Miliusanes, A Class of Cytotoxic Agents from *Miliusa sinensis*

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Bioassay-directed fractionation of the leaves, twigs, and flowers of *Miliusa sinensis* Finet and Gagnep. (Annonaceae) led to the isolation of a new class of potential anticancer lead molecules. They are a cluster of compounds composed of a C_{18} carbon skeleton, a known but heretofore unnamed type, which we have designated as miliusane. Two known (**1** and **2**) as well as 20 new miliusanes (**3**−**22**) have been isolated and identified. They belong to two substructural classes of miliusanes. One subclass (**1**−**19**) was determined to be composed of a γ-lactone spiro-ring system, the opening of which led to the second group of compounds (**21** and **22**) containing a tetrahydrofuran ring system. Compounds **1**−**3**, **5**, **8**, **9**, **18**, **20**, and **21** demonstrated significant cytotoxic activity in our cancer cell line panel comprising KB, Col-2, LNCaP, Lu-1, MCF-7, and HUVEC. The structures were determined by spectroscopic and chemical methods. The structure of miliusate was further confirmed by X-ray crystallographic analysis. The absolute stereochemistry of miliusanes was established by the Mosher ester method. Forty-two modified miliusane derivatives were also prepared and evaluated for their cytotoxic activities.

Introduction

Miliusa sinensis Finet and Gagnep. (Annonaceae), a tree up to 6 m tall, is found in southern Asia including Vietnam and Southern China at 500−5000 m altitude.[1] This plant was investigated as part of our International Cooperative Biodiversity Group (ICBG) project, which was designed to address the related issues of biodiversity conservation, economic growth, and promotion of health through the discovery of anticancer, antihuman immunodeficiency virus (anti-HIV), antimalarial, and antitubercular (anti-TB) natural products through collaboration with institutions in Vietnam, Laos, and the United States.[2] More than 3000 plant samples have been collected from Vietnam and Laos, and extracts of which have been tested for cytotoxic potential using in vitro bioassay systems. Extracts were chosen for further investigation if they inhibited cancer cell growth more than 50% at a concentration of 4 μg/mL. A dichlormethane extract prepared from *M. sinensis* collected in the Cuc Phuong National Park (Nho Quan District, Ninh Binh Province, Vietnam) exhibited cytotoxicity against KB cells with an IC_{50} value of 2.0 μg/mL during initial bioassay. A search of the literature revealed no prior phytochemical or pharmacological reports on this plant. A 5.5 kg sample of dried leaves, twigs, and flowers of this plant was, therefore, recollected for bioassay-directed isolation studies aimed at identifying novel anticancer agents. As a result, 22 compounds, including 20 new ones, were isolated from the CH_2Cl_2 extract of *M. sinensis* using the in vitro KB cell cytotoxicity assay as a monitor. All of these compounds belong to a C_{18} carbon skeleton. Although two compounds in this class were reported previously from another *Miliusa* species,[3,4] their structural type, absolute stereochemistry, and biological activity were not reported. The current paper describes the isolation, identification, and biological evaluation of this series of compounds, which we have designated as miliusanes from the title species. The absolute stereochemistry of the prototype was determined by Mosher esters method and various diagnostic chemical reactions, in addition to X-ray crystallographic analysis. A putative biosynthetic pathway is proposed. In an effort to improve the cytotoxic potential of these compounds, 42 miliusane derivatives were also synthesized by esterification of the C-5 hydroxy of miliusol (**2**).

Results and Discussion

A sample consisting of the dried leaves, twigs, and flowers (5.5 kg) of *M. sinensis* was milled, extracted with CH_2Cl_2, and evaporated in vacuo to afford an extract (173 g). Bioassay-directed fractionation of the CH_2Cl_2 extract by repeated flash column chromatography on Si gel and RP-18 Si gel, followed by preparative high-performance liquid chromatography (HPLC), led to the isolation of **1** (miliusate),[3] **2** (miliusol),[4] and 20 new miliusanes (**3**−**22**). All of the isolates, except for **1**, were purified as colorless gums from bioactive fractions. Compound **1** was isolated as crystalline flakes, which allowed us to confirm its and the other miliusane structures by X-ray crystallographic analysis.

X-ray Structure of Miliusate. Compound **1** was crystallized in space group $P2_12_12_1$ from MeOH. The X-ray crystal structure, high-resolution time-of-flight mass spectrometry (TOFMS) analysis, and NMR studies revealed that the compound has a molecular formula of $C_{20}H_{26}O_5$. It was first isolated from *M.*

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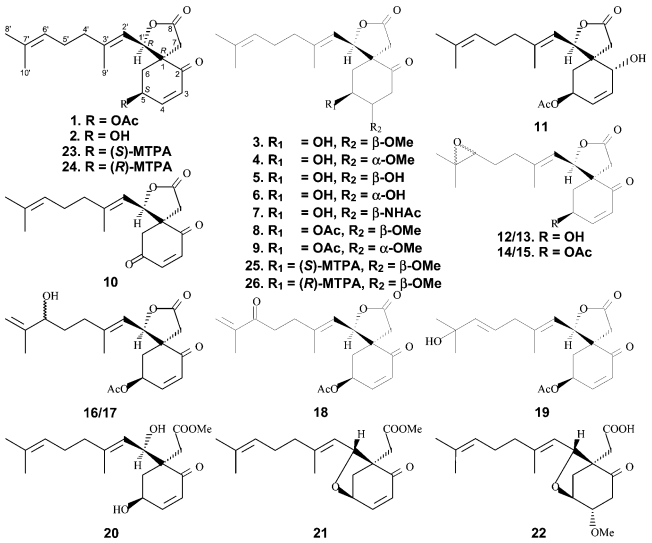


balansae[3] and represents the prototype of a group of compounds belonging to a novel carbon skeleton, which we have designated as the "miliusanes". The X-ray crystal structure (Figure 1)

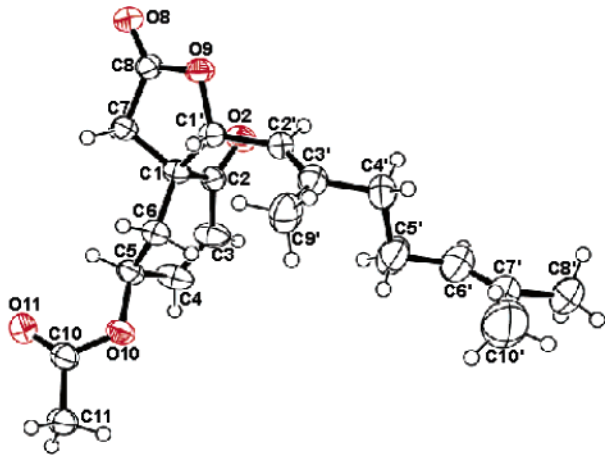


Figure 1. ORTEP drawing of one molecule of **1**.

confirmed the structure of miliusate to be 9β-acetoxy-1β-(*E*-2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione (**1**). The structure containing a six-membered ring fused with a five-membered ring has a half-chair conformation. The bond lengths and angles, generated by an MM2 energy minimization, are in agreement with those from the X-ray structure. Compound **2** was the second miliusane reported from nature and was also obtained from *M. balansae*.[4] Acetylation of **2** with pyridine-Ac_2O produced (+)-miliusate (**1**) (Figure 2), thus providing confirmational evidence for the structure of **2** as 9β-hydroxy-1β-(*E*-2,6-dimethyl-hepta-1, 5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione.

Structures of 3−11. Compounds **3**−**11**, colorless gums, showed very similar NMR data to those of **1** and **2** (Tables 1 and 2), suggesting that their structures are similar. The IR bands of ν_{max} 1764−1783 cm^{-1} indicated a γ-lactone in compounds **3**−**11**.

Analysis of the $^1H-^1H$ correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond correlation (HMBC) spectral data (Figure 3) determined **3** (miliusane I) and **4** (miliusane II), **5** (miliusane III) and **6** (miliusane IV), and **8** (miliusane VI) and **9** (miliusane VII) to be three pairs of epimers at C-4, of which the ^{13}C NMR spectral data were very similar to one another, respectively. The epimerization of the 4-oxy groups from β to α led to only maximum downfield shifts of $\Delta\delta$ 2.3 ppm for the ^{13}C NMR signals at C-4. However, the ^1H NMR data showed much greater differences between the α- and the β-epimers. The proton signals of H-4 and -6α of the α-epimer were more significantly shifted downfield than the β-epimer, while the protons signals of H-5 and -6β were notably shifted upfield. The ^1H NMR coupling patterns of H-4 were also significantly different between the α- and the β-epimers. The ^1H NMR signal of H-4 in the β-epimers was split into a doublet of doublets of doublets by H-3β ($J \approx 12$ Hz), H-3α ($J = 4.4-5.2$ Hz), and H-5α ($J = 2.9-3.4$ Hz), whereas the ^1H NMR signal of H-4 in the α-epimers was first split into a quartet by three protons [H-3β, -3α, and -5α ($J \approx 3.4$ Hz)] and then to a quartet of doublets by H-6β ($J \approx 1.5$ Hz) through a *W*-coupling.

Epimers **3** and **4** differ structurally from **2** only at C-3 and -4. The $\Delta^{3,4}$ double bond in **2** was substituted by a methoxy group at C-4 in cases of **3** and **4**, which resulted in significant downfield shifts of the ^{13}C NMR signals of C-1, -2, -3, and -4 in **3** and **4** from those of **2**. The methoxy group in **3** was β-oriented due to the presence of a ROE correlation between H-4 and H-6α (Figure 4), which, in turn, determined the methoxy group in **4** as α-oriented. Compounds **3** and **4** were thus determined to be 8β-methoxy-9β-hydroxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione and 8α-methoxy-9β-hydroxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione, respectively.

Compounds **5** and **8** shared the same NMR coupling patterns of **3** at H-4, suggesting that the 4-oxy groups of **5** and **8** are also β-oriented. This was confirmed by the ROE correlations between the two protons, H-4 and H-6α. Similarly, **6** and **9** showed the same NMR coupling patterns as **4** at H-4, thus suggesting an α-configuration for the C-4 oxy groups for the two compounds. Compounds **5**, **6**, **8**, and **9** were thus determined to be 8β,9β-dihydroxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione, 8α,9β-dihydroxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione, 8β-methoxy-9β-acetoxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione, and 8α-methoxy-9β-acetoxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione, respectively. Acetylation of **3** and **4** produced (+)-miliusanes VI (**8**) and VII (**9**) (Figure 2), repectively, which further confirmed the structures of **3**, **4**, **8**, and **9**.

Compound **7** (miliusane V) was shown to have a molecular formula of $C_{20}H_{29}NO_5$ by HRTOFMS and NMR studies. The nitrogen atom formed an acetylamino group in **7**, which was assigned to a β-configuration at C-4 due to the presence of the ROE correlations between H-4 and H-6α and the presence of HMBC correlations between the C-4 and the aceyl proton signals. In comparison with compound **3**, the ^{13}C NMR signal of C-4 in **7** was shifted upfield by $\Delta\delta$ 28.9 ppm, due to the presence of the acetylamino group at C-4. Accordingly, **7** was established as 8β-acetylamino-9β-hydroxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione.

Compound **10** (miliusane VIII) is an oxidized isomer of **2**. An additional carbonyl carbon was observed at δ 194.5 ppm in the ^{13}C NMR spectrum of **10** and was assigned to C-5 due to its HMBC correlations to H-3, -4, -6α, and -6β. The existence of the carbonyl group at C-5 led to significant changes of ^1H and ^{13}C NMR chemical shifts of H-3, -6α, -6β, -1′, and -2′ and C-1, -3, -4, -5, and -6 in comparison with those of **2** (Tables 1 and 2). Oxidation of (+)-miliusol (**2**) by pyridinium chlorochromate (PCC) afforded (+)-miliusane VIII (**10**) (Figure 2),

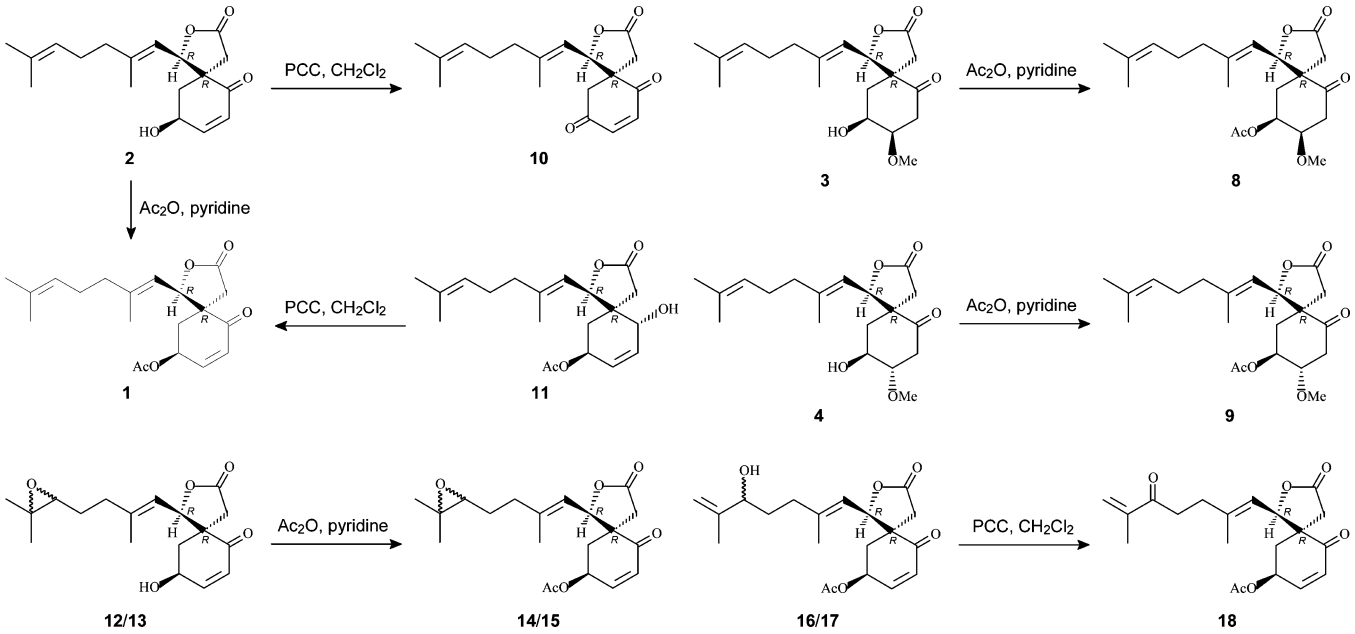


Figure 2. Chemical conversion of miliusanes.

which confirmed it to be 1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6,9-trione.

Compound **11** (miliusane IX) showed no ketone carbonyl carbon signal in the ^{13}C NMR spectrum. However, additional oxy-methine signals [δ 3.94 (brt, $J = 5.2$ Hz); δ 66.9 (d)] were observed in the NMR spectra. The presence of HMBC correlations of the oxy-methine proton signal with C-1, -3, -4, -6, -7, and -1′ placed the oxy-methine group at C-2. The reduction of the C-2 carbonyl group to a hydroxy group resulted in dramatic upfield shifts of the NMR signals of H-4, -7β, and -1′ and C-1, -2, -4, and -6 in comparison with those of **1** (Tables 1 and 2). An α-orientation was assigned to the C-2 oxy-group due to the presence of ROE correlations of H-2 to H-2′ and H-7β. The structure of **11** was also confirmed by its chemical conversion to (+)-miliusate (**1**) by PCC oxidation (Figure 2). Compound **11** (miliusane IX) was thus elucidated to be 6α-hydroxy-9β-acetoxy-1β-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3-one.

Structures of 12−17. Three pairs of inseparable miliusanes [**12**/**13** (miliusanes X/XI), **14**/**15** (miliusanes XII/XIII), and **16**/**17** (miliusanes XIV/XV)] were isolated in approximately a 1.0:1.1 ratio each. This occurrence of compound pairs in mixtures of almost equal parts mirrors the situation that we encountered in our work on litseaverticillols F and G,[5] which also could not be separated despite the use of a variety of separation and chemical derivation techniques.

Although we were unable to separate the three mixture pairs, the ^{1}H and ^{13}C NMR chemical shifts of **12−17** were clearly distinctive and can be assigned to each compound through analysis of their ^{1}H−^{1}H COSY, HMQC, HMBC, and integration data (Tables 3 and 4). The similarity of the NMR data of compounds **12−17** to those for **1** and **2** suggested that these compounds were also miliusanes. However, it was noticed that the $\Delta^{6',7'}$ double bond signals, which occurred in compounds **1−11**, did not appear in the NMR spectra of **12−15**. Instead, epoxy group signals were observed [δ 58.34−58.51 (s) and 63.48−68.97 (d)]. The epoxy groups were assigned to C-6′ and C-7′ due to the presence of HMBC correlations of H-8′ and H-10′ to C-6′ and C-7′.

Acetylation by Ac_2O/pyridine transformed **12**/**13** to **14**/**15** (Figure 2), thus linking the structures of the two pairs to each other. The epimers **12**/**13** and **14**/**15** were thus determined to be 9β-hydroxy-1β-[4-(3,3-dimethyl-1β,2β-oxiranyl)-2-methyl-but-1-enyl]-2-oxa-spiro[4.5]dec-7-ene-3,6-dione/9β-hydroxy-1β-[4-(3,3-dimethyl-1α,2α-oxiranyl)-2-methyl-but-1-enyl]-2-oxa-spiro[4.5]dec-7-ene-3,6-dione and 9β-acetoxy-1β-[4-(3,3-dimethyl-1β,2β-oxiranyl)-2-methyl-but-1-enyl]-2-oxa-spiro[4.5]dec-7-ene-3,6-dione/9β-acetoxy-1β-[4-(3,3-dimethyl-1α,2α-oxiranyl)-2-methyl-but-1-enyl]-2-oxa-spiro[4.5]dec-7-ene-3,6-dione, respectively.

