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Full Papers

Antimycobacterial Compounds from *Piper sanctum*†

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Bioassay-guided chromatographic separation of the antimycobacterial extract of the leaves of *Piper sanctum* afforded 14 new compounds, identified as 2-oxo-12-(3′,4′-methylenedioxyphenyl)dodecane (**1**), 2-oxo-14-(3′,4′-methylenedioxyphenyl)tetradecane (**2**), 2-oxo-16-(3′,4′-methylenedioxyphenyl)hexadecane (**3**), 2-oxo-18-(3′,4′-methylenedioxyphenyl)octadecane (**4**), 2-oxo-14-(3′,4′-methylenedioxyphenyl)-*trans*-13-tetradecene (**5**), 2-oxo-16-(3′,4′-methylenedioxyphenyl)-*trans*-15-hexadecene (**6**), 2-oxo-18-(3′,4′-methylenedioxyphenyl)-*trans*-17-octadecene (**7**), 2-oxo-16-phenyl-*trans*-3-hexadecene (**8**), methyl [6-(10-phenyldecanyl)tetrahydropyran-2-yl]acetate (**9**), methyl 2-(6-tridecyltetrahydro-2*H*-pyran-2-yl)acetate (**10**), methyl 2-(5-tetradecyltetrahydro-2-furanyl)acetate (**11**), 2-oxo-14-(3′,4′-methylenedioxyphenyl)-*trans*-3-tetradecene (**12**), 2-oxo-16-(3′,4′-methylenedioxyphenyl)-*trans*-3-hexadecene (**13**), and 2-oxo-16-phenyl-3-hexadecane (**14**). In addition, *p*-eugenol (**15**), methyleugenol (**16**), *Z*-piperolide (**17**), demethoxyyangonin (**18**), 5,6-dehydro-7,8-dihydromethysticin (**19**), cepharanone B (**20**), piperolactam A (**21**), cepharadione B (**22**), *N*-*trans*-feruloyltyramine (**23**), and *N*-*trans*-(*p*-coumaroyl)tyramine (**24**) were obtained from the anti-TBC stem extract of the plant. GC-MS and HPLC analyses of the essential oils of the leaves and stem revealed that safrol (**25**) was the major component of the oils. Compounds **2**, **3**, **6**, **18**−**21**, and **24** inhibited the growth of *Mycobacterium tuberculosis* when tested by the MABA assay, with MIC values ranging from 4 to 64 μg/mL.

As part of the project Bioactive Agents from Dry Land Biodiversity of Latin America,[1] *Piper sanctum* (Miq.) Schl. (Piperaceae) was selected for bioassay-guided fractionation on the basis of its significant activity against *Mycobacterium tuberculosis* in the Microplate Alamar Blue Assay (MABA).[2,3] Herein, we describe the isolation and structure elucidation of the antimycobacterial constituents from the stem and leaves of *P. sanctum*. In addition, the composition of the essential oils prepared from both parts of the plant was established. This species is very abundant in the south-central region of Mexico, where it is commonly known as "acuyo", "hierba santa", and "hoja santa". The palm-sized, velvety leaves of this anise-scented, bushy perennial species make fragrant wrappers for grilled or steamed fish, as well as for chicken, shrimp, and other Mexican dishes.[4−6] As a home remedy, the leaves prepared as a tea are employed for treating stomach cramps, coughs, bronchitis, tuberculosis, asthma, and colds. As a poultice, they are used for skin irritations and arthritis. The decoction of the roots is highly valued for curing toothaches.[4−6]

Previous phytochemical studies on the roots of *P. sanctum* resulted in the isolation and characterization of aporfinic alkaloids,[7,8] several kawapyrones,[8−12] and a few

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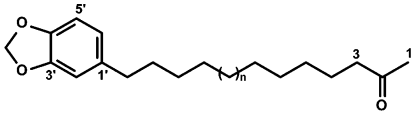
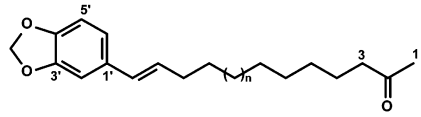
piperolides.[12−16] To our knowledge there is no pharmacological investigation on this plant.

Results and Discussion

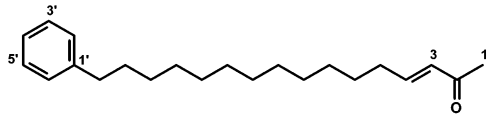
The extract prepared from the leaves inhibited by 99% the growth of *M. tuberculosis* when tested at 100 μg/mL, while the stem extract at the same concentration inhibited by 95% the growth of the microorganism. Bioassay-guided chromatographic separation of the active extract of the leaves afforded 14 new compounds, identified as 2-oxo-12-(3′,4′-methylenedioxyphenyl)dodecane (**1**), 2-oxo-14-(3′,4′-methylenedioxyphenyl)tetradecane (**2**), 2-oxo-16-(3′,4′-methylenedioxyphenyl)hexadecane (**3**), 2-oxo-18-(3′,4′-methylenedioxyphenyl)octadecane (**4**), 2-oxo-14-(3′,4′-methylenedioxyphenyl)-*trans*-13-tetradecene (**5**), 2-oxo-16-(3′,4′-methylenedioxyphenyl)-*trans*-15-hexadecene (**6**), 2-oxo-18-(3′,4′-methylenedioxyphenyl)-*trans*-17-octadecene (**7**), 2-oxo-16-phenyl-*trans*-3-hexadecene (**8**), methyl [6-(10-phenyldecanyl)-tetrahydro-pyran-2-yl]acetate (**9**), methyl 2-(6-tridecyltetrahydro-2*H*-pyran-2-yl)acetate (**10**), methyl 2-(5-tetradecyltetrahydro-2-furanyl)acetate (**11**), 2-oxo-14-(3′,4′-methylenedioxyphenyl)-*trans*-3-tetradecene (**12**), 2-oxo-16-(3′,4′-methylenedioxyphenyl)-*trans*-3-hexadecene (**13**), and 2-oxo-16-phenyl-3-hexadecane (**14**). In addition, *p*-eugenol (**15**),[17]



n= 1 **1**
n= 3 **2**
n= 5 **3**
n= 7 **4**



n= 3 **5**
n= 5 **6**
n= 7 **7**



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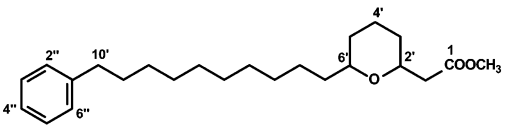

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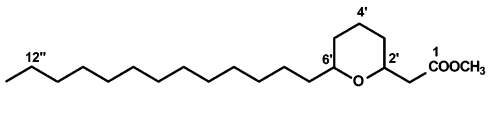

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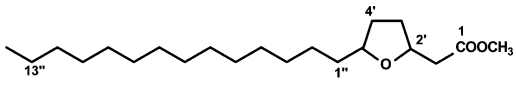

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methyleugenol (**16**),[18] *Z*-piperolide (**17**),[12−16] demethoxyyangonin (**18**),[19,20] 5,6-dehydro-7,8-dihydromethysticin (**19**),[19,20] cepharanone B (**20**),[21] piperolactam A (**21**),[21] cepharadione B (**22**),[21] *N-trans*-feruloyltyramine (**23**),[22] and

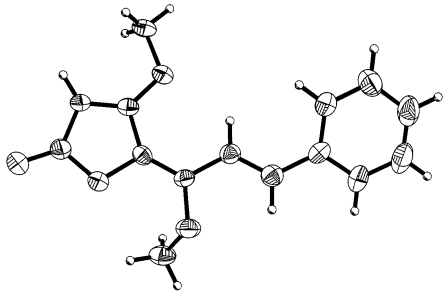
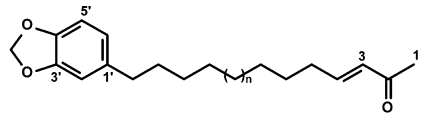
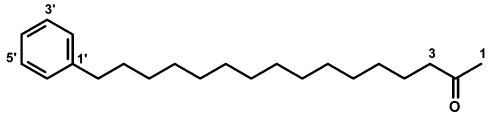


Figure 1. Crystal structure of **17**.

