Isolation of Two Highly Methylated Polyketide Derivatives from a Yew-Associated *Penicillium* Species

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Two selective antifungal agents were produced by a *Penicillium* sp. isolated from the inner bark of the Pacific yew tree, *Taxus brevifolia*. The structures of these highly methylated polyketide derivatives were deduced by detailed analysis of both 1D and 2D NMR and difference NOE spectra. Both compounds were active against the plant pathogen, *Sclerotinia sclerotiorum* in the standard disk assay.

Fungi and bacteria are a prolific source of metabolites with significant biological activities. Many important anticancer, antifungal, and antibacterial chemotherapeutics are either microbial metabolites or semisynthetic derivatives. But existing drugs do not adequately meet our pharmaceutical demands. The increasing incidence of drug resistance in pathogenic microbes as well as the increasing frequency of infectious diseases in immunocompromised individuals necessitate the discovery of new anti-infective agents. Investigating the secondary metabolites of microorganisms isolated from unusual or specialized ecological niches may increase the chance of finding novel compounds. For the past seven years we have been investigating the endosymbiotic microorganisms of medicinal plants, particularly plants used by North American indigenous peoples. Plant endosymbionts are usually nonpathogenic in nature, but generally produce bioactive compounds that enable them to survive in the competitive world of interstitial space.

The first plant targeted in our study was the Pacific yew tree, *Taxus brevifolia*.[1,2] Specimens sampled in Montana, Washington, Oregon, and Idaho harbored many different fungi and bacteria in their inner bark tissues (phloem) and needles. One of these, a *Penicillium* sp. isolated from the root tip of a healthy yew tree growing in Hungry Horse, Montana, has yielded several interesting bioactive metabolites, including the two novel polyketides described here.

The organic extracts of both the medium and the mycelial mat of *Penicillium* sp. isolate H1RE (Medium A) showed potent antibiotic activity against the bacteria *Staphylococcus aureus* and *Vibrio harveyii* and against the plant-pathogenic fungus *Sclerotinia sclerotiorum* in a standard disk assay. Bioassay-guided fractionation of the organic broth extract using antibacterial activity as the guide, yielded furanone and a variety of phomopsolides, which have been described previously.[3] These compounds were responsible for all of the *S. aureus* and *V. harveyii* activities observed. Antifungal bioassay-guided fractionation yielded two new polyketide derivatives, **1** and **5,** that were responsible for the inhibition of *Sclerotinia* growth.

The molecular formula $C_{25}H_{34}O_5$ was established for **1** by HREIMS, requiring nine units of unsaturation. The UV absorption at 296 nm ($\epsilon = 23\,800$) suggested an extended chromophore system. Compound **1** readily formed a methyl ester when treated with diazomethane, and a diacetate when treated with acetic anhydride−pyridine. This established the presence of a carboxylic acid and two alcohol moieties. The ^{13}C NMR spectrum showed two carbonyl absorptions: a ketone carbon at 200.3 ppm and the carboxylic acid carbon at 169.8 ppm. ^{13}C NMR also indicated the presence of six olefinic methines and four quaternary olefinic carbons. Seven double-bond equivalents were exhausted by sp^2 carbons, suggesting the presence of two rings.

NMR correlation spectroscopy, especially $^1H-^1H$ COSY, HMBC, and HMQC, were instrumental in establishing the structure of **1**. The doublet centered at δ 5.84 ($J = 15.5$ Hz) in the ^1H NMR spectrum was coupled to a doublet at δ 7.32 (see Table 1). The large vicinal coupling constant indicated a trans-disubstituted double bond. These two protons were attached to carbons at δ 116.6 and 151.1, respectively, indicating an unsaturated carbonyl system. The HMBC spectrum established the correlation of these two protons to the δ 169.8 acid resonance, indicating an α,β-unsaturated acid moiety. The COSY spectrum established a correlation between the two olefinic protons at δ 7.32 and 6.29, which appeared as a broad singlet. The δ 6.29 signal was, in turn, long-range coupled to a broadened methyl singlet at δ 1.91 and to another broad doublet at δ 5.41. The δ 5.41 doublet ($J = 8.5$ Hz) was also coupled to a broadened methyl singlet at δ 2.05 and a broad doublet at δ 5.34 ($J = 8.5$ Hz). These data strongly suggested the presence of fragment A shown in Figure 1.