In comparing the ^{13}C NMR spectral data of **16**/**17** to **1**, significant differences were observed at C-4′, C-5′, C-6′, C-7′, and C-8′. These positions were determined to be methylene, oxy-methine, quaternary olefinic, and olefinic methylene moieties, respectively, in **16**/**17**. The absence of a methyl NMR signal in **16**/**17** was attributed to the shift of the double bond at $\Delta^{6',7'}$ in **1** to $\Delta^{7',8'}$ in **16**/**17**. An extra oxy-methine group in **16**/**17** was assigned to C-6′ due to the HMBC correlations of C-6′ to H-8′ and H-10′, respectively. The epimers **16**/**17** were thus determined to be 9β-hydroxy-1β-(5β-hydroxy-2,6-dimethyl-hepta-1,6-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione/9β-hydroxy-1β-(5α-hydroxy-2,6-dimethyl-hepta-1,6-dienyl)-2-oxa-spiro-[4.5]dec-7-ene-3,6-dione.

Structures of 18 and 19. Compound **18** (miliusane XVI) was shown to have a molecular formula of $C_{20}H_{24}O_6$ according to HRTOFMS, which indicated one double bond equivalent more than that of the **16**/**17** pair. Compound **18** was determined to have a very similar structure to **16**/**17** with the only difference being at C-6′. The substituent at this position in **18** was found to be a carbonyl instead of the hydroxyl group found in **16**/**17**. Both H_2-4′ and H_2-5′ showed long-range coupling with δ 200.7 (s) in the HMBC spectra, which established C-6′ as a carbonyl carbon. Further evidence for the structural relationship between **16**/**17** and **18** was established by treatment of **16**/**17** with PCC to yield a derivative (Figure 2), which was shown to be identical to **18** by comparison of their ^{1}H NMR spectra and optical rotation data. Compound **18** was thus elucidated as 9β-acetoxy-1β-(2,6-dimethyl-5-oxo-hepta-1,6-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione.

Compound **19** (miliusane XVII) was found to be another isomer of **1**. It differs from **1** by the shift of the double bond at $\Delta^{6',7'}$ in **1** to $\Delta^{5',6'}$ in **19** and the substitution of an additional hydroxyl group at C-7′, which were determined by the presence

Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**11** (500 or 360 MHz, $CDCl_3$, *J* in Hz)

	δ					
position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]	**6**[a]
H-2						
H-3	6.01 dd (10.2, 1.2)	5.96 dd (10.2, 1.4)				
H-3α			2.61 ddd (13.0, 5.1, 1.0)	2.57 ddd (14.1, 3.5, 1.2)	2.50 ddd (13.2, 4.5, 0.9)	2.39 ddd (14.4, 3.7, 1.2)
H-3*β*			2.54 brt (12.5)	2.68 dd (14.2, 2.9)	2.61 brt (12.6)	2.79 dd (14.6, 3.1)
H-4	6.77 ddd (10.2, 3.9, 1.0)	6.87 ddd (10.1, 3.9, 0.8)	3.41 ddd (11.7, 5.2, 2.9)	3.77 brqd (3.4, 1.0)	3.88 ddd (12.1, 4.7, 3.4)	4.29 brq (3.2)
H-5	5.52 brdtd (5.3, 4.3, 1.3)	4.54 brqu[e]d (4.8, 0.9)	4.32, brqd (3.2, 0.8)	4.21 brq (2.9)	4.20 brq (3.2)	4.10 brq (2.8)
H_2-6						
H-6α	2.22 dd (14.6, 5.5)	2.18 dd (14.1, 4.8)	1.45[f] ddd (15.1, 2.5, 1.8)	1.96 dd (14.6, 3.5)	1.49 dd (15.1, 3.2)	2.05 dd (14.4, 3.5)
H-6*β*	2.34 ddd (14.6, 4.3, 1.1)	2.30 ddd (14.2, 4.8, 1.2)	2.63 dd (15.0, 3.0)	2.36 ddd (14.5, 2.5, 1.5)	2.54 (15.3, 3.4)	2.33 brd (14.7)
H-7α	2.23 d (17.7)	2.28 d (17.4)	1.93 d (18.1)	2.01 d (18.0)	1.93 d (17.9)	2.04 d (18.1)
H-7*β*	3.27 d (17.6)	3.15 d (17.2)	3.54 d (18.2)	3.54 d (18.0)	3.53 d (18.0)	3.53 d (18.0)
H-1′	5.41 d (10.1)	5.55 d (10.0)	6.04 d (10.7)	5.99 d (10.6)	6.08 d (10.5)	6.02 d (10.6)
H-2′	5.09 dse[c] (10.2, 1.1)	5.12 dse (10.2, 1.3)	4.91 dse (10.7, 1.2)	4.96 dse (10.7, 1.2)	4.90 brd (10.8)	4.98 dse (10.6, 1.4)
H_2-4′	1.95 m	1.99 m	1.99 m	1.98 m	1.98 m	1.98 m
H_2-5′	1.99 m	2.02 m	2.02 m	2.02 m	2.02 m	2.01 m
H-5a						
H-5b						
H-6′	4.93 tsep[d] (6.4, 1.1)	4.96 tsep (6.8, 1.3)	4.95 tsep (6.7, 1.5)	4.96 m	4.94 brt (6.6)	4.96 tsep (6.7, 1.2)
Me-8′	1.62 d (1.2)	1.64 d (1.3)	1.66 d (0.9)	1.66 s	1.64 s	1.65 s
Me-9′	1.64 d (1.3)	1.68 d (1.5)	1.77 d (1.5)	1.77 d (1.2)	1.76 d (1.4)	1.76 d (1.2)
Me-10′	1.53 d (1.2)	1.55 d (1.5)	1.56 (1.1)	1.56 s	1.55 s	1.56 s
Ac	2.10 s					
OH		2.69 brs	2.54 overlap[g]	2.19 brs		
OMe			3.38 s	3.32 s		
NH						

	δ				
position	**7**[b]	**8**[a]	**9**[a]	**10**[b]	**11**[a]
H-2					3.94 brt (5.2)
H-3				6.69 d (10.2)	5.94 ddd (10.1, 5.1, 1.7)
H-3α	2.48 dd (12.8, 4.8, 1.0)	2.58 ddd (13.4, 4.6, 1.0)	2.56 ddd (14.2, 3.5, 1.5)		
H-3*β*	2.54 brt (12.7)	2.49 dd (13.3, 12.2)	2.42 dd (14.4, 3.1)		
H-4	4.20 dddd (12.8, 7.9, 4.9, 2.6)	3.43 ddd (12.0, 4.4, 3.0)	3.78 brqd (3.4, 1.8)	6.73 d (10.3)	5.82 dd (10.1, 2.5)
H-5	4.23 brq (3.0)	5.61 brqd (3.4, 1.1)	5.21 brtdd (3.9, 2.7, 1.2)		5.33 tddd (8.2, 2.6, 1.7, 1.0)
H_2-6					2.10 m
H-6α	1.63 dd (14.7, 3.2)	1.62 dd (15.3, 3.6)	2.05 dd (15.3, 4.2)	2.98 ABd (16.6)	
H-6*β*	2.45 dd (14.9, 2.7)	2.46 dd (15.4, 3.5)	2.35 ddd (15.3, 4.3, 2.0)	3.03 ABd (16.6)	
H-7α	1.97 d (18.0)	1.95 d (18.0)	1.97 d (18.0)	2.31 d (17.3)	2.30 d (17.4)
H-7*β*	3.60 d (18.1)	3.44 d (18.0)	3.51 d (18.1)	3.30 d (17.5)	2.46 d (17.6)
H-1′	6.18 d (10.5)	5.59 d (10.3)	5.64 d (10.9)	5.04 d (10.2)	4.92 d (10.5)
H-2′	4.93 brd (10.4)	4.95 dse (10.2, 1.2)	4.93 dse (10.6, 1.3)	4.89 dse (10.3, 1.2)	5.29 brd (10.5)
H_2-4′	1.98 m	1.96 m	1.96 m	1.95 m	2.12 m
H_2-5′	2.02 m	1.99 m	1.99 m	1.99 m	
H-5a					2.22 m
H-5b					2.13 m
H-6′	4.94 m	4.91 tsep (6.4, 1.3)	4.91 tsep (6.3, 1.4)	4.94 tsep (6.5, 1.3)	5.01 tsep (6.4, 1.4)
Me-8′	1.65 d (0.9)	1.61 d (0.9)	1.61 d (1.2)	1.65 s	1.68 s
Me-9′	1.76 d (1.4)	1.78 d (1.4)	1.77 d (1.1)	1.59 d (1.2)	1.73 d (1.4)
Me-10′	1.56 d (1.0)	1.52 d (0.9)	1.52 d (1.1)	1.54 s	1.60 d (1.0)
Ac	1.98 s	2.14 s	2.12 s		2.04 s
OH					2.12 brs
OMe		3.29 s	3.30 s		
NH	6.16 brd (8.0)				

[a] 500 MHz. [b] 360 MHz. [c] se represents sextet. [d] sep represents septet. [e] qu represents quintet. [f] If the concentration of **3** in $CDCl_3$ was increased to ca. 30%, the signal turned out to be clear doublet of doublets (J = 15.2, 3.1 Hz) due to no W-coupling between 5-OH and H-6α. [g] Shifted downfield to δ 2.82 ppm (brs).

of HMBC correlations of H-8′ and H-10′ to the oxy-quaternay carbon of C-7′ and to the $\Delta^{5',6'}$ double bond carbons. Compound **19** was thus established as 9*β*-acetoxy-1*β*-(6-hydroxy-2,6-dimethyl-hepta-1,4-dienyl)-2-oxa-spiro[4.5]dec-7-ene-3,6-dione.

Structure of 20. Compound **20** (miliusane XVIII) was obtained as a colorless gum. Its NMR data are very similar to those of **2**. However, in the HMBC spectrum, **20** showed no J^3 coupling of H-1′ to the ester carbonyl carbon at δ 171.5 (s) that was observed in compounds **1**−**19**, which implies an opening of the γ-lactone ring. This opening of the γ-lactone ring resulted in significant upfield shifts of the NMR signals of H-1′ and C-1′ (Table 4). The presence of HMBC correlation of

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**11** (125 or 90 MHz, $CDCl_3$)

	δ										
position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[a]	**8**[b]	**9**[b]	**10**[a]	**11**[b]
C-1	52.1 s	52.4 s	55.1 s	55.9 s	54.8 s	56.0 s	55.2 s	55.2 s	55.4 s	56.2 s	46.8 s
C-2	194.8 s	196.4 s	203.8 s	205.0 s	203.9 s	205.3 s	203.0 s	202.7 s	203.3 s	195.8 s	66.9 d
C-3	130.6 d	128.9 d	40.6 t	39.8 t	43.7 t	43.3 t	41.0 t	42.2 t	40.4 t	140.6 d	130.3 d
C-4	144.0 d	148.8 d	79.9 d	82.2 d	71.0 d	73.7 d	51.0 d	78.1 d	79.0 d	141.2 d	130.0 d
C-5	64.9 d	63.2 d	66.2 d	66.5 d	68.4 d	69.1 d	67.2 d	66.4 d	68.0 d	194.5 s	67.0 d
C-6	36.3 t	39.5 t	37.2 t	38.7 t	37.7 t	38.2 t	40.2 t	35.8 t	36.2 t	46.8 t	31.3 t
C-7	37.0 t	38.0 t	36.2 t	36.6 t	36.3 t	36.9 t	36.4 t	36.3 t	36.1 t	37.0 t	36.0 t
C-8	174.4 s	175.1 s	175.5 s	177.0 s	176.4 s	176.1 s	176.7 s	174.5 s	174.9 s	173.4 s	174.9 s
C-1′	80.9 d	82.2 d	80.4 d	81.1 d	80.8 d	80.9 d	80.6 d	79.5 d	79.6 d	82.8 d	83.2 d
C-2′	118.3 d	118.3 d	118.1 d	118.1 d	117.8 d	118.3 d	117.8 d	117.8 d	118.3 d	118.1 d	118.1 d
C-3′	144.4 s	145.5 s	145.5 s	145.5 s	145.9 s	145.4 s	145.9 s	145.3 s	144.8 s	146.1 s	144.9 s
C-4′	39.5 t	39.7 t	39.6 t	39.6 t	39.6 t	39.6 t	39.6 t	39.5 t	39.4 t	39.6 t	39.8 t
C-5′	25.9 t	26.0 t	26.0 t	25.9 t	25.9 t	26.0 t	25.9 t	25.8 t	25.8 t	25.8 t	25.6 t
C-6′	123.1 d	123.2 d	123.1 d	123.1 d	123.0 d	123.1 d	123.0 d	122.8 d	122.8 d	123.0 d	123.3 d
C-7′	132.0 s	132.2 s	132.2 s	132.2 s	132.2 s	132.2 s	132.3 s	132.1 s	132.2 s	132.4 s	133.4 s
C-8′	25.6 q	25.7 q	25.7 q	25.6 q	25.6 q	25.7 q	25.7 q	25.5 q	25.5 q	25.6 q	25.7 q
C-9′	16.6 q	16.9 q	16.6 q	16.5 q	16.6 q	16.6 q	16.6 q	16.6 q	16.5 q	16.8 q	16.5 q
C-10′	17.6 q	17.7 q	17.7 q	17.7 q	17.7 q	17.7 q	17.7 q	17.6 q	17.6 q	17.7 q	17.8 q
AcO-Me	20.9 q	23.2 q	21.1 q	21.1 q	21.1 q						
AcO−C═O	169.6 s	169.5 s	169.3 s	169.1 s	170.3 s						
OMe	56.6 q	56.6 q	57.1 q	56.8 q							

[a] 90 MHz. [b] 125 MHz.

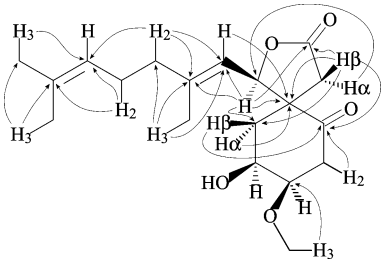


Figure 3. Selected HMBC correlations for **3**.

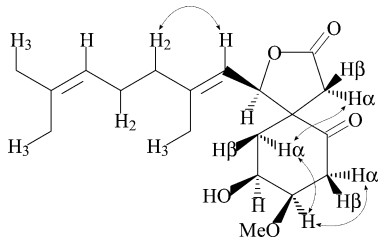


Figure 4. Selected ROESY correlations for **3**.

the methoxy protons to the carbonyl carbon at δ 171.5 (s) confirmed the structure of **20** as [5β-hydroxy-1β-(1α-hydroxy-3,7-dimethyl-octa-2,6-dienyl)-2-oxo-cyclohex-3-enyl]acetic acid methyl ester.

Structures of 21 and 22. Compound **21** (miliusane XIX) also showed no J^3 coupling of H-1′ to the ester carbonyl carbon at δ 172.0 (s) in the HMBC spectrum (Figure 5). Unlike **20**, J^3 long-range correlations between H-1′ and C-5 and between H-5 and C-1′ were clearly observed in **21**, which suggested the formation of a tetrahedrofuran ring between C-1′ and C-5. The ^{13}C NMR signal of C-5 in **21** shifted approximately 10 ppm to downfield in comparison with those of **1**−**20** due to the formation of the tetrahedrofuran ring. Compound **21** was thus determined to be [7α-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxo-6β-oxa-bicyclo[3.2.1]oct-3-en-1-yl]acetic acid methyl ester.

Compound **22** (miliusane XX) is established to be another miliusane with a tetrahedrofuran ring between C-1′ and C-5 by analysis of the NMR data. It differs from **21** by the substitution of a methoxy group to C-4 of the $\Delta^{3,4}$ double bond of **21**. The methoxy group was assigned to an α-orientation due to the spin−spin coupling pattern of H-4 (brtt, J = 5.8, 1.7 Hz) and the presence of ROESY correlations of C-4 methoxy protons to H-3α, 6α, and H-5 (Figure 6). Compound **22** was thus determined to be [4α-methoxy-7α-(2,6-dimethyl-hepta-1,5-dienyl)-2-oxo-6β-oxa-bicyclo[3.2.1]oct-1-yl]acetic acid methyl ester.

Absolute Structures of Miliusanes. The absolute configurations of the miliusanes were determined by preparing Mosher esters[6,7] of **2** and **3**. The hydroxyl groups at C-5 of **2** and **3** were converted to (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) esters to yield **23**−**26**, respectively. Distribution of the positive and negative δ values of the MTPA esters established the chiral centers of both C-1 and -1′ in the *R*-configuration (Table 5).

Successful chemical conversions of **2** to (+)-miliusate (**1**) and (+)-miliusane VIII (**10**), **11** to (+)-miliusol (**2**), **3** to (+)-miliusane VI (**8**), and **4** to (+)-miliusane VII (**9**) confirmed that compounds **1**, **4**, and **8**−**11** possess the same chiral centers at C-1 and -1′ as in **2** and **3**. According to the rules of biogenesis, we presume other miliusanes should also have *R*-configurations at C-1 and -1′. Additionally, the double bond at $\Delta^{2',3'}$ of the miliusanes was established as *E*-configurated due to the observed ROE correlations between H-1′ and H-9′, between H-7β and H-2′, and between H-2′ and H-4′ in the ROESY spectra (Figures 4 and 6).

