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Gonioheptolides A and B: Novel Eight-Membered-Ring Lactones from *Goniothalamus giganteus* (Annonaceae)

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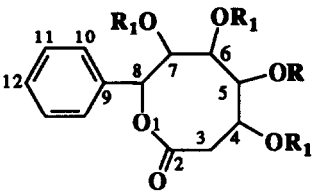
Key Words: Goniothalamus giganteus; gonioheptolides A and B; brine shrimp lethality; human tumor cytotoxicity.

Abstract: Two novel eight-membered-ring lactones, gonioheptolides A (1) and B (3), have been isolated from the bark of *Goniothalamus giganteus*. The structures were elucidated by IR, MS, ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra. 1 and 3 showed marginal cytotoxicities to certain human solid tumor cells in culture. A biogenetic pathway of the 14 styryllactones found in *G. giganteus* is proposed.

INTRODUCTION

In our previous bioactivity-directed studies, two major types of bioactive natural products have been found in the bark extract of *Goniothalamus giganteus* Hook. f. & Thomas (Annonaceae) which originally showed significant murine toxicity in the 3PS lymphocytic leukemia system.[1] The potent cytotoxic Annonaceous acetogenins: goniothalamicin, annonacin,[2] gigantecin,[3] gigantetrocin, gigantriocin,[4] giganenin, 4-deoxygigantecin,[5] gigantetronenin, and gigantrionenin,[6] and the less cytotoxic styryllactones: altholactone (syn: goniothalenol), goniothalamin[7] goniotriol,[8] goniopypyrone, goniofufurone, 8-acetylgoniotriol,[9] 9-deoxygoniopypyrone, 7-epigoniofufurone, goniodiol,[10] goniobutenolides A and B, and goniofupyrone[11] have been reported. Two novel eight-membered-ring lactones, named gonioheptolides A (1) and B (3), have now

been obtained in our continuing search for cytotoxic compounds of this plant. The structures of **1** and **3** were elucidated by IR, MS, ^1H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY specta as well as chemical derivatization. These compounds possess an unusual saturated eight-membered lactone moiety; a similar ring system, with less extensive substitution, was recently reported as the cytotoxic metabolite, octalactin A, from a marine bacterium.[12] Although **1** and **3** were not significantly toxic to brine shrimp[13,14] (BST LC_{50} > 400 ppm), they are both marginally cytotoxic against human tumor cell lines[15-17] [**1**, ED_{50} 4.25, 72.76, and 13.68 μg/ml and **3**, ED_{50} 24.31, 10.93, and 6.91 μg/ml in A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), and HT-29 (human colon adenocarcinoma), respectively]. Compounds showing cytotoxic ED_{50} values less than 4 μg/ml are considered significantly active in the search for new antitumor drugs; however, borderline cytotoxicity may be an indication of other useful bioactivities.