N-trans-(*p*-coumaroyl)tyramine (**24**)[22] were obtained from the active stem extract. The structures of the known compounds were identified by comparison of their spectroscopic data with those values reported previously. Compounds **15**, **16**, **18**, **20**, and **21**−**24** are new to the species. In addition, the structure of *Z*-piperolide (**17**) was confirmed by X-ray analysis (Figure 1). The essential oil of the leaves and the stem were prepared by hydrodistillation. HPLC purification of the oils allowed the isolation of safrol (**25**).[23]



n= 3 **12**
n= 5 **13**



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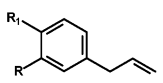
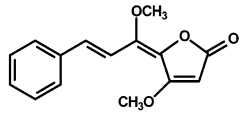
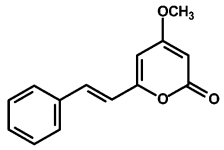
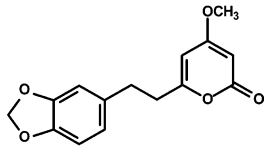




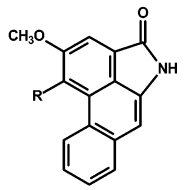
	R	R_1
15	OCH_3	OH
16	OCH_3	OCH_3

17





18 **19**



	R
20	OCH_3
21	OH

Compounds **1**−**4** had the molecular formulas of $C_{19}H_{28}O_3$, $C_{21}H_{32}O_3$, $C_{23}H_{36}O_3$, and $C_{25}H_{40}O_3$, respectively. They exhibited almost identical IR, UV, and NMR spectra, suggesting their close structural relationship. The IR and UV spectra were consistent with an aromatic compound. In all cases the NMR spectra indicated the presence of a piperonyl[24] moiety [δ_H/δ_C ~6.72 (dd, J = 7.8, 0.3 Hz, H-5′)/ ~108.0

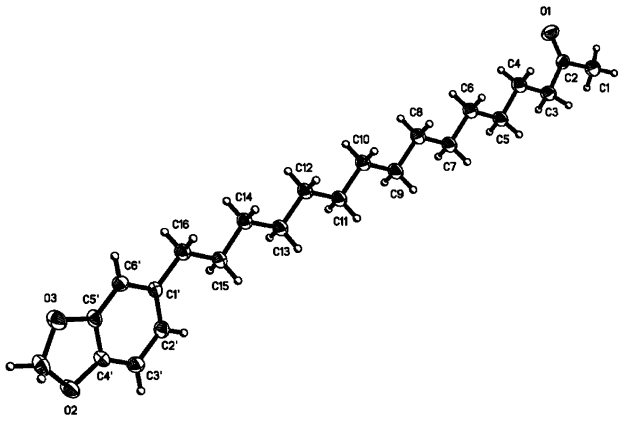


Figure 2. Crystal structure of **3**.

(C-5′); ~6.67 (dd, J = 1.8, 0.3 Hz, H-2′)/~109.0 (C-2′); ~6.61 (dd, J = 8.0, 1.8 Hz, H-6′)/~120.0 (C-6′); ~148.5 (C-3′); ~147.1(C-4′); ~136.2 (C-1′); ~5.91 (s, OCH_2O)/~100.0 (OCH_2O)] attached to an unbranched alkyl chain with a methyl ketone group [δ_H/δ_C ~2.51 (t, J = 7.5 Hz, H-12, H-14, H-16, or H-18)/34.0 (C-12, C-14, C-16, or C-18); 2.41 (t, J = 7.1 Hz, H-3)/42.0 (C-3); ~2.13 (s, CH_3CO)/29.0 (CH_3-CO); 1.52−1.60 and 1.22−1.30 (m, H-4−H-11)/32.0, 26.0, 28.0, 22.8 (C-4−C-11); 209.2 (CO)]. In all cases the substitution pattern of the aromatic ring was confirmed by the correlations observed in the NOESY and HMBC spectra. In addition, the structure of **3** was corroborated by X-ray analysis (Figure 2).

Compounds **5**−**7** were the *trans* dehydro derivates of **2**−**4**, respectively. Their NMR spectra showed important similarities with those of **2**−**4**, differing mainly in the chemical shift of the aromatic hydrogens, which were paramagnetically shifted in **5**−**7** due to the conjugation of the aromatic ring with a double bond. The presence of signals for a conjugated *trans* double bond [δ_H/δ_C ~6.04 (d, J = 15.6 Hz, H-14, H-16, or H-18)/129.3 (C-14, C-16, or C-18); ~6.28 (m, H-13, H-15, or H-17)/129.3 (C-13, C-15, or C-17)] was the second important difference. The fragmentation pattern[24] in the mass spectra (Figure 3) was consistent with the placement of the double bond between C-13/C-14; C-15/C-16, and C-17/C-18 in **5**, **6**, and **7**, respectively.

Compounds **12** and **13** had the composition $C_{21}H_{30}O_3$ and $C_{23}H_{34}O_3$, respectively. The NMR spectra of **12** and **13** were similar to those of **2** and **3** and suggested that they were the *trans* $\Delta^{3,4}$ dehydro derivatives of **2** and **3**, respectively. The most obvious differences between the NMR spectra resulted from the presence of signals for an α,β-unsaturated methyl ketone in **12** and **13** [δ_H/δ_C ~6.82 (dt, J = 15.6, 6.9 Hz, H-4)/148.2 (C-4); ~6.0 (dt, J = 15.6, 1.8 Hz, H-3)/130.0 (C-3); ~2.25 (s, H-1)/~29.0 (CH_3CO); 198.8 (CO)] instead of the absorptions for the saturated methyl ketone moiety in **2** and **3**. The IR absorption frequency for the carbonyl group (1677 cm^{-1}) as well as the mass spectral information were consistent with this proposal.[24]

Compounds **8** and **14** analyzed for $C_{22}H_{34}O$ and $C_{22}H_{36}O$, respectively. The NMR, IR, and MS spectra were similar to those of **13** and **3**, respectively. The analysis of the δ_H 5.0−7.5 and δ_C 100.0−148.0 regions of the NMR spectra revealed the absence of the methylenedioxy signals and the presence of resonances for a monosubstituted benzene ring [δ_H/δ_C 7.1−7.3 (m, H-2′−H-6′)/142.9 (C-1′); 128.4 (C-3′, C-5′); 128.2 (C-2′, C-6′); 125.5 (C-4′)].[25] Thus, **8** and **14** possess a monosubstituted aromatic ring instead of the piperonyl unit of **13** and **3**. The ion at m/z = 91, which arises from a benzylic cleavage followed by the generation of the tropilium ion,[26] further supports the presence of a phenyl ring attached to an alkyl chain. Therefore, **8** and **14** were characterized as 2-oxo-16-phenyl-*trans*-3-hexadecene and 2-oxo-16-phenyl-3-hexadecane, respectively.