Proposed Biogenetic Pathway of Miliusanes. The miliusanes belong to a novel class of natural products consisting of 18 carbons in their skeletons. A plausible biogenetic pathway for miliusanes is proposed as shown in Figure 7. An intermediate, 5-oxo-demethyl-miliusane XVIII, appears to be the first miliusane generated by an alkylation reaction between homogentisic acid and geranyl diphosphate. An electrophilic addition between the resonationg isomer (**A**) of homogentisic acid and a resonance-stabilized allylic carbocation would produce an intermediate cation (**B**), which would be quenched by water to form compound **C**. The C-5 carbonyl group in compound **C** would be subsequently reduced to a hydroxyl group to produce compound **D**, which could then be transformed to **2** through the formation of a γ-lactone group between the 1′-OH group and the 7-COOH group. Compounds **3**−**9** could then be produced from either **2** or its acetylated derivative (**1**) through a Michael type nucleophilic addition of a hydroxy

Table 3. ^{1}H NMR Spectral Data of Compounds **12**−**22** (500 or 360 MHz, $CDCl_3$, *J* in Hz)

	δ					
position	**A12/13**[a]	**B13/12**[a]	**A14/15**[a]	**B14/15**[a]	**A16/17**[a]	**B16/17**[a]
H-3	5.98 d (10.3)	5.97 d (10.3)	6.07 dd (10.0, 1.1)	6.06 dd (10.3, 1.0)	6.07 dd (10.2, 1.3)	6.06 dd (10.2, 1.1)
H-3α						
H-3*β*						
H-4	6.85 ddd (10.3, 4.3, 1.2)	6.87 ddd (10.0, 4.2, 1.2)	6.81 ddd (10.2, 4.0, 0.8)	6.81 ddd (10.2, 4.0, 0.8)	6.81 ddd (10.2, 4.2, 1.2)	6.82 ddd (10.0, 4.5, 1.2)
H-5	4.50 brq (4.2)	4.55 brq (4.1)	5.55 brqd (4.3, 1.3)	5.55 brqd (4.3, 1.3)	5.56 brqd (4.0, 1.3)	5.56 brqd (4.0, 1.3)
H-6α	2.20 dd (14.5, 4.6)	2.18 dd (14.8, 5.0)	2.24 dd (14.5, 5.1)	2.24 dd (14.5, 5.1)	2.24 dd (15.0, 5.8)	2.24 dd (15.0, 5.8)
H-6*β*	2.35 ddd (14.4, 4.3, 1.0)	2.34 ddd (14.6, 3.9, 0.9)	2.41 ddd (14.6, 4.3, 1.0)	2.42 ddd (14.6, 4.2, 1.2)	2.41 ddd (14.9, 3.9, 1.2)	2.42 ddd (14.9, 3.7, 1.4)
H-7α	2.29 d (17.4)	2.26 d (17.5)	2.25 d (17.6)	2.24 d (17.9)	2.24 d (17.4)	2.22 d (17.5)
H-7*β*	3.14 d (17.5)	3.20 d (17.6)	3.34 d (17.8)	3.35 d (17.7)	3.35 d (17.5)	3.38 d (17.5)
H-7a						
H-7b						
H-1′	5.59 d (9.7)	5.52 d (10.2)	5.47 d (10.2)	5.48 d (10.1)	5.48 d (9.9)	5.51 d (10.0)
H-2′	5.15 dse[c] (10.1, 1.3)	5.16 dse (9.8, 1.2)	5.18 dse (10.2, 1.3)	5.16 dse (10.2, 1.3)	5.14 dse (10.0, 1.4)	5.14 dse (10.0, 1.4)
H_2-4′	2.17 brt (7.3)	2.12 brt (7.3)	2.16 m	2.11 m	2.06 m	2.00 m
H-4′a						
H-4′b						
H_2-5′/H-5′			1.60 m	1.58 m	1.60 m	1.56 m
H-5′a	1.69 m	1.64 m				
H-5′b	1.55 m	1.52 m				
H-6′	2.58 dd (7.6, 4.2)	2.65 dd (7.4, 5.3)	2.60 t (6.2)	2.64 t (6.3)	3.98 brt (6.6)	3.93 brt (6.3)
H-8′a					4.93 qu[d] (0.9)	4.91 qu (1.1)
H-8′b					4.834 q (1.5)	4.828 q (1.3)
Me-8′	1.276 s	1.281 s	1.29 s	1.29 s		
Me-9′	1.71 d (1.4)	1.73 d (1.0)	1.705 d (1.0)	1.711 d (1.1)	1.693 d (1.6)	1.686 d (1.3)
Me-10′	1.23 s	1.24 s	1.23 s	1.25 s	1.698 s	1.696 s
Ac			2.14 s	2.14 s	2.136 s	2.138 s
OMe						

	δ				
position	**18**[a]	**19**[a]	**20**[a]	**21**[a]	**22**[a]
H-3	6.06 dd (10.1, 1.2)	6.06 dd (10.2, 0.7)	6.14 d (10.2)	6.08 dd (9.5, 0.9)	
H-3α					2.57 brd (16.2)
H-3*β*					2.79 dd (16.3, 6.0)
H-4	6.81ddd (10.0, 4.0, 1.1)	6.81 ddd (10.1, 4.2, 1.2)	6.89 ddd (10.2, 4.7, 1.1)	7.24 ddd (9.5, 5.7, 1.3)	3.77 brtt (5.8, 1.7)
H-5	5.55 brqd (4.1, 1.5)	5.55 brqd (4.5, 1.4)	4.39 brs	4.74 brt (5.3)	4.48 brt (5.0)
H-6α	2.24 dd (14.8, 5.6)	2.24 dd (14.9, 5.8)	2.42 dd (14.9, 5.3)	2.73 d (11.8)	2.48 d (13.2)
H-6*β*	2.40 ddd (14.7, 4.2, 1.0)	2.41 ddd (14.9, 4.2, 1.2)	2.21 ddd (14.8, 3.3, 1.0)	2.04 ddd (11.8, 5.0, 1.20	2.04 brdd (12.7, 5.0)
H-7α	2.25 d (17.7)	2.23 d (17.5)			
H-7*β*	3.33 d (17.8)	3.36 d (17.4)			
H-7a			2.83 d (16.6)	2.79 d (17.1)	2.63 d (17.1)
H-7b			2.23 d (16.8)	2.23 d (17.2)	2.48 d (16.9)
H-1′	5.45 d (9.9)	5.48 d (9.9)	4.53 d (9.8)	4.39 d (9.4)	4.87 d (9.8)
H-2′	5.10 dse (10.1, 1.4)	5.14 dse (10.1, 1.1)	5.31 dse (9.9, 1.1)	5.04 dse (9.6, 1.2)	5.05 dse (9.6, 1.0)
H_2-4′	2.29 brt (7.9)		2.06 m	2.07 m	2.02 m
H-4′a		2.68 ABdd (14.8, 6.7)			
H-4′b		2.64 ABdd (14.8, 6.9)			
H_2-5′/H-5′		5.48 dt (15.5, 6.8)	2.11 m	2.10 m	2.08 m
H-5′a	2.77 dt (16.9, 8.2)				
H-5′b	2.71 dt (17.1, 8.3)				
H-6′		5.59 dt (15.6, 1.1)	5.02 tsep[e] (7.2, 1.4)	5.03 tsep (6.3, 1.4)	5.01 tsep (7.0, 1.3
H-8′a	5.94 brs				
H-8′b	5.78 qu (1.0)				
Me-8′		1.666 s	1.65 d (1.3)	1.66 d (1.2)	1.66 s
Me-9′	1.72 d (1.2)	1.88 d (1.1)	1.69 d (1.5)	1.61 d (1.5)	1.63 d (1.4)
Me-10′	1.86 s	1.662 s	1.59 d (0.7)	1.58 d (1.1)	1.58 s
Ac	2.14 s	2.14 s			
OMe			3.60 s	3.63 s	3.31 s

[a] 500 MHz. [b] 360 MHz. [c] se represents sextet. [d] qu represents quintet. [e] sep represents septet.

group, a methoxy group, or an acetylamide group to an α,*β*-unsaturated ketone. Oxidation of the 5-OH group of **2** would lead to **10**, while the reduction of the C-2 carbonyl carbon of **1** could produce **11**. Moreover, the oxidation of the $\Delta^{6',7'}$ double bond in the side chain of **2** or **1** would lead to their corresponding derivatives (**12**−**19**). Cyclization between the 5-OH and the 1′-OH through the loss of a H_2O molecule in **20** will then result in an altered structural type of miliusane such as **21**, whose demethyl isomer would produce **22** by a Michael nucleophilic addition of a methoxy group to an α,*β*-unsaturated ketone.

Biological Activity of 1−**22.** Isolates **1**−**22** constitute a new class of cytotoxic compounds. The source plant extract was identified through our screening effort in a panel of human cancer cell lines (KB, LNCaP, Lu-1, Col-2, and HUVEC), and compounds **1**−**22** were subsequently isolated by bioassay-directed fractionation using cytotoxicity to KB cells as a monitor.

Table 4. ^{13}C NMR Spectral Data of Compounds **12**−**22** (125 or 90 MHz, $CDCl_3$)

	δ										
position	**A12/13**[a]	**B13/12**[a]	**A14/15**[a]	**B14/15**[a]	**A16/17**[a]	**B16/17**[b]	**18**[a]	**19**[a]	**20**[a]	**21**[a]	**22**[a]
C-1	52.29 s	52.00 s	52.26 s	52.26 s	52.25 s	52.31 s	52.2 s	52.2 s	50.4 s	58.5 s	57.3 s
C-2	196.98 s	196.19 s	194.68 s	194.78 s	194.84 s	194.84 s	194.6 s	194.8 s	201.7 s	200.8 s	208.6 s
C-3	129.04 d	129.09 d	130.83 d	130.83 d	130.85 d	130.85 d	130.8 d	130.8 d	130.8 d	129.5 d	40.6 t
C-4	148.33 d	148.28 d	143.98 d	143.90 d	143.93 d	143.86 d	144.0 d	143.9 d	147.7 d	150.4 d	79.5 d
C-5	62.87 d	63.15 d	64.95 d	64.89 d	64.91 d	64.81 d	64.9 d	64.9 d	62.2 d	72.6 d	75.3 d
C-6	39.76 t	39.54 t	36.50 t	36.50 t	36.50 t	36.46 t	36.5 t	36.5 t	33.7 t	42.5 t	34.5 t
C-7	38.38 t	37.90 t	37.40 t	36.99 t	37.01 t	36.91 t	37.04 t	36.9 t	39.6 t	34.6 t	33.8 t
C-8	174.68 s	174.78 s	174.27 s	174.27 s	174.34 s	174.34 s	174.2 s	174.3 s	171.5 s	172.0 s	174.7
C-1′	82.38 d	81.85 d	80.72 d	80.68 d	80.82 d	80.72 d	80.7 d	80.6 d	73.4 d	75.8 d	78.7 d
C-2′	119.54 d	119.00 d	119.12 d	118.89 d	118.64 d	118.89 d	118.6 d	119.4 d	121.7 d	120.2 d	121.0 d
C-3′	144.12 s	144.40 s	143.55 s	143.73 s	147.25 s	147.14 s	143.6 s	143.2 s	142.2 s	142.1 s	142.1 s
C-4′	37.16 t	36.33 t	36.50 t	36.31 t	35.55 t	35.55 t	33.6 t	42.0 t	39.9 t	39.9 t	40.0 t
C-5′	26.54 t	27.31 t	26.84 t	27.29 t	32.55 t	32.46 t	35.3 t	123.3 t	26.2 t	26.1 t	26.2 t
C-6′	63.97 d	63.70 d	63.48 d	63.56 d	75.17 d	74.91 d	200.7 s	140.9 d	123.6 d	123.7 d	123.7 d
C-7′	58.51 s	58.51 s	58.34 s	58.34 s	144.30 s	144.02 s	144.5 s	70.6 s	132.1 s	132.0 s	132.0 s
C-8′	24.792 q	24.792 q	24.79 q	24.79 q	111.40 t	111.24 t	124.8 t	29.8 q	25.7 q	25.6 q	25.6 q
C-9′	16.61 q	17.28 q	16.73 q	17.05 q	16.84 q	16.91 q	16.9 q	16.9 q	17.1 q	16.7 q	16.4 q
C-10′	17.277 q	17.277 q	18.73 q	18.71 q	17.56 q	17.61 q	17.6 q	29.8 q	17.7 q	17.7 q	17.7 q
AcO-Me	21.00 q	21.00 q	21.01 q	21.01 q	21.0 q	21.0 q					
AcO−C═O	169.66 s	169.66 s	169.67 s	169.67 s	169.6 s	169.7 s					
OMe	51.7 q	51.6 q	57.0 q								

[a] 125 MHz. [b] 90 MHz.

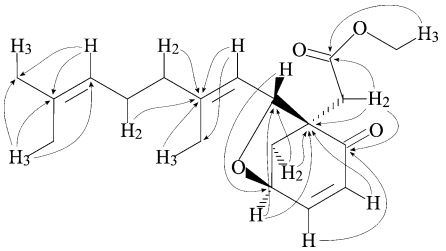


Figure 5. Selected HMBC correlations for **21**.

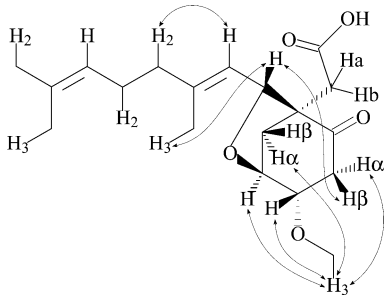


Figure 6. Selected ROESY correlations for **22**.

Among the miliusane derivatives, **1**−**3**, **5**, **20**, and **21** proved to be most cytotoxic (Table 6). Different functional groups affected the cytotoxicity of these compounds to various extents. It is evident from the two pairs of epimeric compounds (**3**/**4** and **5**/**6**) that a significant reduction in cytotoxicity occurred when the 4β-OH was substituted with a 4α-OH.

The introduction of an acetylamide group at C-4 (**7**) rendered the compound nontoxic at 20 μg/mL (IC_{50} > 55 μg/mL). A similar effect was obtained upon the reduction of the carbonyl carbon at C-2 (**1**) to a β-hydroxy group (**11**). The cytotoxic potency also decreased more than 20-fold when the C-5 OH group in **2** was oxidized to a carbonyl group in **10**. The addition of oxy groups in the side chain failed to enhance the cytotoxicity of the miliusanes (**12**−**19**). Interestingly, we have observed that the cytotoxicity was not reduced to any extent when the γ-lactone ring was opened (**20** and **21**). The potent cytotoxic activity of select members of this class of novel and chemically diversified natural product molecules (Table 6) attests to their utility as lead compounds for anticancer drug development.

Compounds **1**−**3** are presently being evaluated in the murine hollow fiber assay that has been validated and applied successfully by the National Cancer Institute as an in vivo screening model to quantitatively define anticancer activity.

Preparation and Biological Activity of Miliusane Derivatives. A semisynthetic effort was initiated in an attempt to improve the biological activity of compound **2**. To that end, 42 ester derivatives were prepared by esterification of the 5-OH group of the compound. The resulting ester derivatives showed in vitro cytotoxicity against our panel of cancer cell lines with IC_{50} values ranging from 0.8 to 18 μM (Table 7).

Among these derivatives, oxy-substituted benzoyl esters (**2ab**, **2ad**−**2ai**) [except for *m*-anisoyl- and piperonyloyl-miliusols (**2ac** and **2aj**)] demonstrated equivalent or marginally better cytotoxic activity than **2** in one or more cell lines. However, it was observed that the nonoxy-substituted benozyl esters, exemplified by benzoyl- and three toluoyl-esters (**2aa** and **2ak**−**2am**) were much less cytotoxic than methoxybenzoyl esters.

The halogenated benzoyl ester derivatives (**2ao**−**2ax**, **2az**, and **2bb**−**2bg**) significantly diminished the cytotoxic activity of **2**, with the exception of 2-fluorobenzoyl-, 4-chlorobenzoyl-, 2,6-dichlorobenzoyl-, and 4-iodobenzoyl-miliusols (**2an**, **2ay**, **2ba**, and **2bh**) that exhibited antitumor activity that is equivalent or marginally superior to that of the parent compound in one or more of the cell lines used.

We have also prepared several nonbenzoyl esters of **2**, but none was observed to enhance cytotoxic activity. Compound **2bk** was observed to be generally equivalent or slightly less cytotoxic than **2**.

The 42 derivatives synthesized failed to produce a clinically significant improvement in the antitumor potential of compound **2**. Nevertheless, the activity profiles of derivatives **2ad**, **2af**, **2ag**, **2ai**, and **2ba** verified that the cytotoxic response was retained or marginally enhanced by our present synthetic approach.

