in the same manner as in the case of isomelodienone (7). The cis isomer (isomelodienone)(7) was more active on human tumor cell lines than the trans isomer (melodienone)(9).

It has been noted that a unique characteristic of the chemistry of *Uvaria* in the Annonaceae is the ability to employ benzyl or benzoyl groups to substitute a number of different types of secondary metabolites.[7] The same feature was observed in the chemistry of *Goniothalamus*[8,9] and now in *Melodorum*. Thus, this capability might be extended to include still more genera of the Annonaceae. A close chemical relationship is obvious among acetylmelodorinol (5), melodienone (9), and isomelodienone (7); they have benzoyl and $C_7$ moieties in common. The $C_7$ (heptene) moieties are reminiscent of the $C_7N$ units of antibiotics from species of *Streptomyces* (pactamycin,[10] geldanamycin,[11] streptonigrin,[12] validamycin,[13] lincomycin A[14]), and a $C_7$ unit is omnipresent in several of the aromatic compounds of *Annonaceae* (altholactone,[15] goniofufurone, goniopypyrone, 8-acetyl goniotriol,[8] goniotriol,[9] goniothalamin,[16] goniothalamin oxide,[17] epizeylenol,[18] zeylena, zeylenol,[19] seneol, senepoxide,[20] pipoxide,[21] and goniodiol[9]). Hence common biosynthetic origins of these $C_7$ units of *Annonceae* aromatic compounds via pathways similar to that of $C_7N$ units might be suspected.

## EXPERIMENTAL

***Plant material.*** Stem bark of *Melodorum fruticosum* Lour. was collected in Thailand where voucher specimens are maintained at the Herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

***General.*** Melting points were determined on a Mettler FP5 and are uncorrected. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. IR spectra were obtained by Beckman IR-33 or Perkin-Elmer 1600 instruments as KBr discs, as liquids or in nujol oil. $^{1}H$-Nmr spectra were recorded on Chemagnetics A-200 or Varian VXR-5000S instruments operating at 200 MHz and 500 MHz, respectively. $^{13}C$-Nmr spectra were recorded on the Chemagnetics A-200 operating at 50.2 MHz or Varian VXR-5000S operating at 125.7 MHz. Proton coupled $^{13}C$-nmr spectra were obtained by gated decoupling (gated decoupler off during acquisition) to allow nuclear Overhauser enhancement. COSY and COLOC experiments were performed using a pulse sequence program supplied with the Varian software. Chemical shifts were reported relative to the residual solvents peaks. Low resolution ms were obtained on a Finnigan 4000 and high resolution ms were obtained on a Kratos MS 50 through peak matching. Circular dichroism was measured by Jasco J600 spectropolarimeter.

***Biological evaluations.*** The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae. The brine shrimp lethality bioassay[4] was performed in our laboratory. In vitro antitumor activities on the three human tumor cell lines were determined in the Purdue Cell Culture Laboratory, Purdue Cancer Center, following protocols established by the National Cancer Institute. The potato disc assay

for inhibition of crown gall tumors was performed as previously reported.[5]

***Isolation of the compounds.*** The air dried and ground stem bark of *Melodorum fruticosum* (4.1 Kg) was extracted with 95% EtOH and concentrated to a residue (700 g, 17 %) (F001) under vacuum. A portion of F001 (200 g) was partitioned between $H_2O$ (F002) and $CH_2Cl_2$ (F003). An insoluble interface formed (F004). The $CH_2Cl_2$ solubles were condensed and partitioned between 90% MeOH (F005) and hexane (F006). The 90% MeOH partition residue (F005) was most toxic to brine shrimp larvae ($LC_{50}$ 67 ppm), so a portion (40 g) was chromatographed through 1.13 kg of Si gel (60-200 mesh) in an open column using a gradient of $CH_2Cl_2$ (50-100%) in hexane. A total of 73 fractions were collected. Compound A crystallized from the fractions 33-35 ($LC_{50}$ > 1000 ppm) and recrystallized from hexane/acetone as colorless bioactive crystals (120 mg, $LC_{50}$ 246 ppm) (Table 1). A pool of fractions 27-30 (900 mg), which was toxic to the brine shrimp larvae ($LC_{50}$ 39 ppm), was chromatographed on a Si gel column with a gradient starting from hexane:$CH_2Cl_2$ (20:1). Resultant fraction 20 (24 mg) was toxic to brine shrimp larvae ($LC_{50}$ 10 ppm) and human tumor cells (compound B) (Table 1).