The molecular formula of **9** was established as $C_{24}H_{38}O_3$ from the molecular ion peak at m/z 374.2821 in the HREIMS. It was isolated as a colorless optically active oil. The six degrees of unsaturation in this formula could be partially accounted for by one carbonyl group and a benzene ring; hence **9** was bicyclic. Three partial structures could be constructed that fully accounted for all the atoms in **9**. First, a monosubstituted benzene ring moiety attached to a decanyl chain was apparent from the ^1H NMR data [δ_H 7.1−7.3 (m, H-2‴−H-6‴), 2.6 (t, J = 7.8 Hz, H-10″), 1.0−1.8 (m, H-1″−H-9″)]; these protons correlated with the signals at δ_C 142.0 (C-1‴), 128.3 (C-2‴, C-6‴), 128.1 (C-3‴, C-5‴), 125.4 (C-4‴), 36.0 (C-10″), and 23.4−31.5 (C-1″−C-9″), respectively. The characteristic ion at m/z 91 again supported this proposal. The second partial structure consisted of a terminal carboxymethyl unit [δ_H/δ_C 3.6 (s,

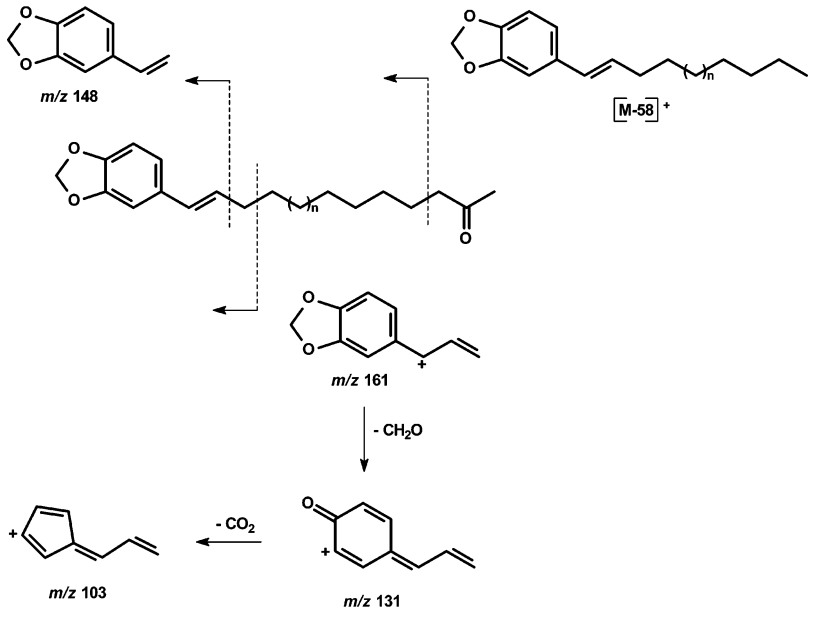


Figure 3. Characteristic fragmentation patterns observed in EIMS of **5**−**7**.

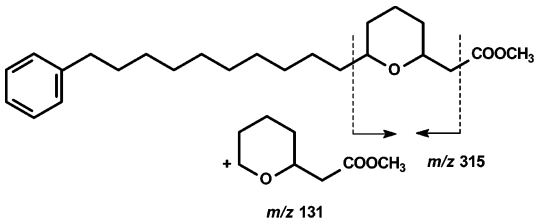


Figure 4. Characteristic fragmentation patterns observed in EIMS of **9**.

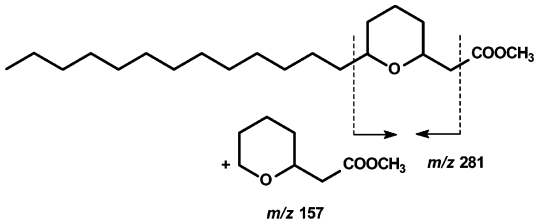


Figure 5. Characteristic fragmentation patterns observed in EIMS of **10**.

OCH_3)/51.5 (OCH_3); 172.0 (C-1)] attached to a diasterotopic methylene group [δ_H/δ_C 2.51 (dd, J = 14.7, 7.8 Hz, H-2b); 2.36 (dd, J = 14.7, 5.7 Hz, H-2a)/41.6 (C-2)]. Finally, the third partial structure was defined as a 2,6-disubstituted tetrahydropyran ring[25] [δ_H/δ_C 3.7 (dddd, J = 13.2, 7.8, 5.7, 1.8 Hz, H-2′)/74.4 (C-2′); 3.2 (tdd, J = 11.1, 4.8, 1.8 Hz, H-6′)/78.0 (C-6′); 1.0−1.8 (m, H-3′−H-5′)/23.4−31.5 (C-3′−C-5′)]. The connection between the three partial structures in **9** was made on the basis of the analysis of the HMBC and mass fragmentation (Figure 4) data. The key HMBC correlations C-2′/H-6′, H-2a, H-2b, H-4′, and H-3′; C-6′/H-5′, H-4′, and H-3′; and C-1/-OMe and H-2′ clearly indicated that the methylene-carboxymethyl moiety was linked to the tetrahydropyran unit at C-2′, whereas the decanylphenyl grouping was attached to C-6′. On the other hand, the strong NOESY interaction between H-2′ and H-6′ revealed the *syn* relationship of both groups and, therefore, the *cis* relative configuration of the tetrahydropyran ring.[25]

Compound **10** was obtained as a colorless, optically active oil and had the composition $C_{21}H_{40}O_3$. The NMR spectra were similar to those of **9**, except for the lack of signals for the phenyl ring. Thus, as in the case of **9**, the NMR data, including NOESY and HMBC information, as well as the fragmentation pattern in the mass spectrum (Figure 5) were consistent with the presence in **10** of a *cis*-2,6-disubstituted tetrahydropyran ring [δ_H/δ_C 3.74 (dddd, J = 10.8, 5.4, 3.0, 1.8 Hz, H-2′)/74.0 (C-2′); 3.27 (tdd, J = 10.7, 7.8, 1.5 Hz, H-6′)/78.0 (C-6′); 1.4−1.6 (m, H-3′−H-5′)/ 22.6−31.9 (C-3′−C-5′)] with a methylene-carboxymethyl unit linked at C-2′. However, **10** possesses at C-6′ a tridecanyl moiety [δ_H/δ_C 0.9 (t, J = 7 Hz, H-13″)/14.0 (C-13″); 1.4−1.6 (m, H-1″−H-12″); 1.2 (br s, H-1″−H-12″)/22.6−31.9 (C-1″−C-12″)] instead of the phenyldecanyl grouping of **9**.

Finally, the structure of **11** was determined as methyl 2-(5-tetradecyltetrahydro-2-furanyl)acetate by the assignment of various NMR data and fragment ion peaks in the EIMS (Figure 6) which were very similar to those of **10**. In this case, however, the 2,6-disubstituted tetrahydropyran unit found in **10** was replaced by a 2,5-disubstituted tetrahydrofuran[27] ring [δ_H/δ_C 4.35 (m, H-2′)/75.0 (C-2′); 4.21 (H-5′)/79.8 (C-5′); 1.4−1.6 (m, H-3′, H-4′)/22.6−31.9 (C-3′, C-4′)] with a *cis* relative configuration according to the H-2′/H-5′ correlation observed in the NOESY spectrum.

GC-MS analysis of the essential oils prepared from the leaves and stems revealed that in both cases the major component was safrol (**25**) (86.75 and 81.11%, respectively).

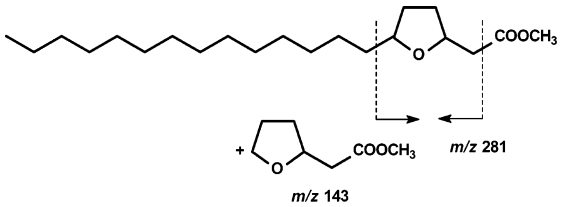
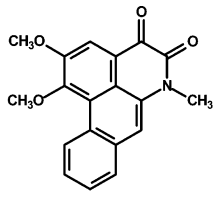


Figure 6. Characteristic fragmentation patterns observed in EIMS of **11**.

Other important constituents identified in both oils were 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene (**26**), myristicin (**27**), methyl 3-phenylpropanoate (**28**), ethyl 3-phenylpropanoate (**29**), and isosafrol (**30**).