Interestingly, of all of the natural and semisynthetically produced miliusane derivatives that we have obtained, **2bi** is the only compound that demonstrated antitumor selectivity in our panel of cancer cell lines comprising KB, Col2, Lu1, LNCaP, and MCF-7. MCF-7 (IC_{50} = 1.70 μM) was observed to be 9−15-fold more susceptible to **2bi** than the other four

Table 5. ^{1}H NMR Spectral Data of Mosher Esters of Compounds **2** and **3** (500 or 360 MHz, $CDCl_3$, *J* in Hz)

	δ					
position	(*R*)-MTPA ester of **2**	(*S*)-MTPA ester of **2**	$\Delta(\delta_S - \delta_R)$ of **2**	(*R*)-MTPA ester of **3**	(*S*)-MTPA ester of **3**	$\Delta(\delta_S - \delta_R)$ of **3**
H-3	6.04 dd (10.1, 1.2)	6.10 dd (10.1, 1.3)	+0.06			
H-3α				2.51 ddd (12.7, 4.0, 1.0)	2.63 ddd (12.9, 4.3, 1.1)	+0.12
H-3*β*				2.18 t (12.7)	2.45 t (12.8)	+0.27
H-4	6.66 ddd (10.1, 4.2, 1.3)	6.76 ddd (10.1, 4.1, 1.3)	+0.10	3.51 ddd (12.5, 4.1, 2.8)	3.52 ddd (12.6, 4.2, 3.3)	+0.01
H-5	5.83 brdtd (5.5, 3.5, 1.1)	5.80 brdtd (5.6, 3.7, 1.2)	−0.03	5.92 brq (3.6)	5.91 brq (3.3)	−0.01
H-6α	2.32 dd (15.2, 5.7)	2.26 dd (15.1, 5.7)	−0.06	1.72 dd (15.6, 3.4)	1.64 dd (15.8, 3.6)	−0.08
H-6*β*	2.48 ddd (15.2, 3.3, 1.4)	2.38 ddd (15.1, 3.8, 1.2)	−0.10	2.52 dd (15.7, 3.0)	2.36 dd (15.7, 3.2)	−0.16
H-7α	2.18 d (17.7)	2.16 d (17.6)	−0.02	1.96 d (18.1)	1.88 d (18.0)	−0.08
H-7*β*	3.42 d (17.7)	3.37 d (17.7)	−0.05	3.45 d (18.1)	3.40 d (18.2)	−0.05
H-1′	5.33 d (10.0)	5.12 d (10.1)	−0.21	5.57 d (10.2)	4.91 d (10.4)	−0.66
H-2′	4.97 dse[a] (10.1, 1.0)	4.97 dse (10.0, 1.2)	0	4.82 brd (10.0)	4.74 dse (10.2, 1.1)	−0.08
H_2-4′	1.88 m	1.91 m	+0.03	1.85 m	1.88 m	+0.03
H_2-5′	1.94 m	1.97 m	+0.03	1.90 m	1.95 m	+0.05
H-6′	4.91 tsep[b] (6.8, 1.4)	4.92 tsep (6.5, 1.2)	+0.01	4.85 m	4.89 brt (6.5)	+0.04
Me-8′	1.63 d (1.1)	1.64 d (1.0)	+0.01	1.62 s	1.64 d (0.9)	+0.02
Me-9′	1.24 d (1.2)	1.38 d (1.4)	+0.14	1.28 d (1.4)	1.43 d (1.3)	+0.15
Me-10′	1.53 d (1.0)	1.54 d (0.8)	+0.01	1.50 s	1.54 s	+0.04
OH						
OMe	3.55 brs	3.53 brs		3.40 s	3.40 s	
				3.61 brs	3.65 brs	
phenyl	7.38−7.51 m	7.39−7.51 m		7.33−7.57 m	7.36−7.55 m	

[a] se represents sextet. [b] sep represents septet.

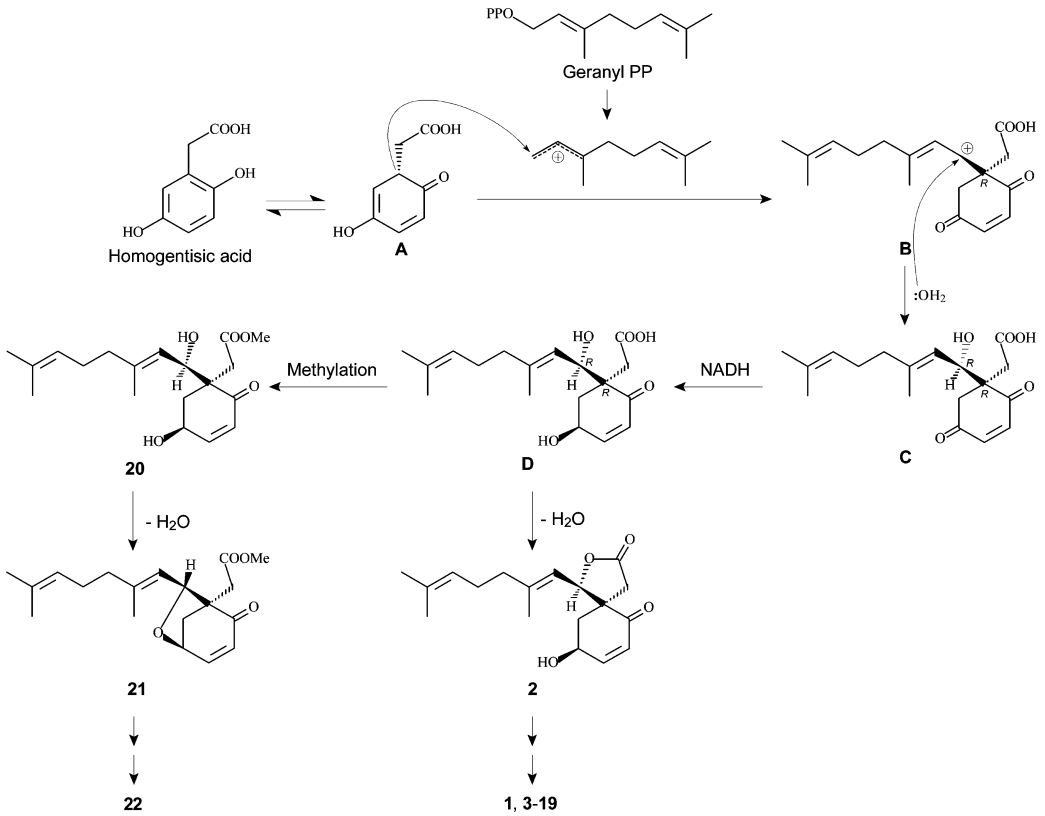


Figure 7. Proposed biogenetic pathway for miliusanes.

cell lines in the panel [IC_{50} = 16.4 (KB), 15.6 (Col2), 25.3 (Lu1), and >26.6 (LNCaP) μM].

In summary, the current paper discusses the bioassay-directed isolation, structure identification/elucidation, and synthesis of miliusol derivatives along with preliminary cytotoxic data. The identified miliusane compounds from *M. sinensis* represent a new class of anticancer agents. Studies defining the molecular target(s) and mechanism of action of miliusanes are currently ongoing. Synthesis of additional miliusane compounds is also currently under way in an attempt to establish structure−activity relationship (SAR), which will facilitate our efforts in search of new miliusanes with the desired biological activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. IR spectra were run on a Jasco FT/IR-410 spectrometer, equipped with a Specac Silver Gate ATR system by applying a film on a Germanium plate. One- and two-dimensional NMR spectra were recorded on a Bruker Avance-500 MHz or a Bruker Avance-360 MHz spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. All NMR data were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on silica gel (200−400 mesh, Natland International Corporation), and reversed-phase flash chromatography was ac-

Table 6. Cytotoxic Activity of Compounds **1**−**22** in Cell Culture[a]

compound	KB	Lu1	Col2	LNCaP	MCF-7	HUVEC	HL60
1	1.18	2.02	1.56	3.18	3.58	2.89	0.32
2	1.18	1.64	1.35	1.78	3.09	1.32	0.66
3	1.40	2.86	2.92	5.06	2.23	1.79	0.45
4	5.45	5.80	9.40	19.64	21.34	6.55	1.73
5	1.18	4.84	4.29	5.06	2.61		0.56
6	32.17	60.43	38.45	>62	15.78		18.66
7	>55	>55	>55	>55	>55		52.29
8	3.97	6.61	4.23	5.29	4.76		
9	5.82	6.16	3.70	5.82	6.08		
10	47.35	63.58	33.44	43.38	26.42	>10.9	
11	>57.4	>57.4	46.01	>57.4	52.56		
12/13	5.22	21.44	8.03	29.56	5.03		3.28
14/15	54.97	9.31	13.43	51.82	12.18		
16/17	5.28	7.46	5.36	27.62	10.06		
18	6.11	19.94	3.89	6.11	6.39		
19	6.71	14.94	9.48	23.95	10.99		
20	3.07	1.82	2.26	2.41	3.01		0.63
21	2.61	1.82	2.01	1.73	2.26		
22	>59	>59	>59	>59	>59		57.01
vinblastine	0.00037	0.011	0.0043	0.000612	0.0026		

[a] Results are expressed as IC_{50} values (concentration required to inhibit cell growth by 50%) in μM, and data were obtained from triplicate experiments. Vinblastine was used as a positive control.

complished with RP-18 silica gel (40−63 μm, EM Science). Reversed-phase HPLC was carried out on a Waters 600E Delivery System pump, equipped with a Waters 996 photodiode detector, and a Watrex GROM-Saphir 110 C18 column (120 Å,12 μm, 300 mm × 40 mm) or a GROM-SIL ODS column (120 Å, 5 μm, 300 mm × 20 mm), which also resulted in extracting UV spectral data of each purified compound. Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of Silica gel 60. HRTOFMS spectra were recorded on a Micromass QTOF-2 spectrometer or a JEOL GCmate II spectrometer. X-ray diffraction data collection was carried out on a Rigaku/MSC RAPID IP area detector equipped with a Cu sealed-tube X-ray source. The direct methods SIR-92 package was used for structure solution,[8] and the WinGX package[9] was used for completing the structure determination. ORTEP[10] was used to generate Figure 1.

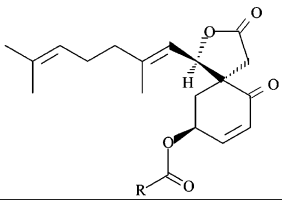
Plant Material. The initial collection of LF + TW + FL sample (SV-0226) of *M. sinensis* Finet and Gagnep (Annonaceae) was made on March 19, 1999 at Cuc Phuong National Park (Vietnam) on a slope northeast of Bong or Park's Center, in a tall primary forest with thick soil cover and occasional exposed limestone rocks (20° 35′ N latitude; 105° 60′ E longitude; 440 m alt.) and was documented by voucher specimens (D.D.S. et al. 10642). A larger amount of the plant sample for the current isolation work, consisting of the same combination of parts (leaves, twigs, and flowers of the plant sample) (SVA-0226, 5.5 kg) was subsequently recollected from plants located in the same area. Duplicate voucher specimens of the initial collection were deposited at the herbaria of Cuc Phuong National Park, Institute of Ecology and Biological Resources of the Vietnamese Academy of Science and Technology in Hanoi, and the John G. Searle Herbarium of the Field Museum of Natural History (Chicago, IL).

Cell Cultures. Human oral epidermoid carcinoma (KB) cell line, human promyelocytic leukemia (HL-60) cell line, human prostate carcinoma (LNCaP) cell line, human breast carcinoma (MCF-7) cell line, human colon carcinoma (Col2) cell line, human lung carcinoma (Lu) cell line, and human umbilical vein endothelial (HUVEC) cell line were obtained from Dr. Heebyung Chai (Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL). Col2 cells were maintained in MEME medium. HUVEC cells were maintained in EGM-2 bullet-kit medium. KB cells were maintained in DMEM medium. HL-60 cells were maintained in RPMI medium. LNCaP cells were maintained in RPMI1640 medium with hormone-free 10% heat-activated FBS (fetal bovine serum) supplemented with 0.1 nM testosterone. Lu1 cells were cultured in MEME medium. MCF-7 cells were maintained in MEME medium containing 10 mg/L insulin. In each case, PSF (100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, and 250 ng/mL amphotericin B) was added. All media were supplemented with 10% heat-inactivated FBS.

Cell Culture Panel Bioassays. Extracts, fractions, and compounds were tested in a KB cell line using established protocols.[11] In addition, all pure compounds were evaluated against the other human cancer cell lines comprising our cytotoxicity screening panel. Cytotoxicity assays involving Col-2, LNCaP, and Lu-1 carcinoma cell lines and HL-60 were performed using sulforhodamine B according to established protocols.[12−14] HUVEC cells were grown in media, and components were supplied in the EGM-2 Bullet-Kit (Clonetics Corporation, Walkersville, MD) with 2% FBS. The HUVEC line constituted a test system to identify samples with potential antiangiogenic activity. MCF-7 cells were maintained and assayed in MEME medium containing 10 mg/L insulin.

Extraction and Isolation. The dried, milled plant material (5.5 kg) was extracted with CH_2Cl_2 to afford 173 g of extract, which was chromatographed over a silica gel column (1.5 kg) and developed by gradient elution with petroleum ether/$CHCl_3$, $CHCl_3$/Me_2CO, and $CHCl_3$/Me_2CO/MeOH solutions to afford 42 fractions [petroleum ether (eluates F1−F4, each 1.5 L); petroleum ether−$CHCl_3$/1:1 (eluates F5−F16, each 2.0 L); $CHCl_3$ (eluates F17−F25, each 2.0 L); $CHCl_3$−Me_2CO/9:1 (eluates F26−F29, each 3.0 L), 8:2 (eluates F30−F32, each 3.0 L); $CHCl_3$−Me_2CO−MeOH/76:19:5 (eluates F33−F36, each 3.0 L), 72:18:10 (eluates F37−F38, each 2.0 L), 65:16:19 (eluates F39−F40, 3.0 L), and 60:13:27 (eluates F40−F42, 4.0 L), respectively]. In vitro bioassay (KB cell line) localized the bioactivity in fractions F13−F19 and F27−F29 with cytotoxic IC_{50} values ranging from 0.4 to 1.3 μg/mL. Fractions F27−F29 (11.5 g) were pooled and chromatographed on a C-18 reverse phase flash column (100 g, gradient elution with Me_2CO and H_2O) to yield 21 fractions [Me_2CO−H_2O/2:8 (eluate F43, 2.0 L), 3:7 (eluates F44−F53, each 1.0 L), 4:6 (eluate F54, each 1.0 L), 5:5 (eluates F55−F63, each 1.0 L), 6:4 (eluates F64−F68, each 1.0 L), 8:2 (eluates F69−F72, each 1.0 L); and Me_2CO (eluate F73, 1.0 L), respectively]. KB active fraction F51 (IC_{50} 0.23 μg/mL) (1.2 g) was subjected to preparative HPLC separation on a GROM-Saphir 110 C18 column (solvent system: MeCN/H_2O 50:50) to afford **2** (672.2 mg), **3** (118.3 mg), **4** (5.9 mg), **5** (6.7 mg), **6** (4.1 mg), **7** (3.6 mg), **10** (4.8 mg), **12**/**13** (3.2 mg), and **22** (1.3 mg). Fractions F13−F19 (25.8 g) were pooled and subjected to a C-18 reverse phase flash column (100 g, gradient elution with Me_2CO and H_2O) to yield 38 fractions [Me_2CO−H_2O/3:7 (eluates F74−F78, each 1.0 L), 4:6 (eluates F79−F84, each 0.5 L), 5:5 (eluates F85−F86, each 0.5 L), 6:4 (eluates F87−F101, each 0.5 L), 8:2 (eluates F102−F109, each 0.5 L), 9:1 (eluate F110, each 1.0 L); and Me_2CO (eluate F111, 1.0 L), respectively]. Bioactive fractions F13−F19 (25.8 g) were pooled and subjected to a C-18

Table 7. Cytotoxic Activity of Derivatives of **2** in Cell Culture[a]



Compound	R	KB	Col2	Lu1	LNCaP	MCF-7	Compound	R	KB	Col2	Lu1	LNCaP	MCF-7
2		1.00	1.00	1.00	1.00	1.00	**2**		1.00	1.00	1.00	1.00	1.00
2aa		2.90	3.56	5.70	2.51	5.13	**2as**		4.37	3.41	8.45	3.88	2.79
2ab		0.96	1.71	0.89	2.37	0.94	**2at**		1.81	3.16	2.10	1.87	1.72
2ac		4.32	2.39	8.06	4.32	3.47	**2au**		5.35	5.06	8.42	3.05	4.70
2ad		0.45	0.75	0.70	1.31	0.45	**2av**		2.81	2.99	8.47	3.24	4.50
2ae		0.84	1.43	0.79	1.05	1.00	**2aw**		4.59	3.53	8.18	3.11	1.94
2af		0.87	0.79	0.80	0.45	0.46	**2ax**		1.67	7.13	10.54	2.20	4.38
2ag		0.79	1.01	0.96	0.67	0.75	**2ay**		1.09	0.63	1.05	1.89	0.68
2ah		0.92	1.05	0.73	0.80	0.84	**2az**		2.01	2.51	2.94	3.36	1.71
2ai		0.70	1.22	0.57	0.49	0.49	**2ba**		0.36	0.43	0.37	0.36	0.52
2aj		2.14	3.11	3.25	2.38	1.57	**2bb**		1.83	3.86	4.39	2.30	1.81
2ak		7.74	5.76	7.86	3.80	4.46	**2bc**		1.40	4.80	6.15	1.36	3.68
2al		3.35	5.37	6.40	2.68	2.06	**2bd**		3.86	3.54	7.72	3.70	1.68
2am		2.75	3.57	3.71	3.36	2.32	**2be**		2.14	6.56	4.23	3.54	4.34
2an		0.87	1.14	1.78	1.59	1.11	**2bf**		2.30	2.04	2.56	0.94	3.26
2ao		2.35	2.78	5.49	2.46	2.31	**2bg**		5.16	5.30	8.88	3.13	3.51
2ap		3.15	3.76	7.58	3.96	2.25	**2bh**		1.00	1.43	1.62	1.98	0.73
2aq		2.92	3.40	6.94	4.99	3.89	**2bi**	MeO-	7.34	8.17	12.70	>6.05	0.82
2ar		3.40	3.13	7.31	3.65	2.48	**2bj**	$Me(CH_2)_4$-	2.45	2.68	2.50	1.54	1.86
2as		4.37	3.41	8.45	3.88	2.79	**2bk**	$Me(CH_2)_9$-	1.52	0.84	0.78	1.07	0.77
2at		1.81	3.16	2.10	1.87	1.72	**2bl**		5.82	4.60	7.90	2.82	3.88
2au		5.35	5.06	8.42	3.05	4.70	**2bm**		2.89	4.48	8.13	3.27	3.34
2av		2.81	2.99	8.47	3.24	4.50	**2bn**		2.01	3.10	4.96	2.75	1.94
2ar		3.40	3.13	7.31	3.65	2.48	**2bo**		1.49	1.73	1.17	1.19	1.12
							2bp		2.19	2.16	**2.73**	3.30	1.56

[a] Bioassay data are expressed relative to IC_{50} value of **2** in the corresponding cell line, and data were obtained from duplicate experiments. Decreasing ratios indicate increasing activity, with a ratio of 1 being equivalent to the cytotoxicity of **2**.

reverse phase flash column (100 g), and fraction F86 (IC_{50} = 0.45 μg/mL) (1.3 g) was subjected to preparative HPLC separation on a GROM-Saphir 110 C18 column (solvent system: MeCN/H_2O 55:45) to afford **1** (746.3 mg), **8** (63.1 mg), **9** (35.1 mg), **11** (3.7 mg), **14**/**15** (1.0 mg), **16**/**17** (2.3 mg), **18** (0.5 mg), **19** (1.6 mg), **20** (1.9 mg), and **21** (5.2 mg).