The HMBC experiment established long-range correlations between the ketone carbon at δ 200.3 and both the isolated methylene moiety at δ 4.29 and 4.23 ($J = 18.0$ Hz) and a broadened olefinic signal at δ 6.32. This olefinic proton was attached to a trisubstituted double bond that was directly attached to a carbon at δ 116.9. This olefinic proton showed COSY correlations to a broadened methyl signal at δ 1.93 and to another olefinic signal at δ 6.00. This indicated the part structure B (Figure 1).

The final part structure for **1** was deduced directly from the HMQC and HMBC experiments. The proton triplet at δ 2.62 ($J = 9.5$ Hz) was attached to a carbon at δ 80.5, which showed long-range correlations to the two methyl doublets at δ 1.11 and 1.00. The coupling in this system indicated a trans-diaxial arrangement of the alcoholic methine and the two adjacent methyl bearing methines in a six-membered ring. This gave the arrangement found in fragment C (Figure 1). We were a little concerned about the assignment of the alcoholic methine to the relatively upfield δ 2.62 proton resonance, but this compared quite nicely to the shift of the alcoholic methine in *trans*-2-*trans*-6-dimethylcyclohexanol at δ 2.52.[4]

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Table 1. Compound **1**, NMR Data [500 MHz, CD_3OD]

carbon	ppm (m)	proton (m, *J* in Hz)	COSY	HMBC (H to C)
1	169.8 (s)			
2	116.6 (d)	5.84 (d, 15.5)	H-3, H-5	C-1, C-4
3	151.1 (d)	7.32 (d, 15.5)	H-2, H-5	C-1, C-2, C-4, C-5, C-21
4	132.4 (s)			
5	143.6 (d)	6.29 (br s)	H-2, H-3, H-7, H-21	C-3, C-6, C-21
6	134.7 (s)			
7	134.4 (d)	5.41 (br d, 8.5)	H-5, H-8, H-22	C-5, C-8, C-17, C-22
8	38.1 (d)	5.34 (dd, 8.5, 2.5)	H-7, H-9	C-6, C-9, C-14, C-17
9	47.7 (d)	1.51 (br d, 11.0)	H-8, H-10, H-14, H-15	C-15, C-17
10	37.8 (d)	1.2[a]	H-9, H-11, H-23	C-9, C-11
11	80.5 (d)	2.62 (t, 9.5)	H-10, H-12	C-12, C-23, C-24
12	35.4 (d)	1.4[a]	H-11, H-13, H-24	
13	38.5 (t)	1.82	H-12, H-13, H-14	C-9, C-11, C-14
		1.3[a]	H-12, H-13, H-14	C-9
14	33.9 (d)	2.72 (br s)	H-9, H-13, H-15, H-25	C-12, C-16
15	142.0 (d)	6.00 (br s)	H-9, H-14, H-18, H-25	C-9, C-13, C-14, C-17, C-18, C-25
16	131.9 (s)			
17	155.2 (s)			
18	116.9 (d)	6.32 (br s)	H-15	C-8, C-16, C-17, C-19
19	200.3 (s)			
20	69.1 (t)	4.29 (d, 18.0)	H-20	C-19
		4.23 (d, 18.0)	H-20	C-19
21	13.0 (q)	1.91 (br s)	H-5	C-3, C-4, C-5, C-6
22	16.2 (q)	2.05 (br s)	H-7	C-4, C-5, C-6, C-7
23	15.0 (q)	1.11 (d, 6.5)	H-10	C-9, C-11
24	18.3 (q)	1.00 (d, 6.0)	H-12	C-11, C-12, C-13
25	18.9 (q)	1.93 (br s)	H-15	C-15, C-17

[a] Estimated from the HMQC experiment.