1 R = Me R_1 = H **2** R = Me R_1 = Ac
3 R = Et R_1 = H **4** R = Et R_1 = Ac

RESULTS AND DISCUSSION

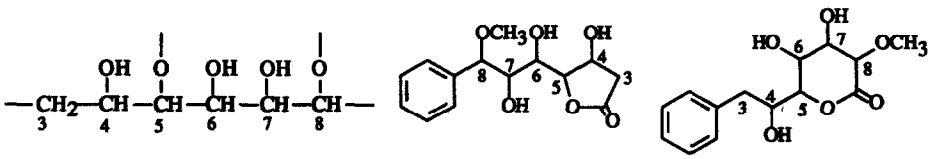
Compound **1** was isolated as a colorless oil. The molecular weight was indicated by a dominant peak at m/z 283 (MH^+) in the CIMS. The presence of three hydroxyl moieties was suggested by three successive losses of water (m/z 18) from the MH^+ in the CIMS and a broad IR absorption band at 3405 cm^{-1}. The molecular weight (M 282) and three hydroxyl groups both were confirmed by the preparation of a triacetate derivative (**2**) which gave peaks at m/z 409 (MH^+), 349 (MH^+ - AcOH), 289 (MH^+ - 2 AcOH), and 229 (MH^+ - 3 AcOH) in the CIMS and three-proton resonances at δ 2.10 (OAc), 2.08 (OAc), and 1.93 (OAc) in the ^1H NMR spectrum. The existence of a phenyl moiety was indicated by the proton resonances at δ 7.46 (d, H-10,14), 7.35 (t, H-11,13), and 7.30 (d, H-12) and carbon peaks at δ 139.4 (C-9), 128.4 (C-11,13), 128.0 (C-12), and 126.4 (C-10,14). A methoxyl moiety was indicated by a single proton peak at δ 3.70 (3 H) and a carbon resonance at δ 52.1 (C-15). Five other oxygen-bearing carbons were also suggested by the ^1H and ^{13}C NMR data (Table 1). A methylene group was indicated by proton resonances at δ 2.88 (dd, H-3a) and 2.64 (dd, H-3b) which coupled to each other with a J_{gem} = 16.7 Hz, and a carbon peak at δ 37.6 (C-3). The existence of an ester bond was supported by a prominant IR absorption at 1726 cm^{-1} and a carbonyl carbon peak at δ 173.2 (C-2). Thus, a molecular formula, $C_{14}H_{18}O_6$, was proposed and confirmed by high resolution CIMS measurement which gave m/z 283.1176 (calcd 283.1182) for the MH^+.

Table 1. NMR Data of Compounds 1 - 4

Atom	^{1}H [δ (J/Hz), 500 MHz, $CDCl_3$] 1	2	3	4	Atom	^{13}C (δ, 125 MHz,$CDCl_3$) 1	2	3
H-3b	2.64 dd (16.7, 3.9)	2.64 dd (15.7, 7.2)	2.62 dd (16.6, 3.9)	2.65 dd (15.5, 7.3)	C-2	173.2	170.3	173.0
					C-3	37.6	35.9	37.8
H-3a	2.88 dd (16.7, 8.8)	2.75 dd (15.7, 5.3)	2.86 dd (16.6, 8.8)	2.75 dd (15.5, 5.5)	C-4	67.8	68.3	67.6
					C-5	79.8	79.0	79.9
H-4	4.43 m	5.55 ddd (7.2, 5.5,5.3)	4.42 m	5.58 ddd (7.3, 5.5, 5.5)	C-6	79.6	76.1	79.4
					C-7	83.8	81.7	83.8
H-5	4.34 t (5.2)	4.51 t (5.5)	4.34 dd (6.3, 5.1)	4.54 dd (5.5, 5.2)	C-8	84.3	83.3	84.1
					C-9	139.4	138.4	139.5
H-6	4.09 dd (6.4, 5.2)	5.37 dd (5.5, 3.7)	4.07 dd (6.5, 6.3)	5.39 dd (5.2, 3.5)	C-10,14	126.4	126.1	126.5
					C-11,13	128.4	128.4	128.5
H-7	4.10 t (6.4)	5.18 dd (4.9, 3.7)	4.08 t (6.5)	5.20 dd (4.8, 3.5)	C-12	128.0	128.2	128.1
					5-OMe	52.1	52.0	61.0
H-8	4.59 d (6.4)	4.88 d (4.9)	4.58 d (6.5)	4.91 d (4.8)	or OEt	—	—	14.0
H-10,14	7.46 d (7.5)	7.46 d (7.5)	7.46 d (7.5)	7.49 d (7.2)	OAc*	—	170.1	—
H-11,13	7.35 t (7.5)	7.33 t (7.5)	7.34 d (7.5)	7.36 t (7.2)			21.0	
H-12	7.30 d (7.5)	7.28 d (7.5)	7.30 d (7.5)	7.30 d (7.2)	OAc*	—	170.0	—
5-OMe	3.70 s (3H)	3.67 s (3H)	4.15 q (7.1, 2H)	4.15 q (7.1, 2H)			20.8	
or OEt			1.26 t (7.1, 3H)	1.27 t (7.1, 3H)	OAc*	—	169.8	—
7-OAc	—	2.10 s (3H)	—	2.13 s (3H)			20.6	
6-OAc	—	2.08 s (3H)	—	2.10 s (3H)				
4-OAc	—	1.93 s (3H)	—	1.95 s (3H)				

* The ^{13}C NMR assignments for three acetyl groups are exchangeable.