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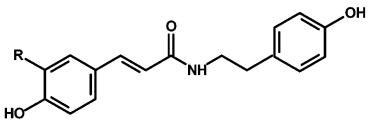
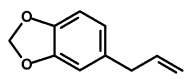


	R
23	OCH_3
24	H



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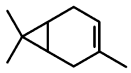

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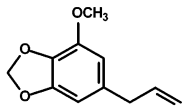

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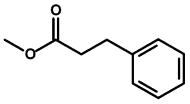

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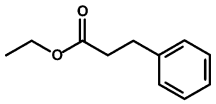

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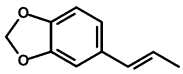

30

Next, the antimycobacterial activity of the principal constituents were examined. As shown in Table 1, the alkaloids **20** and **21**, the kawalactone **19**, as well as **2** and **3** were the most active. The similar activity of **21** and **20** suggests that the free hydroxyl group is not important for activity; however, in the case of **23** and **24**, an extra methoxy group in the *p*-coumaroyl unit lowered the antimycobacterial activity. Of the two kawalactones tested, the dihydro derivative **19** was 4 times more potent than **18**, which is highly conjugated. Finally, of the series **1**−**7**, only **2**, **3**, and **6** were tested, with **2** and **3** showing noted activity. In the Vero cell cytotoxicity assay, compound **19** was the most active, with an IC_{50} value of 41.96 μg/mL. The selectivity index of 10.5 for **19** suggests a nonappreciable toxicity against mammalian cells. The level of activity shown by the isolates is comparable to those previously described for other natural products.[28,29] The use of *P. sanctum* to treat pulmonary diseases, including tuberculosis, could be related with the antimycobacterial properties of the plant demonstrated in this investigation.

Table 1. *M. tuberculosis* Growth Inhibition (MTGI) and Cytotoxicity against Vero Cells of **1**−**24**

compound	MIC (μg/mL) MTGI	IC_{50} (μg/mL) cytotoxicity
1	ND[a]	ND
2	6.25	>102
3	6.25	>102
4, **5**	ND	ND
6	32	ND
7	ND	ND
8−**16**	>128	ND
17	64	>102
18	32	>102
19	4	41.96
20	12	ND
21	8	ND
22	32	>102
23	128	>102
24	32	ND
rifampin	0.25	104.2

[a] ND: not determined due to scarcity of the sample.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Lambda II UV spectrometer in MeOH solution. IR spectra were obtained using KBr disks on a Perkin-Elmer FT 1605 spectrophotometer. NMR spectra, including COSY, NOESY, HMBC, and HMQC experiments, were recorded in $CDCl_3$ or CD_3OD on a Varian Unity INOVA [300 MHz (^{1}H)/75 MHz (^{13}C)] or on a Bruker DMX500 [500 MHz (^{1}H)/125 MHz (^{13}C)] spectrometer using tetramethylsilane (TMS) as an internal standard. Electron-impact mass spectra were registered on a JEOL SX 102 mass spectrometer. Open column chromatography was carried out on silica gel 60 (70−230 mesh, Merck). Analytical and preparative TLCs were performed on precoated silica gel 60 F254 plates (Merck). HPLC was carried out with a Waters HPLC instrument equipped with Waters 996 UV photodiode array detector (900) set at 200−300 nm, using a Nova-Pak HR C_{18} (19 × 300 mm) or μPorasil (19 × 300 mm). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). GC-MS were conducted on a JMX-AX-505H system. The GC column was HP-5MS (30 m × 0.31 mm i.d.). The linear temperature programming was from 60 to 300 °C, at a rate of 20 °C/min, and the carrier gas was He (1 mL/min).

Plant Material. The stems and leaves of *Piper sanctum* (Piperaceae) were collected in May 1999 in San Andrés Tuxtla, State of Veracruz, Mexico. Voucher specimens (GM 423-t and GM 423-h, respectively) have been deposited in the ethnobotanical collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction. Leaves (1.0 kg) and stems (2.95 kg) of *P. sanctum* were separately air-dried, ground, and extracted by maceration with a mixture of CH_2Cl_2−MeOH, 1:1 (12 L × 5, respectively), at room temperature. The extracts were concentrated in vacuo to yield 162.4 and 95 g of dry residues, respectively. Both extracts were evaluated against *Mycobacterium tuberculosis*, and the growth of inhibition as detected by MABA was 99% (leaves) and 95% (stems).

Isolation (leaves extract). The crude extract (160.0 g) was chromatographed over a silica gel (180.0 g) column, eluted with a gradient of hexane−CH_2Cl_2 (10:0 → 0:10) and CH_2Cl_2−MeOH (9:1 → 0:10). Fractions were pooled into eight primary fractions (FL1−FL8) according to their chromatographic profiles observed in the TLC. Fractions FL4 to FL6 were active. The percent of inhibition of *M. tuberculosis* growth as detected by MABA was 100 in all cases.

Active fraction FL4 (4.0 g), eluted with hexane−CH_2Cl_2 (1:1), was subjected to further chromatography on a silica gel (60.0 g) column using gradients of hexane−CH_2Cl_2 (10:0 → 0:10) to afford four secondary fractions (FL4-1−FL4-4). Fraction FL4-3 (1.5 g), eluted with hexane−CH_2Cl_2 (1:1), concentrated the antimycobacterial activity and was further purified by column chromatography on silica gel (60.0 g), eluting with a gradient of hexane−EtOAc (10:0 → 9:1), to yield five tertiary fractions (FL4-3-I−FL4-3-V). Fractions FL4-3-II, FL4-3-III, and FL4-3-IV were found to be active. HPLC separation [Nova-Pak HR C_{18}; acetonitrile−MeOH (80:20); flow rate of 0.3 mL/min] of FL4-3-IV (0.46 g), eluted with hexane−EtOAc (99:1), yielded compounds **1** (2.1 mg; t_R = 10.3 min), **2** (7.0 mg; t_R = 12.8 min), **3** (40.0 mg; t_R = 16.4 min), **4** (2.0 mg; t_R = 19.3 min), **5** (2.1 mg; t_R = 11.6 min), **6** (4.4 mg; t_R = 14.7 min), and **7** (1.0 mg; t_R = 19.6 min) (Figure 7). HPLC purification of FL4-3-II (0.43 g) (Nova-Pak HR C_{18}; acetonitrile; flow rate of 0.3 mL/min), eluted with hexane−EtOAc (99:1), yielded 3.5 mg of **8** (t_R = 20.5 min) and 3.0 mg of **9** (t_R = 14.3 min). Finally, HPLC purification [Nova-Pak HR C_{18}; acetonitrile−MeOH (90:10); flow rate of 7.1 mL/min] of FL4-3-III (0.08 g), eluted with hexane−EtOAc (99:1), resulted in the isolation of 26 mg of **9**.

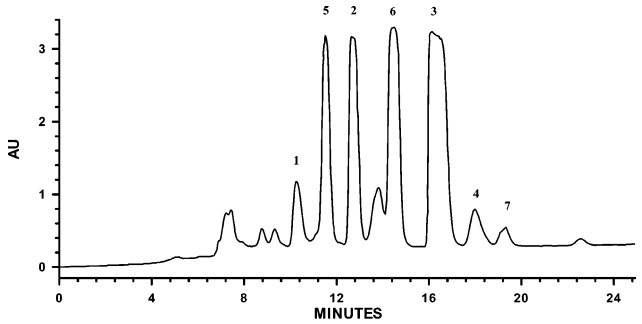

Figure 7. HPLC chromatogram [Nova-Pak HR C_{18}; acetonitrile−MeOH (80:20); flow rate of 0.3 mL/min] of fraction FL4-3-IV containing **1**−**7**.