(+)-Miliusate (1). Crystalline flake, $[\alpha]_D^{20}$ + 72.9° (*c* 5.91, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 223.8 (1.933), 337.6 (0.007) nm. IR (film): ν_{max} 2929, 1782, 1747, 1683, 1376, 1218, 983, 759 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 369.1688 $[M + Na]^+$ (calcd for $C_{20}H_{26}NaO_5$, 369.1678).

X-ray Crystal Structure of 1. A colorless crystal, 0.1 mm on edge, obtained from MeOH. Cell parameters: a = 6.4822(2) Å, b = 8.8824(2) Å, c = 33.9137(11) Å, V = 52.67(1) Å^3, space group $P2_12_12_1$, Z = 4, D_{calcd} = 1.186 g/cm^3, λ = 1.5418 Å, μ(Cu Kα) = 0.683 mm^{-1}, F(000) = 744. A total of 14867 observations were measured yielding 2009 averaged, unique reflections to 2θ = 144.2°; 1912 reflections have intensities greater than 3σ. The structure was refined by full-matrix least-squares on F to $R(3\sigma)$ = 0.0773, R(all) = 0.0826, and GOF = 1.120. Crystallographic data, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 256410. Copies of the information can be obtained, free of charge, on application to CCDC (e-mail: deposit@ccdc.cam.ac.uk).

(+)-Miliusol (2). Colorless gum, $[\alpha]_D^{20}$ + 48.7° (*c* 12.29, $CHCl_3$). UV λmax [AU (absorbance units)] = 240.3 (4.678), 331.6 (0.051) nm. IR (film) ν_{max} 3453 (*br*), 2924, 1777, 1676, 1212, 983, 923.7, 759 cm^{-1}. ^1H and ^{13}C NMR data, see Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 327.1580 $[M+Na]^+$ (calcd for $C_{18}H_{24}NaO_4$, 327.1572).

(+)-Miliusane I (3). Colorless gum, $[\alpha]_D^{20}$ + 47.3° (*c* 10.20, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 208.6 (4.297), 286.4 (0.012) nm. IR (film): ν_{max} 3459 (br), 2929, 1771, 1712, 1176, 1100, 983, 753.1 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 359.1840 $[M + Na]^+$ (calcd for $C_{19}H_{28}NaO_5$, 359.1834).

(+)-Miliusane II (4). Colorless gum, $[\alpha]_D^{20}$ + 50.8° (*c* 1.26, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 191.0 (3.311), 282.0 (0.004) nm. IR (film): ν_{max} 3459 (br), 2929, 1764, 1712, 1176, 1094, 976, 753 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 359.1833 $[M + Na]^+$ (calcd for $C_{19}H_{28}NaO_5$, 359.1834).

(+)-Miliusane III (5). Colorless gum, $[\alpha]_D^{20}$ + 51.5° (*c* 1.86, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 200.4 (3.930), 279.3 (0.023) nm. IR (film): ν_{max} 3453 (br), 2918, 1771, 1712, 1671, 1424, 1176, 1077, 976, 759 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 345.1674 $[M + Na]^+$ (calcd for $C_{18}H_{26}NaO_5$, 345.1678).

(+)-Miliusane IV (6). Colorless gum, $[\alpha]_D^{20}$ + 20.4° (*c* 0.39, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 202.7 (1.733), 278.1 (0.043) nm. IR (film): ν_{max} 3429 (br), 2924, 1765, 1712, 1441, 1235, 1182, 976, 759 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 345.1666 $[M + Na]^+$ (calcd for $C_{18}H_{26}NaO_5$, 345.1678).

(+)-Miliusane V (7). Colorless gum, $[\alpha]_D^{20}$ + 32.2° (*c* 1.99, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 196.9 (1.333), 278.1 (0.060), 377.9 (0.019) nm. IR (film): ν_{max} 3359 (br), 2924, 1771, 1712, 1653, 1535, 1424, 1182, 983, 759 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 386.1932 $[M + Na]^+$ (calcd for $C_{20}H_{29}NNaO_5$, 386.1943).

(+)-Miliusane VI (8). Colorless gum, $[\alpha]_D^{20}$ + 84.0° (*c* 8.42, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 213.3 (1.741), 281.6 (0.015) nm. IR (film): ν_{max} 2924, 1779, 1747, 1718, 1429, 1376, 1224, 1171, 983 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 401.1928 $[M + Na]^+$ (calcd for $C_{21}H_{30}NaO_6$, 401.1940).

(+)-Miliusane VII (9). Colorless gum, $[\alpha]_D^{20}$ + 65.7° (*c* 6.72, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 213.3 (1.676), 292.3 (0.004) nm. IR (film): ν_{max} 2929, 1782, 1747, 1724, 1424, 1371, 1235, 1171, 1106, 988, 759 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HRTOF positive ESIMS *m*/*z* 401.1931 $[M + Na]^+$ (calcd for $C_{21}H_{30}NaO_6$, 401.1940).

(+)-Miliusane VIII (10). Colorless gum, $[\alpha]_D^{20}$ + 99.9° (*c* 1.79, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 200.4 (3.904), 223.3 (1.064), 275.7 (0.041) nm. IR (film): ν_{max} 2929, 1782, 1676, 1212, 1171, 983, 753 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HREIMS *m*/*z* 302.1493 $[M]^+$ (calcd for $C_{18}H_{22}O_4$, 302.1518).

(+)-Miliusane IX (11). Colorless gum, $[\alpha]_D^{20}$ + 27.4° (*c* 0.17, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 207.4 (0.969), 291.1 (0.039) nm. IR (film): ν_{max} 3459 (br), 2929, 1771, 1729, 1376, 1235, 1018, 971, 753 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 1 and 2. HREIMS *m*/*z* 348.1916 $[M]^+$ (calcd for $C_{20}H_{28}O_5$, 348.1937).

(+)-Miliusane X/XI (12/13). Colorless gum, $[\alpha]_D^{20}$ + 96.3° (*c* 0.13, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 192.2 (0.868), 223.0 (0.346), 345.9 (0.005) nm. IR (film): ν_{max} 3435 (br), 2929, 1777, 1683, 1382, 1270, 1206, 983, 753 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 343.1534 $[M + Na]^+$ (calcd for $C_{18}H_{24}NaO_5$, 343.1521).

(+)-Miliusane XII/XIII (14/15). Colorless gum, $[\alpha]_D^{20}$ + 65.1° (*c* 0.09, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 196.9 (0.784), 221.8 (0.479), 325.6 (0.023) nm. IR (film): ν_{max} 2924, 1782, 1741, 1683, 1376, 1221, 983 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 385.1620 $[M + Na]^+$ (calcd for $C_{20}H_{26}NaO_6$, 385.1627).

(+)-Miliusane XIV/XV (16/17). Colorless gum, $[\alpha]_D^{20}$ + 43.3° (*c* 0.11, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 201.6 (1.710), 221.7 (1.340), 335.8 (0.011) nm. IR (film): ν_{max} 3482 (br), 2929, 1782, 1741, 1676, 1376, 1221, 988, 754 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 385.1616 $[M + Na]^+$ (calcd for $C_{20}H_{26}NaO_6$, 385.1627).

(+)-Miliusane XVI (18). Colorless gum, $[\alpha]_D^{20}$ + 42.1° (*c* 0.04, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 198.0 (0.243), 220.3 (0.228), 326.5 (0.004) nm. IR (film): ν_{max} 2924, 1782, 1741, 1676, 1371, 1224, 976 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 383.1473 $[M + Na]^+$ (calcd for $C_{20}H_{24}NaO_6$, 383.1471).

(+)-Miliusane XVII (19). Colorless gum, $[\alpha]_D^{20}$ + 55.1° (*c* 0.09, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 201.6 (1.622), 221.1 (1.139) nm. IR (film): ν_{max} 3465 (br), 2924, 1782, 1741, 1676, 1371, 1218, 971, 753 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 385.1626 $[M + Na]^+$ (calcd for $C_{20}H_{26}NaO_6$, 385.1627).

(+)-Miliusane XVIII (20). Colorless gum, $[\alpha]_D^{20}$ + 75.6° (*c* 0.09, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 205.1 (1.873), 324.4 (0.014) nm. IR (film): ν_{max} 3458 (br), 2958.8, 2924, 1783, 1676, 1441, 1376, 1270, 1212, 1035, 983 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 359.1846 $[M + Na]^+$ (calcd for $C_{19}H_{28}NaO_5$, 359.1834).

(−)-Miliusane XIX (21). Colorless gum, $[\alpha]_D^{20}$ − 32.8 (*c* 0.67, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 213.3 (1.739), 323.2 (0.0147) nm. IR (film): ν_{max} 2918, 1741, 1683, 1435, 1359, 1200, 1182, 1006, 917 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF positive ESIMS *m*/*z* 341.1736 $[M + Na]^+$ (calcd for $C_{19}H_{26}NaO_4$, 341.1729).

(+)-Miliusane XX (22). Colorless gum, $[\alpha]_D^{20}$ + 46.8° (*c* 0.13, $CHCl_3$). UV λ_{max} [AU (absorbance units)] = 199.2 (1.349), 280.1 (0.013) nm. IR (film): ν_{max} 3435 (br), 2929, 1718, 1400, 1206, 1088, 1000, 753 cm^{-1}. ^1H and ^{13}C NMR data: See Tables 3 and 4. HRTOF negative ESIMS *m*/*z* 335.1864 $[M - H]^-$ (calcd for $C_{19}H_{27}O_5$, 335.1859).

Acetylation of 2−4 and 12/13. A sample of **2** (5.11 mg) was placed in a mixture of 1.0 mL each of pyridine and Ac_2O and allowed to react for 40 h at room temperature. The reaction product was evaporated in vacuo to dryness to yield **1** [5.32 mg, $[\alpha]_D^{20}$ + 76.6 (*c* 0.35, $CHCl_3$)]. The same acetylation procedure was applied to **3** (1.24 mg), **4** (1.06 mg), and **12**/**13** (0.79 mg) to yield **8** [1.13 mg, $[\alpha]_D^{20}$ + 82.8 (*c* 0.07, $CHCl_3$)], **9** [0.68 mg, $[\alpha]_D^{20}$ + 60.3 (*c* 0.04, $CHCl_3$)], and **14**/**15** [0.61 mg, $[\alpha]_D^{20}$ + 58.9 (*c* 0.04, $CHCl_3$)], repectively.

Oxidation of 2, 11, and 16/17 with PCC. PCC (39.0 mg) was suspended in CH_2Cl_2 (1.0 mL), and **2** (8.99 mg in 2.0 mL of CH_2-

Cl_2) was rapidly added at room temperature. After 3 h, the reaction mixture was diluted with 10.0 mL of CH_2Cl_2, the solvent was decanted, and the solid residue was washed twice with CH_2Cl_2 (5.0 mL each). The combined solution was evaporated in vacuo to dryness to afford a mixture, which was subjected to preparative HPLC separation on a Watrex GROM-Saphir 110 C18 column (120 Å,12 μm, 300 mm × 40 mm) and eluted with MeCN/H_2O 55:45 to afford **10** [6.33 mg, $[\alpha]_D^{20}$ + 105.3 (*c* 0.40, $CHCl_3$)]. The same oxidation procedure was applied to compound **11** (0.65 mg) and the mixture **16**/**17** (0.58 mg), followed by semipreparative HPLC separation on the GROM-SIL ODS column (120 Å, 5 μm, 300 mm × 20 mm) eluted with a solvent system of MeCN/H_2O 55:45 to yield **1** [0.37 mg, $[\alpha]_D^{20}$ + 67.7 (*c* 0.02, $CHCl_3$)] and **18** [0.26 mg, $[\alpha]_D^{20}$ + 45.3 (*c* 0.02, $CHCl_3$)], respectively.

Preparation of Mosher's Esters of 2 and 3. To a solution of 5.83 mg of **2** in 1.0 mL of dry pyridine was added (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic chloride (18.0 mg, MTPACl). After the mixture was stirred under N_2 at room temperature for 24 h, the reaction product was evaporated to dryness to yield a yellow gum. This gum was purified by preparative HPLC separation on a Watrex GROM-Saphir 110 C18 column (120 Å,12 μm, 300 mm × 40 mm) and eluted with MeCN/H_2O 9:1 to yield (*S*)-MTPA ester (**23**, 4.01 mg). The use of (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetic chloride gave (*R*)-MTPA esters. Workup of the reaction product of **2** (5.25 mg) with (*S*)-MTPACl gave the (*R*)-Mosher's ester (**24**, 6.34 mg). In the same manner, **3** (2.88 mg) also yielded (*S*)-Mosher's ester (**25**, 1.56 mg) by reacting with (*R*)-MTPACl. Compound **3** (7.44 mg) yielded (*R*)-Mosher's ester (**26**, 5.23 mg) by reacting with (*S*)-MTPACl. ^{1}H NMR data of the Mosher esters are prepared in Table 5.

General Method of Preparation of Miliusol Ester Derivatives. The solution of 2.0−5.0 mg of compound **2** (0.0066−0.016 mmol) in 0.5 mL of dry pyridine was pipetted into a solution of selected acyl chloride reagent (0.2 mmol) in 1.0 mL of dry pyridine at 0 °C. The reaction was allowed to proceed at 0 °C for 2 h. The reaction temperature was then raised to room temperature for an additional 16 h, and the reaction product was evaporated in vacuo to dryness to afford a mixture, which was subjected to preparative HPLC separation on a Watrex GROM-Saphir 110 C18 column (120 Å,12 μm, 300 mm × 40 mm) and eluted with MeCN/H_2O 9:1 to afford acyl-miliusol ester. The acyl-miliusol esters were obtained in yields of 14−98%.

Benzoyl-miliusol (2aa). Amount, 0.56 mg; yield, 20.9%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ benzoyl protons [8.03 (2H, d, *J* = 7.0), 7.61 (1H, brt, *J* = 6.9), 7.47 (2H, brt, *J* = 7.3)], 6.98 (1H, brdd, *J* = 10.1, 4.1, H-4), 6.13 (1H, brd, *J* = 9.9, H-3), 5.79 (1H, m, H-5), 5.53 (1H, d, *J* = 10.1, H-1′), 5.18 (1H, brd, *J* = 10.1, H-2′), 4.95 (1H, m, H-6′), 3.35 (1H, d, *J* = 17.5, H-7β), 2.57 (1H, brdd, *J* = 14.8, 4.2, H-6β), 2.37 (1H, d, *J* = 17.7, H-7α), 2.32 (1H, dd, *J* = 14.9, 5.4, H-6α), 2.07 (2H, m, H_2-5′), 2.01 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 1.4, Me-8′), 1.65 (3H, s, Me-9′), 1.55 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 431.1827 $[M + Na]^+$ (calcd for $C_{25}H_{28}NaO_5$, 431.1834).

***o*-Anisoyl-miliusol (2ab).** Amount, 1.04 mg; yield, 36.1%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ anisoyl protons [7.85 (1H, brd, *J* = 7.7), 7.52 (1H, brt, *J* = 6.9), 7.01 (1H, brd, *J* = 7.5), 6.99 (1H, brt, *J* = 8.1)], 6.95 (1H, brdd, *J* = 10.2, 4.4, H-4), 6.08 (1H, brd, *J* = 9.8, H-3), 5.90 (1H, d, *J* = 10.2, H-1′), 5.81 (1H, m, H-5), 5.08 (1H, brd, *J* = 10.5, H-2′), 4.96 (1H, m, H-6′), 3.90 (3H, s, anisoyl OMe), 3.51 (1H, d, *J* = 17.6, H-7β), 2.62 (1H, brdd, *J* = 15.0, 4.5, H-6β), 2.33 (1H, dd, *J* = 15.1, 5.5, H-6α), 2.21 (1H, d, *J* = 17.8, H-7α), 2.00 (2H, m, H_2-5′), 1.95 (2H, m, H_2-4′), 1.65 (3H, s, Me-8′), 1.56 (3H, s, Me-10′), 1.54 (3H, d, *J* = 1.1, Me-9′). HRTOF positive ESIMS *m*/*z* 461.1949 $[M + Na]^+$ (calcd for $C_{26}H_{30}NaO_6$, 461.1940).