The ^{1}H NMR and COSY spectra of the triacetate derivative (**2**) of **1** indicated that the three hydroxyl moieties were located on C-4, C-6, and C-7 according to the obvious down-field shifts of H-4 (δ 4.43 to 5.55), H-6 (δ 4.09 to 5.37), and H-7 (δ 4.10 to 5.18) after acetylation of **1**. The COSY spectrum of **2**, which clearly shows coupling correlations from H-3 through H-8, suggested the existence of fragment A. Considering the presence of a phenyl, a methoxyl, and three hydroxyl moieties as well as an ester bond, a possible structure of compound **1** was a saturated lactone ring with a phenyl group either connecting to C-8 or C-3, a methoxyl at C-5 or C-8, and three hydroxyl groups substituting on C-4, C-6 and C-7. Thus, three possible structures (**1**, **1a**, **1b**) were considered which could match with the above connections. However, a carbonyl IR absorption at 1726 cm^{-1}, a carbonyl carbon peak at δ 173.2 of compound **1** , and a carbonyl carbon peak at δ 170.1 of compound **2** eliminated the possibility of a saturated γ-lactone which usually shows an IR absorption at about 1770 cm^{-1} and a carbonyl carbon peak at about δ 177 to 178;[18-21] this observation excluded **1a**. Based on the possible biogenesis, **1b** then seemed more resonable because several similar styryl δ-lactones have been isolated from this plant.[7-11] However, the HMBC spectrum of **2** provided convincing evidence to support structure **1**; cross peaks were observed between the aromatic protons (H-10,14) and C-8 and between H-8 and C-10,14 (due to a 3-bond heteronuclear coupling), and a cross peak was observed between H-3 and C-2 (due to a 2-bond heteronuclear coupling) (Figure 1A). The NOESY spectrum of **2** also supported structure **1** when we tried to assign relative stereochemistry based on NOE effects. The NOESY spectrum showed cross peaks between some aromatic protons (H-10,14) with H-7 and H-8, respectively, and only **2** could possibly show such NOE effects between these protons (Figure 1B). The NOESY spectrum also showed two small cross peaks between the methoxyl protons and H-5 as well as H-6 to support the location of the methoxyl moiety at the C-5. The ^{13}C NMR spectrum of **1** was assigned by comparison with that of **2** which was assigned based on its HMQC spectrum.