Primary active fraction FL5 (3.11 g), eluted with hexane−CH_2Cl_2 (4:6), was chromatographed on a silica gel (55.0 g) column, eluting with a gradient of hexane−EtOAc (10:0 → 0:10), to yield six secondary fractions (FL5-1−FL5-6). Column chromatography of active fraction FL5-3 (1.0 g) on Si gel (60.0 g), eluted with gradients of hexane−CH_2Cl_2 (10:0 → 0:10) and CH_2Cl_2−EtOAc (10:0 → 0:10), afforded seven tertiary fractions (FL5-3-I−FL5-3-VII). Preparative TLC on silica gel plates (CH_2Cl_2) of fraction FL5-3-V yielded 16 mg of **10**.

Further column chromatography of the active fraction FL6 (5.5 g), eluted with CH_2Cl_2−MeOH (1:9) and CH_2Cl_2, on silica gel (60.0 g), eluting with a gradient of hexane−EtOAc (10:0 → 9:1), afforded nine secondary fractions (FL6-I−F6-IX). Preparative TLC on silica gel [hexane−EtOAc (9:1)] of active fraction FL6-III (113 mg), eluted with hexane−EtOAc (98:2), yielded 25 mg of a mixture, which was further purified by reverse-phase HPLC [Nova-Pak HR C_{18}; acetonitrile−MeOH (90:10); flow rate of 0.3 mL/min] to yield 20 mg of **11**. Column chromatography of active fraction FL6-IV (0.9 g), eluted with hexane−EtOAc (98:2), on Si gel (50.0 g), eluting with a gradient of hexane−EtOAc (10:0 → 9:1) and EtOAc−MeOH (10:0 → 5:5), afforded seven tertiary fractions (FL6-IV-I−FL6-IV-VII). Further purification of fraction FL6-IV-IV, eluted with hexane−EtOAc (98:2), by HPLC [μPorasil; hexane−EtOAc (95:5); flow rate of 0.3 mL/min], resulted in the isolation of 2.0 mg of **12** (t_R = 22.5 min) and 2.0 mg of **13** (t_R = 23.5 min). Preparative TLC, EtOAc−MeOH (95:5), of fraction FL6-V-VI, eluted with hexane−EtOAc (9:1), yielded 20 mg of **14**.

Isolation (stem extract). The crude extract (94 g) was chromatographed over a silica gel (1.2 kg) column, eluted with a gradient of hexane−EtOAc (10:0 → 0:10) and EtOAc−MeOH (9:1 → 0:10). Fractions were pooled into 10 primary fractions (FS1−FS10) according to their chromatographic profiles observed in the TLC. Fractions FS2, FS3, FS5, and FS7 were active. The percent of inhibition of *M. tuberculosis* growth as detected by MABA was 99, 99, 99, and 100%, respectively.

Further column chromatography of the active fraction FS2 (0.95 g), eluted with hexane−EtOAc (8:2), on silica gel (74.0

g), eluting with a gradient of hexane−CH_2Cl_2 (10:0 → 0:10) and CH_2Cl_2−MeOH (10:0 → 5:5), afforded eight secondary fractions (FS2-I −FS2-VIII). Preparative TLC (CH_2Cl_2) of fraction FS2-V, eluted with hexane−CH_2Cl_2 (9:1), yielded 39.1 mg of *p*-eugenol (**15**), 59.6 mg of methyleugenol (**16**), and 85 mg of **3**.

Fraction FS5 (4.0 g), eluted with hexane−EtOAc (5:5 → 4:6), was further fractionated by column chromatography on silica gel (74.0 g), using a gradient of hexane−CH_2Cl_2 (10:0 → 0:10) and CH_2Cl_2−MeOH (10:0 → 4:6), to yield six secondary fractions (FS5-I−FS5-VI). Fractions FS5-III and FS5-V [eluted with hexane−CH_2Cl_2 (3:7) and hexane−CH_2Cl_2 (1:9), respectively] were active. From fraction FS5-III (600 mg) 453 mg of *Z*-piperolide (**17**) crystallized spontaneously, mp 102−103 °C. The mother liquors of fraction FS5-III were subjected to HPLC [μPorasil; hexane−*i*PrOH−MeOH (85:7.5:7.5); flow rate of 7.0 mL/min] to yield 10.2 mg of demethoxyyangonin (**18**) as white crystals (MeOH), t_R = 19.0 min, mp 136−137 °C. Preparative TLC (EtOAc) of fraction FS5-V (70 mg) yielded 6 mg of 5,6-dehydro-7,8-dihydromethysticin (**19**) as yellow crystals (MeOH), mp 150 °C, as well as 5 mg of cepharanone B (**20**), yellow needles (MeOH), mp 264 °C.

From active fraction FS7 (3.29 g), eluted with hexane−EtOAc (2:8), 96 mg of a yellow powder precipitated. The mother liquors were inactive, while the powder showed anti-TBC activity. Preparative TLC [$CHCl_3$−MeOH (9:1)] of this powder afforded 75 mg of cepharadione B (**22**), mp > 300 °C.

From inactive fraction FS8 (3.8 g), 100 mg of a vitreous brown solid precipitated. Preparative TLC [CH_2Cl_2−MeOH (9:1)] of this solid yielded 4 mg of piperolactam A (**21**), mp > 300 °C, 12 mg of *N-trans*-feruloyltyramine (**23**), mp 94 °C, and 13.4 mg of *N-trans*-(*p*-coumaroyl)tyramine (**24**), mp 248 °C.

Essential Oils from the Stems and Leaves. The essential oils of the leaves and stem were prepared by water distillation from 150 g of plant material, yielding 1.35 and 0.27 g, respectively. HPLC separation [Nova-Pak HR C_{18}; acetonitrile−H_2O (70:30); flow rate of 0.3 mL/min] of the oil (150 mg) from the leaves allowed the isolation of **25** (t_R = 12.0, 8 mg). The t_R (min)/percentage composition (%, uncorrected) of the components of the leaves oil are 7.5/86.75 for **25**, 5.4/0.64 for **26**, 9.2/0.49 for **27**, and 9.3/0.50 for **24**. The t_R (min)/percentage composition (%, uncorrected) of the components of the stems oil are 9.8/81.11 for **25**, 6.4/0.29 for **26**, 11.4/1.8 for **27**, 10.4/5.62 for **29**, and 10.2/2.79 for **30**. Major constituents of the essential oils were identified by matching their 70 eV mass spectra with reference libraries. In the case of safrol (**25**) the identity was established by comparison with an authentic sample.

Antimycobacterial Activity. Activity of the crude extract, fractions, and isolated compounds was determined against *M. tuberculosis* H37Rv (ATCC 27294) in the Microplate Alamar Blue Assay (MABA) as previously described.[2] Percentage inhibition for crude extracts and fractions was defined as 1 − (test well fluorescence units/mean fluorescence units triplicate wells containing only bacteria) × 100. The MIC was defined as the lowest drug concentration that affected an inhibition of ≥90% relative to untreated cultures.

Cytotoxicity Assay. Cytotoxicity against Vero cells (ATCC-CCCL-81) in the CellTiter 96 aqueous nonradioactive cell proliferation assay was determined as previously described.[3] The IC_{50} is defined as the reciprocal dilution resulting in 50% inhibition of the Vero cells. Maximum cytotoxicity (100%) was determined by lysing the cells with sodium dodecyl sulfate (Sigma Chemical Co., St. Louis, MO). The selectivity index for **19** was calculated by dividing the IC_{50} value with the MIC value.

X-ray Crystal Structure Determination of 3 and 17.[30] Crystal data of **17**: $C_{15}H_{14}O_4$, MW 258.26, orthorhombic, *Pbca*; a = 8.068(1) Å, b = 7.516(2) Å, c = 44.228(9) Å, V = 2681.9(10) Å^3, Z = 8, $F(000)$ = 1088, D_{calc} = 1.279 g cm^{-3}, absorption coefficient = 0.093 mm^{-1}. A total of 4712 reflections, of which 2356 were independent, were measured at room temperature from a 0.44 × 0.20 × 0.10 mm light yellow parallelpiped crystal using a Siemens P4/PC diffractometer equipped with graphite-monochromated Mo Kα radiation (λ = 1.54178 Å).