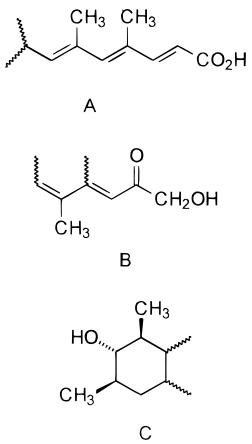


Figure 1. Fragment structures for **1**.

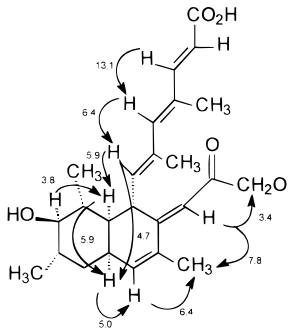


Figure 2. NOE difference data for compound **1**.

These three fragments were assembled by considering a few key resonances. The proton resonating at δ 5.34 was, surprisingly, directly attached to a carbon at δ 38.1. This proton showed COSY correlations to the olefinic proton at δ 5.41 and a methine proton at δ 1.51. The δ 1.51 resonance, in turn, correlated to a methine at δ 1.20, which was attached to the downfield methyl doublet (δ 1.11), to another broad methine singlet at δ 2.72, and to the broad methine singlet at δ 6.00. The broad singlet at δ 2.72 also showed correlations to the δ 6.00 methine and to two methylene protons at δ 1.82 and 1.30. These two methylene protons were coupled to the methine at δ 1.40, that is, in turn, coupled to the methyl doublet at δ 1.00. This gave the proposed bicyclic Decalin system for compound **1**.

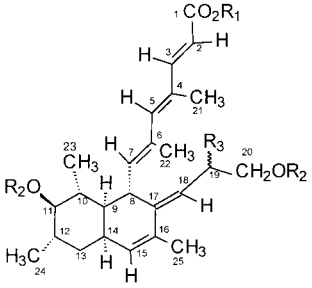
Difference NOE experiments corroborated the proposed structure for **1** and established the relative stereochemistry. Large enhancements for H3 and H5 (13.1%) and H5 and H7 (6.4%) in the NOE experiments (See Figure 2) indicated C4−C5 and C6−C7 stereochemistry was *E*,*E*, respectively. This established the stereochemistry of the trienyl system as *E*,*E*,*E*. Irradiation of the proton at C7 induced enhancement of the two bridgehead methines (to H9, 5.9% and to H14, 4.7%), indicating a general cis arrangement and hence a cis-Decalin system. The rest of the pertinent enhancements are shown in Figure 2.

Extensive molecular modeling studies of compound **1** not only supported the relative conformation but also explained some of the anomalous spectral data. Molecular modeling using HyperChem and AM1 optimization gave a low energy conformation that was consistent with the NOE data. Energy minimization of **1** indicated that the conjugated trienyl system was not planar, apparently due to the H21−H22 methyl−methyl interaction. This explained the hypsochromic shift and low molar absorptivity observed in the UV spectrum for this system. If the trienyl moiety were planar, the λ_{max} should be 319 nm (296 nm observed) with a more intense ϵ value.[5]

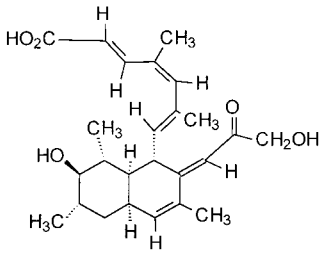
The HMQC experiment indicated another spectral anomaly. The chemical shift of H8 (δ 5.34) seemed unusually low field for a proton attached to a carbon at δ 38.1. Molecular modeling studies showed a close interaction of the ketone oxygen and H8. It was likely that H8 was in the anisotropic deshielding zone of the ketone oxygen. Reduction of the methyl ester diacetate, **2**, with $NaBH_4$ gave selective reduction of the ketone to give a mixture of two diastereomeric alcohols. In one of the diastereomers (**3**), the olefinic protons H7, H15, and H18 appeared as an overlapping multiplet. In the other diastereomer (**4**), the

^{1}H NMR spectrum was fully resolved and assigned with the help of the COSY spectrum. A significant upfield shift of H8 (from δ 5.35 in the ketone, **2**, to δ 3.83 in the reduced alcohol, **4**) supports the anisotropic deshielding argument.