***m*-Anisoyl-miliusol (2ac).** Amount, 1.21 mg; yield, 42.0%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ anisoyl protons [7.61 (1H, brd, *J* = 7.6), 7.54 (1H, brs), 7.37 (1H, brt, *J* = 8.0), 7.14 (1H, dd, *J* = 8.4, 2.6)], 6.95 (1H, ddd, *J* = 10.1, 4.3, 0.9, H-4), 6.11 (1H, dd, *J* = 10.0, 1.1, H-3), 5.78 (1H, m, H-5), 5.52 (1H, d, *J* = 9.8, H-1′), 5.16 (1H, brd, *J* = 10.0, H-2′), 4.97 (1H, m, H-6′), 3.84 (3H, s, anisoyl OMe), 3.35 (1H, d, *J* = 17.7, H-7β), 2.54 (1H, brdd, *J* = 15.1, 4.1, H-6β), 2.36 (1H, dd, *J* = 14.9, 5.6, H-6α), 2.31 (1H, d, *J* = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 0.8, Me-8′), 1.65 (3H, d, *J* = 1.4, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 461.1935 $[M + Na]^+$ (calcd for $C_{26}H_{30}NaO_6$, 461.1940).

***p*-Anisoyl-miliusol (2ad).** Amount, 0.63 mg; yield, 21.9%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ anisoyl protons [7.98 (2H, d, *J* = 8.8), 6.93 (2H, brd, *J* = 8.9)], 6.96 (1H, brdd, *J* = 10.2, 4.3, H-4), 6.10 (1H, dd, *J* = 10.1, 0.8, H-3), 5.76 (1H, m, H-5), 5.53 (1H, d, *J* = 10.2, H-1′), 5.16 (1H, brd, *J* = 10.1, H-2′), 4.97 (1H, m, H-6′), 3.87 (3H, s, anisoyl OMe), 3.36 (1H, d, *J* = 17.5, H-7β), 2.53 (1H, brdd, *J* = 14.7, 4.1, H-6β), 2.35 (1H, dd, *J* = 14.7, 5.5, H-6α), 2.30 (1H, d, *J* = 17.6, H-7α), 2.02 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.64 (3H, d, *J* = 1.1, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 461.1943 $[M + Na]^+$ (calcd for $C_{26}H_{30}NaO_6$, 461.1940).

2,4-Dimethoxybenzoyl-miliusol (2ae). Amount, 3.24 mg; yield, 42.1%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ dimethoxybenzoyl protons [7.90 (1H, d, *J* = 8.8), 6.51 (1H, dd, *J* = 8.9, 2.4), 6.47 (1H, d, *J* = 2.3)], 6.96 (1H, ddd, *J* = 10.2, 4.3, 1.3, H-4), 6.06 (1H, dd, *J* = 10.2, 1.2, H-3), 5.94 (1H, d, *J* = 10.0, H-1′), 5.75 (1H, dtd, *J* = 5.2, 3.2, 1.3, H-5), 5.08 (1H, dse, *J* = 10.0, 1.2, H-2′), 4.96 (1H, tsep, *J* = 7.0, 1.4, H-6′), 3.87 (3H, s, dimethoxybenzoyl OMe), 3.85 (3H, s, dimethoxybenzoyl OMe), 3.51 (1H, d, *J* = 17.9, H-7β), 2.59 (1H, ddd, *J* = 15.0, 2.8, 1.3, H-6β), 2.31 (1H, dd, *J* = 15.1, 5.9, H-6α), 2.19 (1H, d, *J* = 17.8, H-7α), 1.99 (2H, m, H_2-5′), 1.94 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 0.9, Me-8′), 1.553 (3H, d, *J* = 1.1, Me-10′), 1.549 (3H, d, *J* = 1.5, Me-9′). HRTOF positive ESIMS *m*/*z* 491.2049 $[M + Na]^+$ (calcd for $C_{27}H_{32}NaO_7$, 491.2046).

2,6-Dimethoxybenzoyl-miliusol (2af). Amount, 2.78 mg; yield, 36.1%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ dimethoxybenzoyl protons [7.31 (1H, t, *J* = 8.5), 6.55 (2H, d, *J* = 8.5)], 6.87 (1H, ddd, *J* = 10.1, 4.4, 1.3, H-4), 6.09 (1H, dd, *J* = 10.1, 1.2, H-3), 5.83 (1H, dtd, *J* = 5.8, 3.5, 1.0, H-5), 5.72 (1H, d, *J* = 10.3, H-1′), 5.02 (1H, dse, *J* = 10.3, 1.2, H-2′), 4.95 (1H, tsep, *J* = 7.0, 1.1, H-6′), 3.82 (6H, s, dimethoxybenzoyl OMe), 3.53 (1H, d, *J* = 17.8, H-7β), 2.68 (1H, ddd, *J* = 15.1, 2.6, 1.3, H-6β), 2.29 (1H, dd, *J* = 15.2, 5.6, H-6α), 2.18 (1H, d, *J* = 17.9, H-7α), 1.97 (2H, m, H_2-5′), 1.91 (2H, m, H_2-4′), 1.64 (3H, d, *J* = 1.2, Me-8′), 1.54 (3H, d, *J* = 0.9, Me-10′), 1.44 (3H, d, *J* = 1.6, Me-9′). HRTOF positive ESIMS *m*/*z* 491.2050 $[M + Na]^+$ (calcd for $C_{27}H_{32}NaO_7$, 491.2046).

3,4-Dimethoxybenzoyl-miliusol (2ag). Amount, 5.17 mg; yield, 67.2%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ dimethoxybenzoyl protons [7.67 (1H, dd, *J* = 8.5, 2.0), 7.52 (1H, d, *J* = 2.0), 6.89 (1H, d, *J* = 8.3], 6.96 (1H, ddd, *J* = 10.2, 3.7, 0.8, H-4), 6.10 (1H, dd, *J* = 10.1, 1.2, H-3), 5.76 (1H, dtd, *J* = 5.5, 4.2, 1.2, H-5), 5.55 (1H, d, *J* = 9.9, H-1′), 5.16 (1H, dse, *J* = 10.1, 1.3, H-2′), 4.97 (1H, tsep, *J* = 6.8, 1.4, H-6′), 3.94 (3H, s, dimethoxybenzoyl OMe), 3.92 (3H, s, dimethoxybenzoyl OMe), 3.37 (1H, d, *J* = 17.3, H-7β), 2.54 (1H, ddd, *J* = 14.9, 4.2, 0.8, H-6β), 2.30 (1H, dd, *J* = 14.9, 5.6, H-6α), 2.18 (1H, d, *J* = 17.6, H-7α), 2.02 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 0.9, Me-8′), 1.64 (3H, d, *J* = 1.5, Me-9′), 1.56 (3H, d, *J* = 1.2, Me-10′). HRTOF positive ESIMS *m*/*z* 491.2043 $[M + Na]^+$ (calcd for $C_{27}H_{32}NaO_7$, 491.2046).

3,5-Dimethoxybenzoyl-miliusol (2ah). Amount, 4.95 mg; yield, 64.3%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ dimethoxybenzoyl protons [7.15 (2H, d, *J* = 2.3), 6.67 (1H, t, *J* = 2.2)], 6.93 (1H, ddd, *J* = 10.1, 3.6, 0.8, H-4), 6.11 (1H, dd, *J* = 10.1, 1.4, H-3), 5.77 (1H, dtd, *J* = 5.6, 4.2, 1.3, H-5), 5.50 (1H, d, *J* = 10.1, H-1′), 5.16 (1H, dse, *J* = 9.8, 1.3, H-2′), 4.97 (1H, tsep, *J* = 6.9, 1.3, H-6′), 3.81 (6H, s, dimethoxybenzoyl OMe), 3.34 (1H, d, *J* = 17.5, H-7β), 2.53 (1H, ddd, *J* = 14.8, 4.2, 0.8, H-6β), 2.35 (1H, dd, *J* = 14.7, 5.8, H-6α), 2.31 (1H, d, *J* = 17.6, H-7α), 2.02 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 1.4, Me-9′), 1.65 (3H, d, *J* = 1.2, Me-8′), 1.56 (3H, d, *J* = 1.2, Me-10′). HRTOF positive ESIMS *m*/*z* 491.2045 $[M + Na]^+$ (calcd for $C_{27}H_{32}NaO_7$, 491.2046).

3,4,5-Trimethoxybenzoyl-miliusol (2ai). Amount, 6.17 mg; yield, 80.2%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ trimethoxybenzoyl protons [7.27 (2H, s)], 6.95 (1H, ddd, *J* = 10.1, 3.8, 1.1, H-4), 6.11 (1H, dd, *J* = 10.1, 1.3, H-3), 5.77 (1H, dtd, *J* = 5.6, 4.3, 1.3, H-5), 5.53 (1H, d, *J* = 10.1, H-1′), 5.16 (1H, dse, *J* = 10.0, 1.3, H-2′), 4.96 (1H, tsep, *J* = 6.9, 1.5, H-6′), 3.91 (3H, s, trimethoxybenzoyl OMe), 3.88 (6H, s, trimethoxybenzoyl OMe), 3.36 (1H, d, *J* = 17.7, H-7β), 2.54 (1H, ddd, *J* = 14.8, 4.4, 1.1, H-6β), 2.36 (1H, dd, *J* = 14.7, 5.9, H-6α), 2.30 (1H, d, *J* = 17.8, H-7α), 2.02 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 1.2, Me-8′), 1.65 (3H, d, *J* = 1.4, Me-9′), 1.56 (3H, d, *J* = 1.2, Me-10′). HRTOF positive ESIMS *m*/*z* 521.2145 $[M + Na]^+$ (calcd for $C_{28}H_{34}NaO_8$, 521.2151).

Piperonyloyl-miliusol (2aj). Amount, 3.15 mg; yield, 42.4%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ piperonyloyl protons [7.64 (1H, dd, *J* = 8.4, 1.8), 7.43 (1H, d, *J* = 1.8), 6.85 (1H, d, *J* = 8.6], 6.93 (1H, ddd, *J* = 10.1, 3.8, 1.0, H-4), 6.10 (1H, dd, *J* = 10.3, 1.5, H-3), 6.05 (2H, s, pieronyloyl OCH_2), 5.74 (1H, dtd, *J* = 5.4, 4.1, 1.3, H-5), 5.48 (1H, d, *J* = 10.1, H-1′), 5.17 (1H, dse, *J* = 10.2, 1.2, H-2′), 4.97 (1H, tsep, *J* = 6.9, 1.4, H-6′), 3.33 (1H, d, *J* = 17.5, H-7β), 2.51 (1H, ddd, *J* = 14.6, 4.4, 0.9, H-6β), 2.34 (1H, dd, *J* = 14.6, 5.6, H-6α), 2.31 (1H, d, *J* = 17.4, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.65 (3H, s, Me-8′), 1.65 (3H, d, *J* = 1.4, Me-9′), 1.56 (3H, d, *J* = 1.0, Me-10′). HRTOF positive ESIMS *m*/*z* 475.1730 $[M + Na]^+$ (calcd for $C_{26}H_{28}NaO_7$, 475.1733).

***o*-Toluoyl-miliusol (2ak).** Amount, 2.43 mg; yield, 87.5%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ toluoyl protons [7.89 (1H, dd, *J* = 7.9, 1.5), 7.44 (1H, td, *J* = 7.7, 1.5), 7.28 (1H, brd, *J* = 7.5), 7.25 (1H, brt, *J* = 7.4)], 6.95 (1H, ddd, *J* = 10.1, 3.8, 1.0, H-4), 6.11 (1H, dd, *J* = 10.1, 1.4, H-3), 5.78 (1H, dtd, *J* = 5.5, 3.7, 1.1, H-5), 5.47 (1H, d, *J* = 10.1, H-1′), 5.16 (1H, dse, *J* = 10.2, 1.1, H-2′), 4.97 (1H, tsep, *J* = 6.8, 1.4, H-6′), 3.32 (1H, d, *J* = 17.7, H-7β), 2.62 (3H, s, toluoyl Me), 2.51 (1H, ddd, *J* = 14.6, 4.6, 1.0, H-6β), 2.37 (1H, dd, *J* = 14.4, 5.7, H-6α), 2.32 (1H, d, *J* = 17.5, H-7α), 2.01 (2H, m, H_2-5′), 1.97 (2H, m, H_2-4′), 1.65 (3H, d, *J* = 1.2, Me-8′), 1.59 (3H, d, *J* = 1.3, Me-9′),1.56 (3H, d, *J* = 0.9, Me-10′). HRTOF positive ESIMS *m*/*z* 445.1989 $[M + Na]^+$ (calcd for $C_{26}H_{30}NaO_5$, 445.1991).

***m*-Toluoyl-miliusol (2al).** Amount, 2.55 mg; yield, 91.8%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ toluoyl protons [7.84 (1H, brs), 7.83 (1H, brd, *J* = 8.7), 7.42 (1H, brd, *J* = 7.7), 7.35 (1H, brt, *J* = 8.6)], 6.95 (1H, ddd, *J* = 10.1, 4.0, 1.1, H-4), 6.11 (1H, dd, *J* = 10.1, 1.4, H-3), 5.79 (1H, dtd, *J* = 5.6, 4.4, 1.4, H-5), 5.52 (1H, d, *J* = 9.8, H-1′), 5.16 (1H, dse, *J* = 9.7, 1.2, H-2′), 4.97 (1H, tsep, *J* = 6.8, 1.4, H-6′), 3.36 (1H, d, *J* = 17.6, H-7β), 2.54 (1H, ddd, *J* = 14.8, 4.3, 1.2, H-6β), 2.40 (3H, s, toluoyl Me), 2.35 (1H, dd, *J* = 14.8, 5.8, H-6α), 2.30 (1H, d, *J* = 17.5, H-7α), 2.02 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 1.3, Me-8′), 1.66 (3H, d, *J* = 1.3, Me-9′), 1.56 (3H, d, *J* = 1.0, Me-10′). HRTOF positive ESIMS *m*/*z* 445.1995 $[M + Na]^+$ (calcd for $C_{26}H_{30}NaO_5$, 445.1991).

***p*-Toluoyl-miliusol (2am).** Amount, 0.84 mg; yield, 30.3%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz) δ toluoyl protons [7.92 (2H, d, *J* = 8.2), 7.26 (2H, d, *J* = 8.3)], 6.96 (1H, brdd, *J* = 10.1, 4.2, H-4), 6.11 (1H, dd, *J* = 10.2, 1.2, H-3), 5.77 (1H, dtd, *J* = 5.2, 3.7, 1.2, H-5), 5.54 (1H, d, *J* = 10.0, H-1′), 5.17 (1H, dse, *J* = 10.0, 0.8, H-2′), 4.98 (1H, m, H-6′), 3.37 (1H, d, *J* = 17.5, H-7β), 2.54 (1H, brdd, *J* = 14.9, 4.2, H-6β), 2.42 (3H, s, toluoyl Me), 2.36 (1H, dd, *J* = 15.1, 5.8, H-6α), 2.30 (1H, d, *J* = 17.8, H-7α), 2.02 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 1.2, Me-8′), 1.64 (3H, d, *J* = 1.2, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 445.1987 $[M + Na]^+$ (calcd for $C_{26}H_{30}NaO_5$, 445.1991).

2-Fluorobenzoyl-miliusol (2an). Amount, 1.00 mg; yield, 35.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz) δ fluorobenzoyl protons [7.96 (1H, brt, *J* = 7.2), 7.57 (2H, m), 7.16 (1H, dd, *J* = 10.3, 8.6)], 6.95 (1H, brdd, *J* = 10.2, 4.2, H-4), 6.12 (1H, dd, *J* = 10.1, 1.2, H-3), 5.83 (1H, m, H-5), 5.61 (1H, d, *J* = 9.8, H-1′), 5.11 (1H, brd, *J* = 10.1, H-2′), 4.97 (1H, m, H-6′), 3.42 (1H, d, *J* = 17.6, H-7β), 2.59 (1H, brdd, *J* = 15.1, 4.6, 1.0, H-6β), 2.36 (1H, dd, *J* = 15.0, 5.2, H-6α), 2.26 (1H, d, *J* = 17.7, H-7α), 2.01 (2H, m, H_2-5′), 1.96 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.64 (3H, d, *J* = 1.5, Me-9′),1.56 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 449.1744 $[M + Na]^+$ (calcd for $C_{25}H_{27}FNaO_5$, 449.1740).

3-Fluorobenzoyl-miliusol (2ao). Amount, 2.14 mg; yield, 76.4%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz) δ fluorobenzoyl protons [7.83 (1H, brd, *J* = 7.7), 7.70 (1H, brd, *J* = 8.2), 7.45 (1H, m), 7.32 (1H, td, *J* = 8.0, 2.6)], 6.94 (1H, ddd, *J* = 10.0, 3.8, 1.0, H-4), 6.13 (1H, dd, *J* = 10.0, 1.1, H-3), 5.79 (1H, dtd, *J* = 5.1, 3.9, 1.1, H-5), 5.46 (1H, d, *J* = 9.9, H-1′), 5.18 (1H, dse, *J* = 9.8, 1.1, H-2′), 4.97 (1H, tsep, *J* = 6.9, 1.0, H-6′), 3.32 (1H, d, *J* = 17.6, H-7β), 2.53 (1H, ddd, *J* = 15.0, 4.1, 0.8, H-6β), 2.38 (1H, dd, *J* = 14.8, 5.5, H-6α), 2.33 (1H, d, *J* = 17.4, H-7α), 2.03 (2H, m, H_2-5′), 2.00 (2H, m, H_2-4′), 1.658 (3H, s, Me-8′), 1.657 (3H, d, *J* = 1.0, Me-9′), 1.56 (3H, d, *J* = 1.0, Me-10′). HRTOF positive ESIMS *m*/*z* 449.1747 $[M + Na]^+$ (calcd for $C_{25}H_{27}FNaO_5$, 449.1740).