Crystal data of **3**: $C_{23}H_{36}O_3$, MW 360.52, triclinic, $P\bar{1}$ with unit cell parameters (at 25 °C) a = 5.4481(14) Å, b = 5.5186(4) Å, c = 35.294(2) Å, V = 1048.1(1) Å^3, Z = 2, $F(000)$ = 396, D_{calc} = 1.114 g cm^{-3}, absorption coefficient = 0.073 mm^{-1}. A total of 12 548 reflections, of which 3714 were independent, were measured at room temperature from a 0.112 × 0.31 × 0.33 mm colorless prism using a Bruker Smart Apex CCD diffractometer equipped with graphite-monochromated Mo Kα radiation (λ = 1.54178 Å).

The structure of each compound was determined by direct methods (SIR-92 or SIR-97).[31] All atoms except hydrogens were refined anisotropically by full-matrix least-squares methods on F^2 using SHELXL97[32] to give a final R-factor of 0.0709 (R_w = 0.1509 for all data) with a data-restraints-parameters ratio of 2356/0/174 in the case of **17**, and a final R-factor of 0.0600 (R_w = 0.1056 for all data) with a data-restraints-parameters ratio of 3714/0/236 in the case of **3**.

2-Oxo-12-(3′,4′-methylenedioxyphenyl)dodecane (1): colorless oil; UV (MeOH) λ_{max} (log ϵ) 284 (2.90) nm; IR ν_{max} (film) 2926, 1716, 1499, 1490, 1472, 1440, 1361, 940, 802, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (1 H, dd, J = 7.8, 0.3 Hz, H-5′), 6.67 (1 H, dd, J = 1.8, 0.3 Hz, H-2′), 6.61 (1 H, dd, J = 8.0, 1.8 Hz, H-6′), 5.91 (2 H, s, OCH_2O), 2.51 (1 H, t, J = 7.8 Hz, H-12), 2.40 (2 H, t, J = 7.2 Hz, H-3), 2.13 (3 H, s, H-1), 1.52−1.59 (m, H-4, H-11), 1.20−1.30 (m, H-5−H-10); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.5 (C-2), 149.2 (C-3′), 147.2 (C-4′), 136.0 (C-1′), 120.5 (C-6′), 109.0 (C-2′), 108.1 (C-5′), 100.0 (OCH_2O), 42.0 (C-3), 34.0 (C-12), 32.0 (C-11), 29.0 (C-1), 26.0−28.0 (C-5−C-10), 22.8 (C-4); EIMS *m/z* 304 [M$^+$] (100), 289 (1.4), 246 (22.2), 218 (2.7), 204 (1.4), 177(1.4), 161 (1.4), 140 (4.2), 135-(86.1), 105 (2.7), 91 (1.4), 77 (2.7), 55 (1.4), 43 (2.7); HREIMS *m/z* 304.2037 (calcd for $C_{19}H_{28}O_3$, 304.2038).

2-Oxo-14-(3′,4′-methylenedioxyphenyl)tetradecane (2): crystalline solid (MeOH); mp 60−61 °C; UV (MeOH) λ_{max} (log ϵ) 286 (2.82) nm; IR ν_{max} (KBr) 2913, 1713, 1489, 1490, 1476, 1440, 1353, 1228, 945, 918, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.71 (1 H, dd, J = 7.8, 0.3 Hz, H-5′), 6.67 (1 H, dd, J = 1.8, 0.3 Hz, H-2′), 6.60 (1 H, dd, J = 7.8, 1.8 Hz, H-6′), 5.91 (2 H, s, OCH_2O), 2.51 (2H, t, J = 7.8 Hz, H-14), 2.41 (2H, t, J = 7.1 Hz, H-3), 2.13 (3 H, s, H-1), 1.52−1.59 (m, H-4, H-13), 1.22−1.30 (m, H-5−H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.2 (C-2), 149.5 (C-3′), 147.2 (C-4′), 135.0 (C-1′), 120.5 (C-6′), 109.0 (C-2′), 108.1 (C-5′), 100.0 (OCH_2O), 41.8 (C-3), 35.7 (C-14), 34.0 (C-13), 31.8 (C-12), 29.0 (C-1), 26.0−28.0 (C-5−C-11), 23.0 (C-4); EIMS *m/z* 332 [M$^+$] (100), 328 (4.54), 274 (18.1), 177 (1.5), 163 (1.5), 148 (4.5), 135 (90.9), 105 (3.0), 91 (6.0), 77 (1.5), 55 (1.5), 43 (3.0); HREIMS *m/z* 332.2349 (calcd for $C_{21}H_{32}O_3$, 332.2351).

2-Oxo-16-(3′,4′-methylenedioxyphenyl)hexadecane (3): crystalline solid (MeOH); mp 60−61 °C; UV (MeOH) λ_{max} (log ϵ) 286 (2.90) nm; IR ν_{max} (KBr) 2916, 1713, 1492, 1437, 1360, 1228, 918, 812, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (1 H, dd, J = 8.0, 0.3 Hz, H-5′), 6.67 (1 H, dd, J = 1.8, 0.3 Hz, H-2′), 6.61 (1 H, ddt, J = 7.8, 1.8, 0.6 Hz, H-6′), 5.91 (2 H, s, OCH_2O), 2.51 (2 H, br t, J = 7.8 Hz, H-16), 2.41 (2 H, t, J = 7.2 Hz, H-3), 2.13 (3 H, s, H-1), 1.52−1.6 (m, H-4, H-16), 1.22−1.30 (m, H-5−H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.5 (C-2), 149.0 (C-3′), 147.0 (C-4′), 136.0 (C-1′), 120.5 (C-6′), 109.0 (C-2′), 108.0 (C-5′), 100.0 (OCH_2O), 42.0 (C-3), 34.0 (C-16), 32.0 (C-15), 29.0 (C-1), 26.0−28.0 (C-5−C-14), 23.0 (C-4); EIMS *m/z* 360 [M$^+$] (100), 345 (1.7), 302 (22.3), 177 (1.7), 148 (4.4), 135 (89.3), 105 (2.6), 91 (1.7), 77 (2.6), 43 (3.5); HREIMS *m/z* 360.2663 (calcd for $C_{23}H_{36}O_3$, 360.2664).

2-Oxo-18-(3′,4′-methylenedioxyphenyl)octadecane (4): colorless oil; UV (MeOH) λ_{max} (log ϵ) 283 (2.87) nm; IR ν_{max} (film) 2914, 2848, 1718, 1492, 1499, 1490, 1472, 1440, 1361, 940, 802, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (1 H, dd, J = 8.0, 0.3 Hz, H-5′), 6.67 (1 H, dd, J = 1.8, 0.3 Hz, H-2′), 6.61 (1 H, dd, J = 8.0, 1.8 Hz, H-6′), 5.91 (2 H, s, OCH_2O), 2.51 (2 H, t, J = 7.5 Hz, H-18), 2.41 (2 H, t, J = 7.1 Hz, H-3), 2.13 (3 H, s, H-1), 1.52−1.60 (m, H-4, H-17), 1.20−1.30 (m, H-5 − H-16); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.2 (C-2), 148.5 (C-3′), 147.1 (C-4′), 136.2 (C-1′), 120.0 (C-6′), 109.0 (C-2′), 108.0 (C-5′), 100.0 (OCH_2O), 42.0 (C-3), 34.0 (C-18), 32.0 (C-17), 29.0 (C-1), 26.0−28.0 (C-5−C-16), 22.8 (C-4); EIMS *m/z* 388 [M$^+$]

(100), 373 (2.7), 330 (22.2), 302 (22.3), 288 (1.4), 274 (1.4), 177 (1.4), 140 (4.2), 135 (88.8), 105 (1.4), 91 (1.4), 77 (2.7), 43 (3.5); HREIMS *m/z* 388.2976 (calcd for $C_{25}H_{40}O_3$, 388.2977).