A second compound with NMR spectra similar to that of **1** was also isolated. Compound **5** was shown to be isomeric with **1** by HREIMS. The ^{1}H NMR spectrum of **5** had small differences from that of **1**, especially for the protons associated with the conjugated side chain. NOE difference spectra resolved the difference between these compounds. Irradiation of H3 gave 8.9% enhancement of H7. This is consistent with the C2−C3, C4−C5, and C6−C7 stereochemistry as *E*, *Z*, *E*, respectively. Although compounds **1** and **5** are stereoisomers and might be converted by chemical or photochemical isomerization, we have seen no conversion in long-term storage of these compounds. Further, we did not detect compound **5** in any samples of compound **1** that were subjected to isolation conditions.



	R_1	R_2	R_3
1	H	H	=O
2	Me	Ac	=O
3	Me	Ac	OH
4	Me	Ac	OH



5

Compounds **1** and **5** are believed to be highly methylated polyacetate derivatives. We are currently studying the biosynthesis of these compounds.

Many *Sclerotinia* species are potent plant pathogens and are the cause of watery rot on many fruits and vegetables.[6] Both compounds **1** and **5** were active against this pathogenic fungus in the disk assay. Compound **1** had a zone of inhibition of 17 mm when tested at 1.1×10^{-4} μmole/disk, and compound **5** had a zone of 16 mm at 3.4×10^{-4} μmole/disk, which was undiminished after 96 h. Neither compound exhibited toxicity toward bacteria, other fungi (including *Candida albicans, Helminthosporium sativum, Geotrichum candidum, Aspergillus flavus, Aspergillus niger*, and *Fusarium oxysporum*) at 500 μg/disk in the disk assay or toward brine shrimp at 500 μg/mL in our intramural bioassay.

Compounds **1** and **5** have a carbon skeleton and cyclization pattern similar to those of the potent competitive inhibitor of HMG−CoA reductase, lovastatin acid,[7] and the two antifungal metabolites hamigerone and dihydrohamigerone.[8] We are currently investigating other biological activities associated with compounds **1** and **5**.

The organic extract of the mycelial mat also inhibited the growth of *Sclerotinia sclerotiorum*. Two known compounds, oxaline and roquefortine C, were isolated from this extract using bioassay-guided fractionation. Careful examination of the spectral data yielded these two structures, which were confirmed by subsequent comparison with the literature.[9,10]

An interesting aspect of this project has been observing the effects of media alteration on secondary-metabolite production. It is well-known that antibiotic production can be altered by the addition of certain nutrients.[11] We have adopted five different media as part of our general fungal fermentation protocol. All are soytone based; M1S contains sucrose, Mediums A−D contain glucose. Medium A also contains $Mg_3(PO_4)_2$, which has been described as an ammonium trap by Tanaka and Omura.[11] They used "ammonium ion-depressed fermentation" to effect twofold to tenfold increases in the production of the antibiotics tylosin, cerulenin, leucomycin, and cephalosporin.[11]