Fractions 31-32 (817 mg) from the initial column were chromatographed on chromatotron (centrifugal radial thin layer chromatography, 2 mm Si gel rotor x3) with a gradient starting from hexane:$CHCl_3$:acetone(10:1:0.5). Resultant fractions 1-3 (15 mg) were toxic to brine shrimp larvae and human tumor cells. This yellow liquid was mixed with hexane/acetone and kept in freezer to yield bioactive light yellow crystals (compound C, $LC_{50}$ 24 ppm) (Table 1).

***Compound A (acetylmelodorinol).*** Colorless crystal, m.p. 75-76°; $[\alpha]^D$ +209°(c=1, $CHCl_3$); UV $\lambda_{max}$(MeOH): 287 nm; IR $\nu_{max}$(KBr)$cm^{-1}$: 3080, 2950, 1790, 1750, 1735, 1685, 1290, 1275, 1240; elemental analysis: calcd. for $C_{16}H_{14}O_6$, C 63.58 H 4.67 O 31.78, found C 63.66 H 4.64 O 31.70; CD (c=0.23 mg/ml, MeOH) $[\theta]^{25}$ (nm) +0.01 (387.8), +0.99 (339.2), +1.36 (328.6), 0.00 (310.6), -1.49 (302.8), +0.04 (300.2), +17.06 (281.6); EIMS m/z (%): 302 ($M^+$,9), 244 (35), 243 ($M^+$-$OCOCH_3$, 100), 215 (7), 180 (5), 105 (15), 77 ($C_6H_5^+$, 7); CIMS (Ammonia) m/z: 320 ($MNH_4^+$), 244 (3), 243 (21); $^1$H-nmr, see Table 2; $^{13}$C-nmr, see Table 3; COLOC data, see Figure 1.

***Hydrolysis of compound A.*** 40 mg of compound A was dissolved in 4 ml of 2% NaOH and stirred for 24 hours at room temperature. The reaction mixture was washed with ether, and HCl was added to the aqueous layer until it reached pH 2. The resultant precipitate was filtered and recrystallized from acetone to yield crystalline benzoic acid (m.p. 126-127°). IR $\nu_{max}$(KBr) $cm^{-1}$: 3000, 2835, 1684, 1422, 1289, 931; $^1$H-nmr (200 MHz, $CDCl_3$) δ 8.29 (d, 8.8 Hz, 2H), 7.66 (t, 8.3 Hz, 1H), 7.51 (t, 8.8 Hz, 2H); $^{13}$C-nmr (50.2 MHz, $CDCl_3$) δ 172.7, 133.78, 130.20, 129.52, 128.49.

***Compound B (isomelodienone).*** Light yellow liquid; HR EIMS: 274. 0839 ($M^+$, calcd. for $C_{15}H_{14}O_5$ :274.0841), 215.0710 (calcd. for $C_{13}H_{11}^{\bullet}O_3$: 215.0708), 161.0605 (calcd. for $C_{10}H_9O_2$: 161.0602), 113.0242 (calcd. for $C_5H_5O_3$: 113.0238); IR $\nu_{max}$(liquid) $cm^{-1}$: 1720, 1665, 1268, 1221, 707; EIMS m/z (%): 274 ($M^+$,0.2), 258 (0.1), 243 ($M^+$-$OCH_3$, 0.1), 229 (0.2), 215 ($M^+$-$COOCH_3$, 0.6), 197 ($M^+$-$C_6H_5$, 0.1), 169 ($M^+$-$COC_6H_5$, 2), 161 (0.4), 153 ($M^+$-$OCOC_6H_5$, 10), 121 ($C_6H_5COO^+$, 11), 113 (8.1), 105 (100), 77 (72); $^1$H-nmr, see Table 2; $^{13}$C-nmr, see Table 3.

***Oxime derivatization of compound B.*** 0.4 mg of compound B was mixed with 0.1 ml of MOX (2%

solution of methoxyamine HCl in pyridine) in a Reacti-Vial and heated at 60° for 6 hours. The reaction mixture was directly analyzed by low resolution CIMS.

***Compound C (melodienone).*** Light yellow crystal, m.p. 69-70°; IR $\nu_{max}$(nujol) $cm^{-1}$: 2919 1725, 1672, 1267; EIMS m/z (%): 171 (1.3), 169 (1.1), 153 (6.6), 143 (3.3), 125 (4.5), 121 (3.0), 113 (21.1), 105 (100), 77 (67.8); CIMS (isobutane): m/z 275 ($MH^+$, 2.6); HR EIMS: 121.0292 (calcd. for $C_7H_5O_2$: 121.0289), 153.0551 (calcd. for $C_8H_9O_3$: 153.0551); $^1$H-nmr, see table 2; $^{13}$C-nmr, see table 3.