Fragment A 1a 1b

The multiple stable conformations of the eight-membered lactone ring system, which are due to the high flexibility, pseudorotation, and inversion of eight-membered rings,[22] make the determination of the stereochemistry of this type of compound very complicated. X-ray crystallography would be a superior method for stereochemical studies, however, the amount of compound **1** was insufficient to prepare a suitable crystalline derivative. Therefore, we could only propose the possible relative stereochemistry of **1** based on the NOESY spectrum of **2**. The six low-energy conformations of the eight-membered lactone ring have been predicted by previous molecular mechanics calculations,[12,23] and two of them (one boat-chair and crown conformations) were demonstrated to exist in solid state by X-ray crystallography.[12,24] The NOESY spectrum of **2** showed strong cross peaks between H-8/H-6 and H-8/H-5 and small cross peaks between H-6/H-4, respectively, in addition to strong cross peaks for all vicinal protons. Considering those predicted low-energy conformations,[12,23] the six stable conformations of **2** were simulated and calculated for energy minimization by using a Quanta Program in Silicon Graphics. The measurements of the distances between different protons in all of these simulated conformations of **2** indicated that only the *cis* configuration of H-8, H-6, and H-5 could give close space distances between H-8/H-6 and H-8/H-5 and the *cis* configuration of H-6 and H-4 could give close space distance between H-6/H-4 (here, the close distance means it is similar to the distances between vicinal protons) in some stable conformations, respectively. The *trans* configuration between H-7 and H-5 was also suggested since no NOE cross peaks were observed. The measurements of the distances for the *cis* and *trans* configurations of H-7 and H-5 in the simulated stable conformations of **2** showed that the *cis* configuration had close space distance for H-7 and H-5 in some stable conformations and the *trans* configuration gave longer space distance for H-7 and H-5. Although, we can't explain all of the observed NOE effects in just one simulated conformation, the NOE result of **2** may still suggest the possible configuration of **1** when considering the high flexibility, pseudorotation, and inversion of eight-membered rings. As an example, the partial stereochemistry of an eight-membered homocephem compound was assigned by using similar NOE effects.[25] Thus, the possible relative stereochemistry of **1** was proposed as illustrated in Figure 1B.

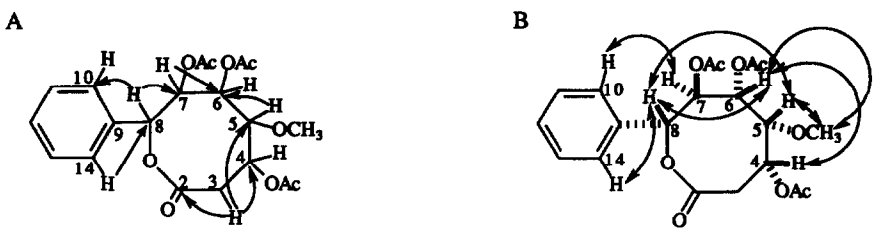
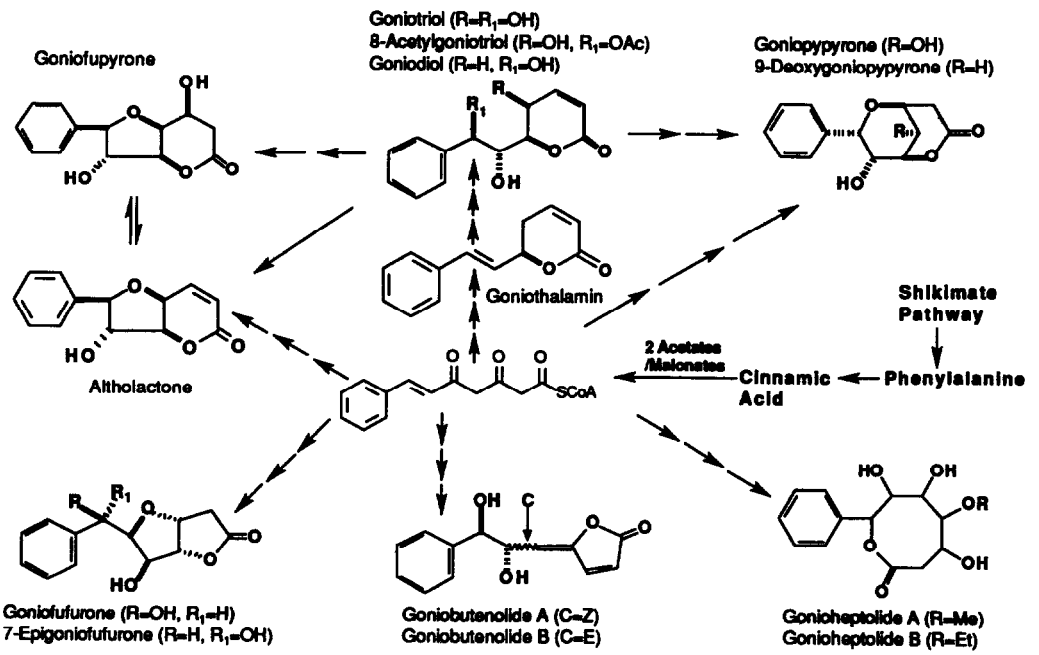


Figure 1. A. Long-range Heteronuclear Coupling Correlations of **2** from HMBC;
B. NOE Correlations of **2** for Non-Vicinal Protons from NOESY.