In comparative fermentation studies, organic extracts of H1RE exhibited the most potent biological activity when cultured in Medium A. Antifungal activity, in particular, increased with the addition of $Mg_3(PO_4)_2$. When H1RE was grown in Medium A, compounds **1** and **5** were found exclusively in the $CHCl_3$ extract of the broth. To compare compound production as a function of growth medium we also grew H1RE in unadulterated mycological broth (Medium D). We could find no evidence of either compound **1** or **5** in the $CHCl_3$ extracts of Medium D, although a small amount was found in the organic extract of the mycelial mat. It appears that **1** is produced at somewhat higher levels and is partitioned directly into the medium when phosphate is added to the broth. Oxaline was not detected when H1RE was grown in Medium D. Roquefortine C was isolated from both media preparations. We are currently investigating the effect of media alteration on other fungi in our collection.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra: $CDCl_3$ on a Bruker DPX-300 or a DRX-500 spectrometer. ^{1}H NMR spectra were recorded at 500 MHz, and the ^{13}C NMR spectra were recorded at 125 MHz, unless otherwise noted. All of the chemical shifts were recorded with respect to the deuterated solvent shift ($CDCl_3$, 7.24 for the proton resonance and δ 77.0 for the carbon spectra, and CD_3OD at δ 3.30 for the proton spectra and δ 47.0 for the carbon spectra). The IR spectra were recorded on a Perkin−Elmer 1310 spectrometer. The optical rotations were recorded on a Perkin−Elmer 241 MC polarimeter using a 1-mL cell. The MS were provided by the Montana State Mass Spectrometer facility at Montana State University. All solvents used were spectral grade.

Extraction and Isolation. The isolation of fungus H1RE from *T. brevifolia* has been previously described.[1] H1RE was identified as a *Penicillium* sp. by Microbial ID, Inc., and is deposited in the Agricultural Research Culture Collection (NRRL# 21208). *Penicillium* sp. was grown in Medium A broth [10 g soytone + 40 g glucose + 10 G $Mg_3(PO_4)_2 \cdot 12\ H_2O$ per L of broth, 14 × 500 mL] in still culture. After 21 days, the mycelia was removed by filtration and the broth extracted with $CHCl_3$ (3 × with 1 L of $CHCl_3$) to give 1.3673 g of a brown oil. This crude extract was fractionated on (CCCC) using hexane−EtOAc−MeOH−H_2O 2:2:2:1 to give several major bioactive fractions. Compounds **1** and **5** were further purified by HPLC on Si gel using hexane−isopropyl alcohol as solvents. The fractions in each of these steps were combined by their activities in the *Sclerotinia* disk assay. The relative amounts of the metabolites in the $CHCl_3$ extract of this growth were **1**, 15 mg (2.1 mg/L); compound **5**, 3.0 mg (0.4 mg/ L).

H1RE was also grown in mycological broth, Medium D, (10 g soytone + 40 g glucose per L of broth, 6 × 2000 mL) in still culture for 21 days and worked up as above. Compounds **1**

and **5** were undetectable in the $CHCl_3$ extracts of this fermentation. The mycelial mat of this growth was air-dried and extracted with 1:1 $CHCl_3$−MeOH (3 × with 1 L), the solvents removed, and the extract partitioned between $CHCl_3$ and H_2O (1 L of each). This $CHCl_3$ extract was purified as above with CCCC and HPLC to give 20 mg (1.7 mg/ L) of **1** and 2.0 mg (0.2 mg/ L) of **5**.

Compound 1: isolated as a colorless oil; $[\alpha]^{20}_D$ +7.2° (*c* 0.0014, MeOH); UV (MeOH) λ_{max} (log ϵ) 296 (4.38), 202 (4.06); IR (film) ν_{max} 2902, 1670, 1607, 1576, 1270, 1050, 900 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* [M^+] 414 (27), 399 (3), 396 (12), 355 (21), 337 (81), 291 (21), 267 (52), 145 (41), 105 (61), 55 (100); HREIMS *m/z* 414.2399 (calcd for $C_{25}H_{34}O_5$, 414.2406).