2-Oxo-14-(3′,4′-methylenedioxyphenyl)-*trans*-13-tetradecene (5): colorless oil; UV (MeOH) λ_{max} (log ϵ) 260 (4.24) nm; IR ν_{max} (KBr) 2916, 2847, 1705, 1502, 1251, 1038, 963, 926, 792, 717 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (1 H, d, J = 1.8 Hz, H-2′), 6.84 (1 H, dd, J = 7.8, 1.8 Hz, H-6′), 6.76 (1 H, d, J = 7.8 Hz, H-5′), 6.28 (1 H, m, H-13), 6.04 (1 H, d, J = 15.6 Hz, H-14), 5.91 (2 H, s, OCH_2O), 2.41 (2 H, t, J = 7.5 Hz, H-3), 2.13 (3 H, s, H-1), 1.50−1.60 (m, H-4, H-12), 1.20−1.40 (m, H-5−H-11); EIMS *m/z* 330 [M$^+$] (100), 272 (11.1), 189 (2.2), 161 (60.0), 148 (35.5), 131 (77.8), 122 (6.7), 103 (26.7), 77 (6.7), 55 (2.2), 43 (8.9); HREIMS *m/z* 330.2190 (calcd for $C_{21}H_{30}O_3$, 330.2194).

2-Oxo-16-(3′,4′-methylenedioxyphenyl)-*trans*-15-hexadecene (6): colorless oil; UV (MeOH) λ_{max} (log ϵ) 265 (4.52) nm; IR ν_{max} (KBr) 2915, 2827, 1704, 1500, 1232, 1037, 926, 792 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.96 (1 H, d, J = 1.5 Hz, H-2′), 6.80 (1 H, dd, J = 7.5, 1.5 Hz, H-6′), 6.72 (1 H, d, J = 7.5 Hz, H-5′), 6.28 (1 H, m, H-15), 6.04 (1 H, d, J = 16.4 Hz, H-16), 5.92 (2 H, s, OCH_2O), 2.42 (2 H, t, J = 7.8 Hz, H-3), 2.10 (3 H, s, H-1), 1.50−1.64 (m, H-5−H-13), 1.21−1.33 (m, H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.5 (C-2), 147.9 (C-3′), 146.5 (C-4′), 132.5 (C-1′), 129.3 (C-15, C-16), 120.2 (C-6′), 108.2 (C-5′), 105.4 (C-2′), 100.0 (OCH_2O), 42.0 (C-3), 32.9 (C-14), 29.0 (C-1), 26.0−28.0 (C-5−C-13), 23.0 (C-4); EIMS *m/z* 358 [M$^+$] (100), 300 (11.1), 161 (35.5), 148 (22.2), 131(44.4), 103 (15.5), 77 (2.2), 43 (6.7); HREIMS *m/z* 358.2499 (calcd for $C_{23}H_{34}O_3$, 358.2507).

2-Oxo-18-(3′,4′-methylenedioxyphenyl)-*trans*-17-octadecene (7): colorless oil; UV (MeOH) λ_{max} (log ϵ) 263 (4.12) nm; IR ν_{max} (KBr) 2916, 2845, 1706, 1511, 1217, 961, 910, 791 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.97 (1 H, d, J = 1.8 Hz, H-2′), 6.81 (1 H, dd, J = 7.5, 1.5 Hz, H-6′), 6.76 (1 H, d, J = 7.5 Hz, H-5′), 6.28 (1 H, m, H-17), 6.04 (1 H, d, J = 16.0 Hz, H-18), 5.91 (2 H, s, OCH_2O), 2.40 (2 H, t, J = 7.8 Hz, H-3), 2.10 (3 H, s, H-1), 1.51−1.62 (m, H-4−H-16), 1.20−1.30 (m, H-5, H-15); EIMS *m/z* 386 [M$^+$] (100), 328 (13.3), 189 (2.2), 161 (35.5), 148 (20.0), 131 (37.8), 122 (4.4), 103 (11.1), 58 (2.2), 43 (4.4); HREIMS *m/z* 386.2808 (calcd for $C_{25}H_{38}O_3$, 386.2820).

2-Oxo-16-phenyl-*trans*-3-hexadecene (8): colorless oil; UV (MeOH) λ_{max} (log ϵ) 286 (4.11) nm; IR ν_{max} (film) 2925, 2915, 2853, 1738, 1677, 1627, 1495, 1463, 1453, 978, 746, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.1−7.3 (5 H, m, H-2′−H-6′), 6.8 (1 H, dt, J = 15.9, 6.9 Hz, H-4), 6.00 (1 H, dt, J = 15.9, 1.5 Hz, H-3), 2.60 (1 H, t, J = 7.5 Hz, H-16), 2.24 (2 H, m, H-5), 2.2 (3 H, s, H-1), 1.6 (m, H-7−H-12), 1.46 (2 H, q, J = 7.2 Hz, H-6), 1.2 (m, H-13−H-15); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 198.8 (C-2), 148.7 (C-4), 131.3 (C-3), 142.9 (C-1′), 128.4 (C-3′, C-5′), 128.2 (C-2′, C-6′), 125.5 (C-4′), 35.9 (C-16), 32.4 (C-15), 31.5 (C-5), 29.2−29.6 (C-1, C-7−C-14), 28.0 (C-1), 26.8 (C-6); EIMS *m/z* 314 [M$^+$] (68.8), 296 (4.4), 245 (5.1), 256 (8.8), 174 (6.6), 160 (6.6), 131 (22.2), 117 (7.7), 104 (51.1), 97 (64.4), 91 (100); HREIMS *m/z* 314.2602 (calcd for $C_{22}H_{34}O$, 314.2609).

Methyl [6-(10-phenyldecanyl)tetrahydropyran-2-yl]acetate (9): colorless oil; $[\alpha]^{20}_D$ −3.0°; UV (MeOH) λ_{max} (log ϵ) 286 (3.50) nm; IR ν_{max} (film) 2926, 2853, 1954, 1743, 1495, 1436, 747, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.1−7.3 (5 H, m, H2‴−H6‴), 3.7 (1 H, dddd, J = 13.2, 7.8, 5.7, 1.8 Hz, H-2′), 3.6 (3 H, s, OCH_3), 3.2 (1 H, tdd, J = 11.1, 4.8, 1.8 Hz, H-6′), 2.6 (2 H, t, J = 7.8 Hz, H-10″), 2.51 (1 H, dd, J = 14.7, 7.8 Hz, H-2b), 2.36 (1 H, dd, J = 14.7, 5.7 Hz, H-2a), 1.0−1.8 (m, H-3′−H-5′, H-1″−H-9″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.0 (C-1), 142.0 (C-1‴), 128.3 (C-2‴, C-6‴), 128.1 (C-3‴, C-5‴), 125.4 (C-4‴), 78.0 (C-6′), 74.4 (C-2′), 51.5 (OCH_3), 41.6 (C-2), 36.0 (C-10″), 23.4−31.5 (C-3′−C-5′, C-1″−C-9″); EIMS *m/z* 374 [M$^+$] (52.8), 356 (6.6), 342 (13.2), 324 (5.6), 314 (1.8), 301 (16.0), 282 (14.1), 256 (5.6), 158 (13.2), 157 (100), 143 (5.6), 125 (56.6), 104 (29.2), 91 (73.6), 69 (8.5), 55 (11.3), 41 (7.5); HREIMS *m/z* 374.2821 (calcd for $C_{24}H_{38}O_3$, 374.2820).