4-Fluorobenzoyl-miliusol (2ap). Amount, 2.09 mg; yield, 74.6%; colorless gum. Amount, 2.55 mg; yield, 91.8%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz) δ fluorobenzoyl protons [8.05 (2H, dd, *J* = 8.8, 5.2), 7.14 (2H, t, *J* = 8.8)], 6.94 (1H, ddd, *J* = 10.1, 3.9, 0.8, H-4), 6.12 (1H, dd, *J* = 10.1, 1.4, H-3), 5.77 (1H, dtd, *J* = 5.5, 4.0, 1.4, H-5), 5.48 (1H, d, *J* = 10.0, H-1′), 5.17 (1H, dse, *J* = 9.9, 1.2, H-2′), 4.97 (1H, tsep, *J* = 6.8, 1.4, H-6′), 3.34 (1H, d, *J* = 17.7, H-7β), 2.53 (1H, ddd, *J* = 14.6, 4.5, 0.9, H-6β), 2.36 (1H, dd, *J* = 14.7, 5.5, H-6α), 2.32 (1H, d, *J* = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.66 (3H, d, *J* = 0.5, Me-8′), 1.64 (3H, d, *J* = 1.3, Me-9′), 1.56 (3H, d, *J* = 0.7, Me-10′). HRTOF positive ESIMS *m*/*z* 449.1735 $[M + Na]^+$ (calcd for $C_{25}H_{27}FNaO_5$, 449.1740).

2,3-Difluorobenzoyl-miliusol (2aq). Amount, 1.19 mg; yield, 40.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz) δ difluorobenzoyl protons [7.72 (1H, brd, *J* = 7.7), 7.40 (1H, brt, *J* = 8.4), 7.19 (1H, m)], 6.92 (1H, ddd, *J* = 9.9, 4.0, 0.8, H-4), 6.13 (1H, dd, *J* = 10.3, 1.4, H-3), 5.83 (1H, m, H-5), 5.54 (1H, d, *J* = 9.8, H-1′), 5.12 (1H, dse, *J* = 9.9, 1.1, H-2′), 4.97 (1H, m, H-6′), 3.39 (1H, d, *J* = 17.7, H-7β), 2.57 (1H, ddd, *J* = 14.7, 4.0, 0.9, H-6β), 2.37 (1H, dd, *J* = 14.8, 5.8, H-6α), 2.27 (1H, d, *J* = 17.7, H-7α), 2.00 (2H, m, H_2-5′), 1.97 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.62 (3H, d, *J* = 1.2, Me-9′), 1.56 (3H, s, Me-10′).

2,4-Difluorobenzoyl-miliusol (2ar). Amount, 0.78 mg; yield, 26.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ difluorobenzoyl protons [8.01 (1H, m), 6.99 (1H, m), 6.89 (1H, dd, *J* = 8.6, 2.2], 6.95 (1H, brdd, *J* = 10.3, 3.9, H-4), 6.11 (1H, dd, *J* = 10.2, 1.3, H-3), 5.82 (1H, m, H-5), 5.57 (1H, d, *J* = 9.8, H-1′), 5.11 (1H, dse, *J* = 9.9, 0.8, H-2′), 4.97 (1H, m, H-6′), 3.40 (1H, d, *J* = 17.5, H-7β), 2.56 (1H, ddd, *J* = 15.0, 3.9, 1.1, H-6β), 2.36 (1H, dd, *J* = 15.0, 5.6, H-6α), 2.26 (1H, d, *J* = 17.3, H-7α), 2.02 (2H, m, H_2-5′), 1.97 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.61 (3H, d, *J* = 1.3, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 467.1652 $[M + Na]^+$ (calcd for $C_{25}H_{26}F_2NaO_5$, 467.1646).

2,5-Difluorobenzoyl-miliusol (2as). Amount, 1.62 mg; yield, 55.5%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ difluorobenzoyl protons [7.64 (1H, m), 7.27 (1H, m), 7.15 (1H, td, *J* = 9.2, 4.0)], 6.91 (1H, ddd, *J* = 9.8, 4.5, 1.2, H-4), 6.13 (1H, dd, *J* = 10.1, 1.7, H-3), 5.82 (1H, m, H-5), 5.56 (1H, d, *J* = 9.9, H-1′), 5.12 (1H, dse, *J* = 10.1, 1.2, H-2′), 4.97 (1H, m, H-6′), 3.39 (1H, d, *J* = 17.5, H-7β), 2.56 (1H, ddd, *J* = 14.8, 3.8, 1.0, H-6β), 2.37 (1H, dd, *J* = 15.0, 5.6, H-6α), 2.26 (1H, d, *J* = 17.7, H-7α), 2.01 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.65 (3H, s, Me-8′), 1.62 (3H, d, *J* = 1.1, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS *m*/*z* 467.1649 $[M + Na]^+$ (calcd for $C_{25}H_{26}F_2NaO_5$, 467.1646).

2,6-Difluorobenzoyl-miliusol (2at). Amount, 1.28 mg; yield, 43.8%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, *J* in Hz): δ difluorobenzoyl protons [7.48 (1H, tt, *J* = 8.2, 2.0), 6.99 (2H, brt, *J* = 8.3)], 6.91 (1H, ddd, *J* = 10.0, 4.2, 0.9, H-4), 6.13 (1H, dd, *J* = 10.1, 1.2, H-3), 5.87 (1H, dtd, *J* = 5.0, 3.9, 1.1, H-5), 5.51 (1H, d, *J* = 10.0, H-1′), 5.09 (1H, dse, *J* = 10.1, 0.7, H-2′), 4.96 (1H, tsep, *J* = 6.8, 1.0, H-6′), 3.39 (1H, d, *J* = 17.6, H-7β), 2.56 (1H,

ddd, J = 14.7, 3.7, 1.0, H-6β), 2.37 (1H, dd, J = 14.8, 5.6, H-6α), 2.27 (1H, d, J = 17.6, H-7α), 1.99 (2H, m, H_2-5′), 1.94 (2H, m, H_2-4′), 1.65 (3H, s, Me-8′), 1.55 (3H, s, Me-10′), 1.54 (3H, d, J = 1.4, Me-9′). HRTOF positive ESIMS m/z 467.1652 $[M + Na]^+$ (calcd for $C_{25}H_{26}F_2NaO_5$, 467.1646).

3,4-Difluorobenzoyl-miliusol (2au). Amount, 0.51 mg; yield, 17.5%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ difluorobenzoyl protons [7.83 (2H, m), 7.28 (1H, m)], 6.92 (1H, brdd, J = 9.9, 3.9, H-4), 6.13 (1H, dd, J = 10.1, 1.5, H-3), 5.77 (1H, m, H-5), 5.42 (1H, d, J = 9.8, H-1′), 5.09 (1H, brd, J = 10.3, H-2′), 4.97 (1H, m, H-6′), 3.31 (1H, d, J = 17.6, H-7β), 2.52 (1H, brdd, J = 14.6, 4.2, 1.0, H-6β), 2.39 (1H, dd, J = 14.8, 5.6, H-6α), 2.33 (1H, d, J = 17.4, H-7α), 2.03 (2H, m, H_2-5′), 2.00 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.54 (3H, s, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS m/z 467.1638 $[M + Na]^+$ (calcd for $C_{25}H_{26}F_2NaO_5$, 467.1646).

3,5-Difluorobenzoyl-miliusol (2av). Amount, 2.13 mg; yield, 72.9%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ difluorobenzoyl protons [7.54 (2H, dd, J = 7.7, 2.2), 7.07 (1H, tt, J = 8.4, 2.3)], 6.92 (1H, brdd, J = 10.0, 3.8, H-4), 6.14 (1H, dd, J = 10.1, 1.0, H-3), 5.79 (1H, dtd, J = 5.1, 3.8, 1.0, H-5), 5.39 (1H, d, J = 10.1, H-1′), 5.19 (1H, dse, J = 9.8, 1.4, H-2′), 4.97 (1H, tsep, J = 6.9, 1.2, H-6′), 3.29 (1H, d, J = 17.5, H-7β), 2.50 (1H, ddd, J = 14.6, 4.9, 0.8, H-6β), 2.38 (1H, dd, J = 14.6, 5.4, H-6α), 2.35 (1H, d, J = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 2.00 (2H, m, H_2-4′), 1.67 (3H, d, J = 1.6, Me-9′), 1.66 (3H, s, Me-8′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS m/z 467.1644 $[M + Na]^+$ (calcd for $C_{25}H_{26}F_2NaO_5$, 467.1646).

2-Chlorobenzoyl-miliusol (2aw). Amount, 1.00 mg; yield, 34.3%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ chlorobenzoyl protons [7.80 (1H, dd, J = 8.0, 1.4), 7.47 (2H, m), 7.35 (1H, ddd, J = 7.7, 5.7, 3.0)], 6.95 (1H, ddd, J = 10.2, 4.1, 0.8, H-4), 6.12 (1H, dd, J = 10.1, 1.1, H-3), 5.83 (1H, brqd, J = 4.4, 1.3, H-5), 5.50 (1H, d, J = 10.0, H-1′), 5.12 (1H, dse, J = 9.9, 1.2, H-2′), 4.95 (1H, tsep, J = 6.9, 1.0, H-6′), 3.35 (1H, d, J = 17.6, H-7β), 2.56 (1H, ddd, J = 14.9, 4.4, 0.8, H-6β), 2.38 (1H, dd, J = 14.6, 5.5, H-6α), 2.31 (1H, d, J = 17.7, H-7α), 1.99 (2H, m, H_2-5′), 1.95 (2H, m, H_2-4′), 1.65 (3H, s, Me-8′), 1.55 (3H, s, Me-10′), 1.54 (3H, d, J = 1.2, Me-9′). HRTOF positive ESIMS m/z 465.1441 $[M + Na]^+$ (calcd for $C_{25}H_{27}ClNaO_5$, 465.1445).

3-Chlorobenzoyl-miliusol (2ax). Amount, 1.48 mg; yield, 50.8%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ chlorobenzoyl protons [8.00 (1H, brt, J = 1.8), 7.92 (1H, brdt, J = 7.9, 1.1), 7.59 (1H, ddd, J = 8.2, 2.2, 1.3), 7.42 (1H, t, J = 7.8)], 6.93 (1H, ddd, J = 10.2, 3.8, 1.0, H-4), 6.13 (1H, dd, J = 10.1, 1.1, H-3), 5.80 (1H, dtd, J = 5.2, 4.0, 1.3, H-5), 5.45 (1H, d, J = 10.0, H-1′), 5.18 (1H, dse, J = 10.1, 1.1, H-2′), 4.97 (1H, tsep, J = 6.7, 1.3, H-6′), 3.33 (1H, d, J = 17.7, H-7β), 2.53 (1H, ddd, J = 14.8, 4.7, 1.2, H-6β), 2.36 (1H, dd, J = 14.7, 5.6, H-6α), 2.33 (1H, d, J = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 2.00 (2H, m, H_2-4′), 1.67 (3H, d, J = 1.3, Me-9′), 1.66 (3H, d, J = 0.8, Me-8′), 1.56 (3H, d, J = 0.8, Me-10′). HRTOF positive ESIMS m/z 465.1438 $[M + Na]^+$ (calcd for $C_{25}H_{27}ClNaO_5$, 465.1445).

4-Chlorobenzoyl-miliusol (2ay). Amount, 1.70 mg; yield, 58.3%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ chlorobenzoyl protons [7.97 (2H, d, J = 7.7), 7.45 (2H, d, J = 7.6)], 6.94 (1H, ddd, J = 10.1, 3.8, 0.9, H-4), 6.12 (1H, dd, J = 10.3, 1.5, H-3), 5.77 (1H, brq, J = 4.1, H-5), 5.47 (1H, d, J = 10.1, H-1′), 5.17 (1H, dse, J = 10.0, 1.1, H-2′), 4.97 (1H, tsep, J = 6.7, 1.1, H-6′), 3.34 (1H, d, J = 17.6, H-7β), 2.53 (1H, ddd, J = 14.8, 4.4, 0.9, H-6β), 2.37 (1H, dd, J = 14.7, 5.7, H-6α), 2.32 (1H, d, J = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.64 (3H, d, J = 1.5, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS m/z 465.1439 $[M + Na]^+$ (calcd for $C_{25}H_{27}ClNaO_5$, 465.1445).

2,4-Dichlorobenzoyl-miliusol (2az). Amount, 0.54 mg; yield, 17.2%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ dicloroorobenzoyl protons [7.79 (1H, d, J = 8.3), 7.51 (1H, d, J = 2.0), 7.34 (1H, dt, J = 8.4, 1.9], 6.93 (1H, ddd, J = 10.0, 4.3, 0.9, H-4), 6.12 (1H, dd, J = 10.3, 1.4, H-3), 5.81 (1H, brq, J = 4.5, H-5), 5.46 (1H, d, J = 9.8, H-1′), 5.13 (1H, dse, J = 9.8, 1.2, H-2′), 4.96 (1H, m, H-6′), 3.34 (1H, d, J = 17.8, H-7β), 2.56 (1H, ddd, J = 15.0, 4.3, 0.8, H-6β), 2.38 (1H, dd, J = 15.0, 5.6, H-6α), 2.31 (1H, d, J = 17.6, H-7α), 2.00 (2H, m, H_2-5′), 1.96 (2H, m, H_2-4′), 1.65 (3H, s, Me-8′), 1.59 (3H, d, J = 1.6, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS m/z 499.1060 $[M + Na]^+$ (calcd for $C_{25}H_{26}Cl_2NaO_5$, 499.1055).

2,6-Dichlorobenzoyl-miliusol (2ba). Amount, 0.46 mg; yield, 14.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ dichlorobenzoyl protons [7.35 (3H, m)], 6.93 (1H, ddd, J = 10.2, 4.3, 1.0, H-4), 6.14 (1H, dd, J = 10.2, 1.3, H-3), 5.89 (1H, brq, J = 4.0, H-5), 5.44 (1H, d, J = 10.1, H-1′), 5.06 (1H, dse, J = 10.1, 1.4, H-2′), 4.94 (1H, tsep, J = 6.8, 1.2, H-6′), 3.39 (1H, d, J = 17.6, H-7β), 2.61 (1H, ddd, J = 14.8, 3.7, 1.2, H-6β), 2.39 (1H, dd, J = 15.0, 5.6, H-6α), 2.29 (1H, d, J = 17.7, H-7α), 1.97 (2H, m, H_2-5′), 1.93 (2H, m, H_2-4′), 1.64 (3H, d, J = 0.8, Me-8′), 1.54 (3H, d, J = 0.6, Me-10′), 1.45 (3H, d, J = 1.3, Me-9′). HRTOF positive ESIMS m/z 499.1057 $[M + Na]^+$ (calcd for $C_{25}H_{26}Cl_2NaO_5$, 499.1055).

3,4-Dichlorobenzoyl-miliusol (2bb). Amount, 2.68 mg; yield, 85.4%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ dichlorobenzoyl protons [8.10 (1H, d, J = 2.1), 7.86 (1H, dd, J = 8.1, 2.1), 7.56 (1H, d, J = 8.2)], 7.92 (1H, ddd, J = 10.1, 3.6, 0.5, H-4), 6.13 (1H, dd, J = 10.1, 1.3, H-3), 5.78 (1H, dtd, J = 5.2, 3.8, 1.3, H-5), 5.41 (1H, d, J = 9.8, H-1′), 5.18 (1H, dse, J = 9.8, 1.4, H-2′), 4.97 (1H, tsep, J = 6.9, 1.3, H-6′), 3.30 (1H, d, J = 17.7, H-7β), 2.51 (1H, ddd, J = 14.8, 4.8, 0.9, H-6β), 2.37 (1H, dd, J = 14.7, 5.6, H-6α), 2.34 (1H, d, J = 17.6, H-7α), 2.03 (2H, m, H_2-5′), 2.00 (2H, m, H_2-4′), 1.67 (3H, d, J = 1.4, Me-9′), 1.66 (3H, d, J = 0.8, Me-8′), 1.56 (3H, d, J = 0.8, Me-10′). HRTOF positive ESIMS m/z 499.1062 $[M + Na]^+$ (calcd for $C_{25}H_{26}Cl_2NaO_5$, 499.1055).

3,5-Dichlorobenzoyl-miliusol (2bc). Amount, 3.06 mg; yield, 97.5%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ dichlorobenzoyl protons [7.88 (2H, d, J = 1.9), 7.59 (1H, t, J = 1.9)], 7.90 (1H, dd, J = 10.2, 4.0, H-4), 6.14 (1H, dd, J = 10.2, 1.5, H-3), 5.80 (1H, dtd, J = 5.4, 3.8, 1.4, H-5), 5.37 (1H, d, J = 10.1, H-1′), 5.19 (1H, dse, J = 10.0, 1.2, H-2′), 4.97 (1H, tsep, J = 6.9, 1.3, H-6′), 3.28 (1H, d, J = 17.3, H-7β), 2.50 (1H, ddd, J = 14.6, 4.9, 0.9, H-6β), 2.37 (1H, dd, J = 14.5, 5.3, H-6α), 2.35 (1H, d, J = 17.4, H-7α), 2.04 (2H, m, H_2-5′), 2.01 (2H, m, H_2-4′), 1.69 (3H, d, J = 1.4, Me-9′), 1.65 (3H, d, J = 0.7, Me-8′), 1.56 (3H, d, J = 0.7, Me-10′). HRTOF positive ESIMS m/z 499.1059 $[M + Na]^+$ (calcd for $C_{25}H_{26}Cl_2NaO_5$, 499.1055).