Compound 5: isolated as a colorless oil; $[\alpha]^{20}_D$ +20.5° (*c* 0.0073, MeOH); UV (MeOH) λ_{max} (log ϵ) 291 (4.74), 205 (4.63); ^1H NMR (CD_3OD) δ 7.73 (d, 1H, J = 15.9, H-3), 6.25 (br s, 1H, H-18), 6.19 (br s, 1H, H-5), 5.94 (br s, 1H, H-15), 5.78 (d, 1H, J = 15.9, H-2), 5.32 (d, 1H, J = 9.0, H-8), 5.13 (br d, 1H, J = 9.0, H-7), 4.24 (s, 2H, H-20), 2.82 (br s, 1H, H-14), 2.60 (t, 1H, J = 9.3, H-11), 1.93 (br s, 3H, H-22), 1.85 (br s, 6H, H-21 and H-25), 1.77 (m, 1H, H-13), 1.53 (br d, 1H, J = 11.4, H-9), 1.3 (m, 2H, H12 and H13), 1.2 (m, 1H, H-10), 1.07 (d, 3H, J = 6.4, H-23), 0.96 (d, 3H, J = 6.0, H-24); ^{13}C NMR (CD_3OD) δ 200.1 (s, C-19), 169.8 (s, C-1), 154.3 (s, C-17), 144.0 (d, C-3), 142.9 (d, C-5), 141.1 (d, C-15), 140.5 (d, C-7), 134.1 (s, C-6), 132.7 (s, C-16), 130.5 (s, C-4), 117.1 (d, C-18), 115.9 (d, C-2), 79.5 (d, C-11), 68.1 (t, C-20), 47.0 (d, C-9), 37.6 (t, C-13), 37.2 (d, C-8), 37.0 (d, C-14), 34.4 (d, C-12), 32.7 (d, C-10), 18.2 (q, C-25), 17.9 (q, C-24), 17.3 (q, C-22), 15.1 (q, C-23), 14.0 (q, C-21); EIMS (70 eV) *m/z* [M^+] 414 (5), 396 (2), 366 (6), 337 (17), 281 (11), 275 (31), 187 (20), 150 (68), 105 (100), 91 (94), 55 (56); HREIMS *m/z* 414.2409 (calcd for $C_{25}H_{34}O_5$, 414.2406).

Methylation and Acetylation of 1 (2). Compound **1** (10 mg) was dissolved Et_2O (3 mL), and a solution of CH_2N_2 in Et_2O was added dropwise until the yellow color persisted. After stirring for 3 min at room temperature, the solvent was removed under a stream of nitrogen. The crude methyl ester was then dissolved in 500 μL of pyridine and 500 μL of Ac_2O and stirred at room temperature for 24 h. After that time the volatiles were removed under reduced pressure. The methyl ester, diacetate was purified by HPLC on Si gel using hexane with increasing amounts of isopropyl alcohol to give **2** as an oil (7.7 mg); ^1H NMR ($CDCl_3$) δ 7.31 (d, 1H, J = 16.0, H-3), 6.24 (br s, 1H, H-5), 6.12 (br s, 1H, H-18), 5.92 (br s, 1H, H-15), 5.83 (d, 1H, J = 16.0, H-2), 5.34 (br d, J = 9.0, H-7), 5.19 (br d, 1H, J = 9.0, H-8), 4.80 (d, 1H, J = 16.5, H-20), 4.68 (d, 1H, J = 16.5, H-20), 4.33 (t, 1H, J = 10.5, H-11), 3.75 (s, 3H, OCH_3), 2.73 (br s, 1H, H-14), 2.19 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.01 (s, 3H, H-22), 1.88 (br s, 6H, H-21 and H-25), 1.79 (m, 1H, H-13), 1.58 (br d, 1H, J = 16.0, H-9), 1.54 (m, 1H, H-12), 1.41 (m, 2H, H-10 and H-13), 0.90 (d, 3H, J = 6.5, H-23), 0.84 (d, 3H, J = 6.5, H-24); ^{13}C NMR ($CDCl_3$) δ 192.7 (s, C-19), 171.0 (s, OAc), 170.0 (s, OAc), 168.6 (s, C-1), 155.4 (s, C-17), 150.8 (d, C-3), 143.5 (d, C-5), 141.8 (d, C-15), 135.3 (s, C-6), 133.4 (d, C-7), 132.1 (s, C-4), 131.6 (s, C-16), 116.4 (d, C-18), 116.0 (d, C-2), 81.7 (d, C-11), 69.1 (t, C-20), 51.4 (q, OCH_3), 46.8 (d, C-9), 38.0 (t, C-13), 37.2 (d, C-8), 35.5 (d, C-12), 33.3 (d, C-14), 33.2 (d, C-10), 20.9 (q, OAc), 20.6 (q, OAc), 19.7 (q, C-25), 18.3 (q, C-24), 16.8 (q, C-22), 15.0 (q, C-23), 13.8 (q, C-21); EIMS (70 eV) *m/z* [M^+] 512 (1.1), 452 (1.9), 359 (52), 351 (15), 299 (13), 169 (12), 101 (11), 93 (11), 91 (12), 55 (15), 43 (100).