***X-ray data.*** A colorless needle was mounted on a glass fiber in a random orientation. Preliminary examination and data collection was performed with Mo Kα radiation (λ=0.71703 Å) on an Enraf-Nonius CAD4 computer controlled kappa axis diffractometer equipped with graphite crystal, incident beam monochromator. Cell constants and an orientation matrix for data collection were obtained from least-square refinement, using the setting angles of 25 reflections in the range $13 < \theta < 20°$, measured by the computer controlled diagonal slit method of centering. The data were collected at a temperature of 20 ± 1° using the ϖ-2θ scan technique. The scan rate varied from 2 to 20°/min (in omega). Data were collected to a maximum 2θ of 50.0°. The scan range (in deg.) was determined as a function of θ to correct for the separation of the Kα doublet; the scan width was calculated as follows: ϖ scan width= 0.50 + 0.350 tan θ. Moving-crystal moving-counter background counts were made by scanning an additional 25 % above and below this range. Thus, the ratio of peak counting time to background counting time was 2:1. The counter aperture was also adjusted as a function of θ. The horizontal aperture width ranged from 1.8 to 2.3 mm; the vertical aperture was set at 4.0 mm. the diameter of the incident beam collimator was 0.7 mm and the crystal to detector distance was 21 cm. For intense reflections an attenuator was automatically inserted in front of the detector; the attenuater factor was 12.9. Lorentz and polarization corrections were applied to the data. The linear absorption coefficient is 1.0 $cm^{-1}$ for Mo Kα radiation. No absorption correction was made. The structure was solved by direct methods using SHELX 86. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located and added to the structure factor calculations but their positions were not refined. The structure was refined in full-matrix least-squares where the function minimized was $\Sigma w(|Fo|-|Fc|)^2$ and the weight w is defined as per the Killean and Lawrence method with terms of 0.020 and 1.0. Scattering factors were taken from Cromer and Waber.[22] Anomalus dispersion effects were included in Fc; the values for δf' and δf'' were those of Cromer. Only the 1024 reflections having intensities greater than 3.0 times their standard deviation were used in the refinements. The final cycle of refinement included 198 variable parameters and converged (largest parameter shift was 0.00 times is esd) with unweighted and weighted agreement factors of: $R1 = \Sigma |Fo-Fc| / > Fo = 0.038$, $R2 = SQRT(\Sigma w (Fo-Fc)^2 / \Sigma w Fo^2) = 0.045$. All calculation were performed on a VAX computer using SDP/VAX. Crystal data and data collection parameters are summarized below.

## ACKNOWLEDGMENTS

This work was supported by R01 grant no. CA 30909 from the National Cancer Institute, National Institute of Health. We thank Peggy Criswell for the cell culture data, J. G. Leeds for mass spectral data, J. E. Anderson for COLOC experiment, and Dr. K. V. Wood for helpful discussions on the MS data.

| | | | |
|---|---|---|---|
| formula | $O_6C_{16}H_{14}$ | scan method | $\omega$-2$\theta$ |
| formula weight | 302.29 | h, k, l limits | 0-8, 0-13, -11-10 |
| space group | $P2_1$ (No. 4) | 2$\theta$ range, deg | 4.00-50.00 |
| a, Å | 7.278(3) | scan width, deg | 0.50 + 0.35tan($\theta$) |
| b, Å | 11.676(2) | take-off angle, deg | 3.15 |
| c, Å | 9.351(3) | programs used | Enraf-Nonius SDP |
| $\beta$, deg | 111.13(1) | $F_{000}$ | 316.0 |
| V, Å$^3$ | 741.1(7) | p-factor used in weighting | 0.040 |
| Z | 2 | data collected | 1399 |
| $d_{calc}$, g cm$^{-3}$ | 1.355 | unique data | 1399 |
| crystal demension, mm | 0.46x0.17x0.11 | data with I>3.0 $\sigma$ (I) | 1024 |
| temperature, deg C | 20 | number of variables | 198 |
| radiation (wavelength) | Mo$K_\alpha$ (0.71073 Å) | largest shift/ | 0.00 |
| monochromator | graphite | esd in final cycle | |
| linear abs coef, cm$^{-1}$ | 0.98 | R | 0.038 |
| absorption correction | none | $R_w$ | 0.045 |
| diffractometer | Enraf-Nonius CAD4 | goodness of fit | 1.111 |

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