Methyl 2-(6-tridecyltetrahydro-2*H*-pyran-2-yl)acetate (10): colorless glassy solid; UV(MeOH) λ_{max} (log ϵ) 275 (3.35) nm; IR ν_{max} (film) 2924, 2853, 1744, 1456, 1436, 1345, 1286, 1197, 1089, 1074, 1048, 721; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.74 (1 H, dddd, J = 10.8, 5.4, 3.01, 1.8 Hz, H-2′), 3.6 (3 H, s, OCH_3), 3.27 (1 H, tdd, J = 10.7, 7.8, 1.5 Hz, H-6′), 2.51 (1 H, dd, J = 14.4, 7.0 Hz, H-2b), 2.4 (1 H, dd, J = 14.2, 5.1 Hz, H-2a), 1.4−1.6 (m, H-1″−H-12″, H-3′−H-5′), 1.2 (br s, H-1″−H-12″), 0.9 (3H, t, J = 7.0 Hz, H-13″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.0 (C-1), 78.0 (C-6′), 74.0 (C-2′), 51.5 (OCH_3), 41.6 (C-2), 22.6−31.9 (C-3′−C-5′, C1″−C12″), 14.0 (C-13″); EIMS *m/z* 340 [M$^+$] (19.3), 322 (6.4), 267 (19.3), 248 (6.4), 222 (3.2), 185 (4.8), 157 (100), 129 (30.6), 125 (62.9), 116 (62.9), 97 (20.9), 69 (14.5), 43 (6.45); HREIMS *m/z* 340.2975 (calcd for $C_{21}H_{40}O_3$, 340.2977).

Methyl 2-(5-tetradecyltetrahydro-2-furanyl)acetate (11): colorless oil; UV (MeOH) λ_{max} (log ϵ) 271 (3.5) nm; IR ν_{max} (film) 2921, 2850, 1741, 1457, 1436, 1345, 1287, 1196, 1089, 1072, 1046, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.35 (1 H, m, H-2′), 4.21 (1 H, m, H-5′), 3.68 (3 H, s, OCH_3), 2.63 (1 H, dd, J = 15.0, 6.6 Hz, H-2b), 2.45 (1 H, dd, J = 15.3, 6.6 Hz, H-2a), 1.4−1.6 (m, H-3′, H-4′), 1.2 (br s, H-1″−H-13″), 0.88 (3 H, s, H-14″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.7 (C-1), 79.8 (C-5′), 75.0 (C-2′), 51.5 (OCH_3), 41.0 (C-2), 22.6−31.9 (C-3′, C-4′, C-1″−C-13″), 14.0 (C-14″); EIMS *m/z* 340 [M$^+$] (4.9), 322 (3.27), 267 (16.3), 240 (1.6), 240 (1.6), 143 (100), 116 (73.7), 111 (50.8), 97 (16.3), 55 (18), 43 (14.75); HREIMS *m/z* 340.2975 (calcd for $C_{21}H_{40}O_3$, 340.2977).

2-Oxo-14-(3′,4′-methylenedioxyphenyl)-*trans*-3-tetradecene (12): colorless oil; UV (MeOH) λ_{max} (log ϵ) 255 (4.11) nm; IR ν_{max} (film) 2913, 1645, 1491, 1489, 1474, 1453, 1442, 1359, 1251, 1167, 978, 914, 798 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.82 (1 H, dt, J = 15.6, 6.9 Hz, H-4), 6.72 (1 H, d, J = 8.1 Hz, H-5′), 6.67 (1 H, dd, J = 1.8, 0.3 Hz, H-2′), 6.61 (1 H, ddt, J = 8.1, 1.8, 0.6 Hz, H-6′), 6.0 (1 H, dt, J = 15.6, 1.8 Hz, H-3), 2.6 (2 H, t, J = 7.5 Hz, H-14), 2.25 (3 H, s, H-1), 2.23 (2 H, m, H-5), 1.6 (2 H, m, H-13), 1.47 (2 H, q, J = 7.2 Hz, H-6), 1.26 (m, H-7−H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 198.8 (C-2), 149.4 (C-3′), 148.2 (C-4), 147.0 (C-4′), 134.0 (C-1′), 130.0 (C-3), 120.0 (C-6′), 109.2 (C-2′), 108.0 (C-5′), 100.0 (OCH_2O), 35.7 (C-14), 34.0 (C-13), 30.4 (C-12), 29.0 (C-1), 26.0−28.0 (C-5−C-11); EIMS *m/z* 330 [M$^+$] (91), 272 (2.0), 189 (1.8), 136 (53), 135 (100), 105 (3.3), 97 (17.1), 77 (12.0); HREIMS *m/z* 330.2192 (calcd for $C_{21}H_{30}O_3$, 330.2194).

2-Oxo-16-(3′,4′-methylenedioxyphenyl)-*trans*-3-hexadecene (13): colorless oil; UV (MeOH) λ_{max} (log ϵ) 251 (4.15) nm; IR ν_{max} (film) 2913, 1645, 1474, 1491, 1489, 1474, 1453, 1439, 1351, 1251, 1169, 978, 914, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.8 (1 H, dt, J = 15.6, 6.9 Hz, H-4), 6.74 (1 H, d, J = 8.0 Hz, H-5′), 6.64 (1 H, dd, J = 1.8, 0.3 Hz, H-2′), 6.6 (1 H, ddt, J = 8.0, 1.8, 0.6 Hz, H-6′), 6.1 (1 H, dt, J = 15.6, 1.8 Hz, H-3), 2.6 (2 H, t, J = 7.5 Hz, H-16), 2.24 (3 H, s, H-1), 2.23 (2 H, m, H-5), 1.6 (2 H, m, H-15), 1.44 (2 H, q, J = 7.2 Hz, H-6), 1.24 (m, H-7−H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 198.8 (C-2), 149.0 (C-3′), 147.8 (C-4), 147.1 (C-4′), 134.0 (C-1′), 131.2 (C-3), 120.0 (C-6′), 109.0 (C-2′), 108.2 (C-5′), 100.0 (OCH_2O), 35.2 (C-16), 34.3 (C-15), 30.1 (C-14), 29.0 (C-1), 26.0−28.0 (C-5−C-13); EIMS *m/z* 358 [M$^+$] (89), 343 (1.8), 288 (1.8), 175 (1.2), 136 (22), 135 (100), 96 (8.1), 77 (3.0); HREIMS *m/z* 358.2501 (calcd for $C_{23}H_{34}O_3$, 358.2507).

2-Oxo-16-phenyl-3-hexadecane (14): yellow oil; UV (MeOH) λ_{max} (log ϵ) 275 (3.10) nm; IR ν_{max} (film) 2910, 1712, 1451, 972, 736, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.1−7.3 (5 H, m, H-2″−H-6″), 2.6 (2 H, t, J = 7.8 Hz, H-16), 2.4 (2 H, t, J = 7.5 Hz, H-3), 2.13 (3 H, s, H-1), 1.2 (1 H, br s, H-5′−H-14′), 1.0 (m, H-4′, H-6′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.3 (C-2), 141.5 (C-1′), 128.2 (C-2′, C-3′, C-5′, C-6′), 125.5 (C-4′), 43.8 (C-3), 35.9 (C-16), 31.5 (C-15), 29.3−29.8 (C-1, C-4 − C-14), 29.1 (C-1); EIMS *m/z* 316 [M$^+$] (7.69), 298 (7.6), 298 (46.1), 273 (2.5), 258 (30.7), 216 (5.12), 188 (7.69), 131 (10.2), 105 (33.3), 91 (100), 71 (28.2), 55 (25.6), 43 (28.2); HREIMS *m/z* 316.2764 (calcd for $C_{22}H_{36}O$, 316.2766).

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