Reduction of 2. Compound **2** (5.0 mg) was dissolved in MeOH (2.0 mL), the solution cooled to 0 °C, and $NaBH_4$ (10.0 mg) was added. After stirring for 10 m, Me_2CO (2 mL) was added to destroy the excess reducing agent and the solvents removed under reduced pressure. The residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was separated and the solvent removed. The reduced products were purified by HPLC on Si gel using hexane with increasing amounts of isopropyl alcohol to give two major diastereomeric products, **3** and **4**, as oils.

Compound 3: ^1H NMR ($CDCl_3$) δ 7.28 (d,1H, J = 15.4, H-3), 6.19 (br s, 1H, H-5), 5.81 (d, 1H, J = 15.4, H-2), 5.45 (m, 3H, H-7, H-15 and H-18), 4.63 (dt, 1H, J = 8.3, 3.2, H-19), 4.32 (t, 1H, J = 10.2, H-11), 4.16 (dd, 1H, J = 11.3, 3.2, H-20), 3.97 (dd,1H, J = 11.3, 8.3, H-20), 3.82 (br d, 1H, J = 8.8, H-8), 3.73 (s, 3H, OCH_3), 2.59 (br s, 1H, H-14), 2.10 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.91 (br s, 3H, H-22), 1.88 (br s, 3H, H-21), 1.82 (br s, 3H, H-25), 1.74 (dm, 1H, J = 13.3, H-13), 1.6 (m, 1H, H-12), 1.5 (m, 2H, H-9 and H-10), 1.33 (dt, 1H, J = 13.3, 4.9, H-13), 0.93 (d, 3H, J = 6.3, H-23), 0.81 (d, 3H, J = 6.3, H-24); EIMS (70 eV) *m/z* [$M^+ - H_2O$] 496 (1), 454 (2), 436 (3), 363 (3), 307 (8), 281 (9), 228 (10), 199 (20), 169 (20), 91 (20), 43 (100).

Compound 4: ^1H NMR ($CDCl_3$) δ 7.29 (d, 1H, J = 15.6, H-3), 6.20 (br s, 1H, H-5), 5.82 (d, 1H, J = 15.6, H-2), 5.46 (br s, 1H, H-15), 5.45 (d, 1H, J = 8.4, H-18), 5.39 (d, 1H, J = 9.0, H-7), 4.63 (dt, 1H, J = 8.2,3.4, H-19), 4.31 (t, 1H, J = 10.3, H-11), 4.02 (dd, 1H, J = 11.2,3.4, H-20), 3.95 (dd, 1H, J = 11.2,8.1, H-20), 3.86 (br d, 1H, J = 9.0, H-8), 3.72 (s, 3H, OCH_3), 2.59 (br s, 1H, H-14), 2.07 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.89 (br s, 3H, H-22), 1.87 (br s, 3H, H-21), 1.81 (br s, 3H, H-25), 1.74 (dt, 1H, J = 13.4,3.4, H-13), 1.5 (m, 2H, H-9 and H-10), 1.6 (m, 1H, H-12), 1.33 (dt, 1H, J = 13.4,5.3, H-13), 0.93 (d, 3H, J = 6.3, H-23), 0.81 (d, 3H, J = 6.3, H-24); EIMS (70 eV) *m/z* [$M^+ - H_2O$] 496 (0.3), 436 (1), 376 (2), 351 (3), 281 (6), 228 (7), 213 (16), 169 (16), 91 (18), 43 (100).

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