2-Bromobenzoyl-miliusol (2bd). Amount, 1.42 mg; yield, 44.3%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ bromobenzoyl protons [7.75 (1H, m), 7.68 (1H, m), 7.51 (1H, m), 7.38 (1H, m)], 6.96 (1H, ddd, J = 10.1, 4.0, 1.0, H-4), 6.12 (1H, dd, J = 9.9, 1.2, H-3), 5.82 (1H, dtd, J = 5.2, 4.0, 1.1, H-5), 5.49 (1H, d, J = 10.0, H-1′), 5.12 (1H, dse, J = 10.0, 1.3, H-2′), 4.95 (1H, tsep, J = 6.7, 1.1, H-6′), 3.35 (1H, d, J = 17.5, H-7β), 2.58 (1H, ddd, J = 14.9, 4.3, 1.2, H-6β), 2.38 (1H, dd, J = 14.9, 5.7, H-6α), 2.31 (1H, d, J = 17.6, H-7α), 1.99 (2H, m, H_2-5′), 1.95 (2H, m, H_2-4′), 1.65 (3H, d, J = 0.9, Me-8′), 1.55 (3H, d, J = 0.8, Me-10′), 1.52 (3H, d, J = 1.5, Me-9′). HRTOF positive ESIMS m/z 509.0931 $[M + Na]^+$ (calcd for $C_{25}H_{27}BrNaO_5$, 509.0940).

3-Bromobenzoyl-miliusol (2be). Amount, 1.05 mg; yield, 32.8%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ bromobenzoyl protons [8.16 (1H, brt, J = 1.7), 7.97 (1H, ddd, J = 7.9, 1.4, 1.0), 7.74 (1H, ddd, J = 8.2, 1.8, 0.9), 7.36 (1H, t, J = 8.2)], 6.92 (1H, ddd, J = 10.0, 3.6, 0.8, H-4), 6.13 (1H, dd, J = 10.2, 1.4, H-3), 5.80 (1H, dtd, J = 5.3, 3.7, 1.2, H-5), 5.45 (1H, d, J = 10.2, H-1′), 5.18 (1H, dse, J = 9.9, 1.0, H-2′), 4.98 (1H, m, H-6′), 3.33 (1H, d, J = 17.5, H-7β), 2.53 (1H, ddd, J = 14.9, 4.8, 1.1, H-6β), 2.36 (1H, dd, J = 14.8, 5.6, H-6α), 2.33 (1H, d, J = 17.6, H-7α), 2.03 (2H, m, H_2-5′), 2.01 (2H, m, H_2-4′), 1.68 (3H, d, J = 1.3, Me-9′), 1.66 (3H, d, J = 0.8, Me-8′), 1.56 (3H, d, J = 0.6, Me-10′). HRTOF positive ESIMS m/z 509.0947 $[M + Na]^+$ (calcd for $C_{25}H_{27}BrNaO_5$, 509.0940).

4-Bromobenzoyl-miliusol (2bf). Amount, 71.51 mg; yield, 89.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ bromobenzoyl protons [7.86 (2H, d, J = 8.8), 7.58 (2H, d, J =

8.9)], 6.92 (1H, brdd, J = 10.2, 3.7, H-4), 6.09 (1H, dd, J = 10.1, 1.6, H-3), 5.75 (1H, brq, J = 4.1, H-5), 5.42 (1H, d, J = 9.9, H-1′), 5.15 (1H, dse, J = 10.0, 1.2, H-2′), 4.94 (1H, tsep, J = 6.8, 1.4, H-6′), 3.27 (1H, d, J = 17.6, H-7β), 2.49 (1H, brdd, J = 14.8, 4.9, H-6β), 2.36 (1H, dd, J = 14.7, 5.7, H-6α), 2.32 (1H, d, J = 17.5, H-7α), 2.00 (2H, m, H_2-5′), 1.96 (2H, m, H_2-4′), 1.63 (3H, s, Me-8′), 1.62 (3H, d, J = 1.3, Me-9′), 1.53 (3H, d, J = 0.6, Me-10′).^{13}C NMR (360 MHz, $CDCl_3$, J in Hz) δ bromobenzoyl carbons [164.7 (s), 132.0 (d, 2C), 131.1 (d, 2C), 129.0 (s), 127.8 (s)], 194.8 (s, C-2), 174.2 (s, C-8), 144.8 (s, C-3′), 144.0 (d, C-4), 132.0 (s, C-7′), 130.8 (d, C-3), 123.1 (d, C-6′), 118.1 (d, C-2′), 80.9 (d, C-1′), 66.1 (d, C-5), 52.2 (s, C-1), 39.5 (t, C-4′), 37.0 (t, C-7), 36.3 (t, C-6), 25.8 (t, C-5′), 25.8 (q, C-8′), 17.6 (q, C-10′), 16.8 (q, C-9′). HRTOF positive ESIMS m/z 509.0946 $[M + Na]^+$ (calcd for $C_{25}H_{27}BrNaO_5$, 509.0940).

2-Iodobenzoyl-miliusol (2bg). Amount, 6.18 mg; yield, 70.4%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ iodobenzoyl protons [8.00 (1H, dd, J = 8.0, 0.8), 7.75 (1H, ddd, J = 7.9, 1.8), 7.43 (1H, td, J = 7.8, 0.8), 7.20 (1H, td, J = 7.9, 1.7)], 6.99 (1H, brdd, J = 10.3, 3.5, H-4), 6.13 (1H, dd, J = 10.2, 1.2, H-3), 5.81 (1H, brq, J = 4.1, H-5), 5.46 (1H, d, J = 9.9, H-1′), 5.13 (1H, brd, J = 9.9, H-2′), 4.95 (1H, tsep, J = 6.8, 1.1, H-6′), 3.33 (1H, d, J = 17.4, H-7β), 2.59 (1H, brdd, J = 14.4, 4.5, H-6β), 2.39 (1H, dd, J = 14.5, 5.4, H-6α), 2.33 (1H, d, J = 17.6, H-7α), 1.99 (2H, brdd, J = 12.3, 6.4, H_2-5′), 1.94 (2H, brdd, J = 12.2, 4.3, H_2-4′), 1.65 (3H, s, Me-8′), 1.55 (3H, s, Me-10′), 1.53 (3H, d, J = 1.3, Me-9′). HRTOF positive ESIMS m/z 557.0812 $[M + Na]^+$ (calcd for $C_{25}H_{27}INaO_5$, 557.0801).

4-Iodobenzoyl-miliusol (2bh). Amount, 4.27 mg; yield, 48.6%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ iodobenzoyl protons [7.83 (2H, d, J = 8.7), 7.72 (2H, d, J = 8.4)], 6.93 (1H, brdd, J = 10.1, 3.7, H-4), 6.11 (1H, dd, J = 10.1, 1.0, H-3), 5.77 (1H, brq, J = 4.4, H-5), 5.46 (1H, d, J = 9.9, H-1′), 5.17 (1H, dse, J = 10.0, 1.0, H-2′), 4.97 (1H, tsep, J = 6.8, 1.2, H-6′), 3.33 (1H, d, J = 17.8, H-7β), 2.52 (1H, ddd, J = 14.9, 4.4, 0.7, H-6β), 2.36 (1H, dd, J = 14.8, 5.6, H-6α), 2.32 (1H, d, J = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.66 (3H, s, Me-8′), 1.63 (3H, d, J = 1.5, Me-9′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS m/z 557.0807 $[M + Na]^+$ (calcd for $C_{25}H_{27}INaO_5$, 557.0801).

Methoxyacetyl-miliusol (2bi). Amount, 2.48 mg; yield, 40.1%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.80 (1H, brdd, J = 10.0, 4.4, H-4), 6.08 (1H, dd, J = 10.1, 0.9, H-3), 5.68 (1H, m, H-5), 5.46 (1H, d, J = 10.0, H-1′), 5.11 (1H, brd, J = 9.9, H-2′), 4.97 (1H, m, H-6′), 4.09 (2H, s, methoxyacetyl CH_2), 3.46 (3H, s, methoxyacetyl OMe), 3.35 (1H, d, J = 17.6, H-7β), 2.42 (1H, ddd, J = 14.9, 3.8, 0.8, H-6β), 2.28 (1H, dd, J = 14.8, 5.5, H-6α), 2.25 (1H, d, J = 17.7, H-7α), 2.02 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.663 (3H, d, J = 1.1, Me-9′), 1.657 (3H, s, Me-8′), 1.56 (3H, s, Me-10′). HRTOF positive ESIMS m/z 385.1626 $[M + Na]^+$ (calcd for $C_{20}H_{26}NaO_6$, 385.1627).

***n*-Hexanoyl-miliusol (2bj).** Amount, 4.77 mg; yield, 72.1%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.78 (1H, ddd, J = 10.1, 4.0, 1.3, H-4), 6.05 (1H, dd, J = 10.1, 1.2, H-3), 5.57 (1H, dtd, J = 5.4, 4.0, 1.2, H-5), 5.47 (1H, d, J = 10.2, H-1′), 5.11 (1H, dse, J = 10.1, 1.2, H-2′), 4.97 (1H, tsep, J = 6.9, 1.3, H-6′), 3.36 (1H, d, J = 17.8, H-7β), 2.38 (1H, ddd, J = 14.7, 4.2, 1.1, H-6β), 2.36 (2H, td, J = 7.5, 3.1, hexanoyl CH_2), 2.23 (1H, dd, J = 14.6, 5.4, H-6α), 2.23 (1H, d, J = 17.7, H-7α), 2.02 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.67 (3H, d, J = 1.4, Me-9′), 1.66 (3H, d, J = 1.1, Me-8′), 1.56 (3H, d, J = 1.0, Me-10′), 1.37−1.25 (6H, m, hexanoyl CH_2), 0.89 (3H, t, J = 7.2, hexanoyl Me). HRTOF positive ESIMS m/z 425.2301 $[M + Na]^+$ (calcd for $C_{24}H_{34}NaO_5$, 425.2304).

Undecanoyl-miliusol (2bk). Amount, 2.38 mg; yield, 30.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.79 (1H, ddd, J = 10.3, 4.5, 1.2, H-4), 6.05 (1H, dd, J = 10.3, 1.2, H-3), 5.57 (1H, dtd, J = 5.4, 3.8, 1.2, H-5), 5.47 (1H, d, J = 10.0, H-1′), 5.11 (1H, dse, J = 10.1, 1.3, H-2′), 4.97 (1H, tsep, J = 6.9, 1.3, H-6′), 3.36 (1H, d, J = 17.5, H-7β), 2.37 (1H, ddd, J = 14.7, 4.0, 1.2, H-6β), 2.36 (2H, td, J = 7.5, 3.1, undecanoyl CH_2), 2.23 (1H, dd, J = 14.7, 5.4, H-6α), 2.23 (1H, d, J = 17.7, H-7α), 2.02 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.67 (3H, d, J = 1.3, Me-9′), 1.66 (3H, d, J = 1.1, Me-8′), 1.57 (3H, d, J = 1.1, Me-10′), 1.35−1.18 (16H, m, undecanoyl CH_2), 0.86 (3H, t, J = 7.2, undecanoyl Me). HRTOF positive ESIMS m/z 495.3091 $[M + Na]^+$ (calcd for $C_{29}H_{44}NaO_5$, 495.3086).

Cyclopropanecarbonyl-miliusol (2bl). Amount, 1.46 mg; yield, 23.9%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.80 (1H, brdd, J = 10.2, 4.7, H-4), 6.06 (1H, dd, J = 10.1, 1.1, H-3), 5.56 (1H, m, H-5), 5.52 (1H, d, J = 10.1, H-1′), 5.12 (1H, dse, J = 9.9, 1.1, H-2′), 4.98 (1H, m, H-6′), 3.36 (1H, d, J = 17.5, H-7β), 2.62 (1H, brdd, J = 15.1, 5.0, 1.1, H-6β), 2.42 (1H, dd, J = 14.8, 5.6, H-6α), 2.24 (1H, d, J = 17.6, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.69 (3H, d, J = 1.41, Me-9′), 1.66 (3H, s, Me-8′), 1.57 (3H, s, Me-10′), 1.06 (1H, m, cyclopropane CH), 0.83 (4H, m, cyclopropane CH_2). HRTOF positive ESIMS m/z 395.1838 $[M + Na]^+$ (calcd for $C_{22}H_{28}NaO_5$, 395.1834).

Cyclobutanecarbonyl-miliusol (2bm). Amount, 3.33 mg; yield, 52.5%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.80 (1H, ddd, J = 10.0, 4.5, 1.1, H-4), 6.05 (1H, dd, J = 10.1, 1.3, H-3), 5.56 (1H, m, H-5), 5.48 (1H, d, J = 10.1, H-1′), 5.09 (1H, dse, J = 10.2, 1.3, H-2′), 4.97 (1H, tsep, J = m, H-6′), 3.38 (1H, d, J = 17.6, H-7β), 3.19 (1H, qu, J = 8.7, cyclobutane CH), 2.36 (1H, ddd, J = 14.6, 4.2, 1.1, H-6β), 2.22 (1H, dd, J = 14.6, 5.4, H-6α), 2.22 (4H, m, cyclobutane CH_2), 2.21 (1H, d, J = 17.7, H-7α), 2.01 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.93 (2H, m, cyclobutane CH_2), 1.656 (3H, s, Me-8′), 1.652 (3H, d, J = 1.3, Me-9′), 1.56 (3H, d, J = 1.0, Me-10′). HRTOF positive ESIMS m/z 409.1884 $[M + Na]^+$ (calcd for $C_{23}H_{30}NaO_5$, 409.1991).

Cyclopentanecarbonyl-miliusol (2bn). Amount, 1.56 mg; yield, 23.7%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.79 (1H, brdd, J = 10.0, 4.5, H-4), 6.05 (1H, dd, J = 10.1, 1.0, H-3), 5.57 (1H, m, H-5), 5.50 (1H, d, J = 10.1, H-1′), 5.10 (1H, brd, J = 10.0, H-2′), 4.97 (1H, m, H-6′), 3.37 (1H, d, J = 17.5, H-7β), 2.78 (1H, qu, J = 8.0, cyclopentane CH), 2.39 (1H, ddd, J = 14.9, 4.4, 1.1, H-6β), 2.22 (1H, dd, J = 14.7, 5.5, H-6α), 2.21 (1H, d, J = 17.7, H-7α), 2.03 (2H, m, H_2-5′), 1.98 (2H, m, H_2-4′), 1.67 (3H, d, J = 1.0, Me-9′), 1.66 (3H, s, Me-8′), 1.57 (3H, s, Me-10′), 1.50−2.00 (8H, m, cyclopentane CH_2). HRTOF positive ESIMS m/z 423.2151 $[M + Na]^+$ (calcd for $C_{24}H_{32}NaO_5$, 423.2147).

Cyclohexanecarbonyl-miliusol (2bo). Amount, 2.38 mg; yield, 35.0%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ 6.77 (1H, ddd, J = 10.0, 4.4, 0.8 H-4), 6.05 (1H, dd, J = 10.0, 10.9, H-3), 5.56 (1H, m, H-5), 5.48 (1H, d, J = 10.0, H-1′), 5.11 (1H, dse, J = 10.1, 1.2, H-2′), 4.97 (1H, tsep, J = 6.7, 1.2, H-6′), 3.36 (1H, d, J = 17.6, H-7β), 2.35 (1H, brdd, J = 14.8, 4.4, H-6β), 2.31 (1H, tt, J = 11.0, 3.8, cyclohexane CH), 2.22 (1H, dd, J = 15.0, 5.5, H-6α), 2.22 (1H, d, J = 17.8, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.90 (2H, m, cyclohexane CH_2), 1.75 (2H, m, cyclohexane CH_2), 1.67 (3H, d, J = 1.3, Me-9′), 1.66 (3H, s, Me-8′), 1.57 (3H, s, Me-10′), 1.20−1.50 (5H, m, cyclohexane CH_2). HRTOF positive ESIMS m/z 437.2313 $[M + Na]^+$ (calcd for $C_{25}H_{34}NaO_5$, 437.2304).

2-Thiophenecarbonyl-miliusol (2bp). Amount, 2.21 mg; yield, 32.5%; colorless gum. ^{1}H NMR (360 MHz, $CDCl_3$, J in Hz): δ iodobenzoyl protons [7.86 (1H, dd, J = 3.9, 1.2), 7.63 (1H, dd, J = 5.1, 1.2), 7.14 (1H, dd, J = 5.0, 3.8)], 6.91 (1H, brdd, J = 10.1, 3.8, H-4), 6.11 (1H, dd, J = 10.2, 1.1, H-3), 5.77 (1H, brq, J = 4.4, H-5), 5.55 (1H, d, J = 10.0, H-1′), 5.13 (1H, brd, J = 9.9, H-2′), 4.98 (1H, tsep, J = 6.8, 1.2, H-6′), 3.39 (1H, d, J = 17.6, H-7β), 2.55 (1H, brdd, J = 15.0, 4.1, H-6β), 2.34 (1H, dd, J = 14.9, 5.6, H-6α), 2.26 (1H, d, J = 17.8, H-7α), 2.03 (2H, m, H_2-5′), 1.99 (2H, m, H_2-4′), 1.69 (3H, d, J = 1.1, Me-9′), 1.66 (3H, s, Me-8′), 1.57 (3H, s, Me-10′). HRTOF positive ESIMS m/z 437.1387 $[M + Na]^+$ (calcd for $C_{23}H_{26}NaO_5S$, 437.1399).

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Supporting Information Available: One- and two-dimensional NMR spectra of miliusanes (**1**−**22**), ^{1}H NMR spectra of Mosher's esters, and ^{1}H NMR spectral data of miliusol derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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