Compound **3** was also isolated as a colorless oil. The molecular weight of **3** was indicated by a peak at m/z 297 (MH^+) in the CIMS. The high resolution CIMS gave m/z 297.1335 (calcd 297.1338) for the MH^+, corresponding to the molecular formula, $C_{15}H_{20}O_6$. As with **1**, the presence of a phenyl moiety, three hydroxyl groups, a methylene moiety, five oxygen-bearing carbons, and an ester bond were indicated by IR, NMR, and preparation of a triacetate derivative (**4**), but an ethoxyl instead of a methoxyl moiety was evident. The assignments and comparisons of the ^1H and ^{13}C NMR (Table 1) and COSY spectra of **3** with those of **1**, as well as comparisons of ^1H NMR and COSY spectra of **4** with those of **2**, suggested that compound **3** had exactly the same structural skeleton as **1** but with an ethoxyl instead of a methoxyl group at C_5. The NOESY spectrum of **4** showed the same cross peak correlations as those of **2**, suggesting that these two compounds may have the same relative stereochemistry. These are novel natural products and were named gonioheptolides A (**1**) and B (**3**).

Compounds **1** and **3**, as well as 12 styryllactones previously reported from this plant,[7-11] all have a basic C_6-C_3-C_4 skeleton (phenylpropanoid connecting with four carbons). It is predicted that the biosynthesis of these compounds could follow the shikimic acid pathway, through phenylalanine to cinnamic acid (C_6-C_3) with incorporation of two acetate/malonate units to form the C_6-C_3-C_4 backbone.[26] By reductions, hydroxylations, and different cyclizations, the several different styryllactone ring systems can then be generated from this C_6-C_3-C_4 unit. A possible biogenetic pathway, to inter-relate the 14 styryllactones known to be formed in this species, is proposed in Scheme 1.



Scheme 1. Biogenetic Pathway Inter-relating the Styryllactones from *Goniothalamus giganteus*

EXPERIMENTAL

General experimental procedures. Mps were determined on a Mettler FP5 hot-stage apparatus and are uncorrected. The optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were taken in EtOH on a Beckman DU-7 spectrophotometer. IR spectra were obtained on salt plates on a Perkin Elmer 1600 FTIR spectrophotometer. Low resolution MS were recorded on a Finnigan 4000 mass spectrometer. The exact masses were determined on a Kratos 50 mass spectrometer through peak matching. All of 1D and 2D NMR spectra were recorded on a Varian VXR-500S spectrometer, using the Varian software systems.

Plant material. The stem bark of *G. giganteus* (B-826538, PR-50604) was collected in Thailand in Sept. 1978 under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, U.S.D.A., Beltsville, Maryland, where voucher specimens are maintained.

Bioassays. Brine shrimp lethality (BST) [13,14] was performed in our laboratory. The cytotoxicity tests against A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), and HT-29 (human colon adenocarcinoma)[15-17] cells were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with adriamycin as a positive standard control. Adriamycin gave ED_{50} values of 1.05×10^{-3}, 1.00×10^{-1}, and 1.50×10^{-3} μg/ml, respectively, when compounds **1** and **3** were tested.

Extraction and isolation. The crude residue of the 95% EtOH extract of 4 kg of the stem bark was partitioned between H_2O and $CHCl_3$ to give a H_2O layer and a $CHCl_3$ layer; the residue of the $CHCl_3$ layer was partitioned between hexane and 10% H_2O in MeOH to give a MeOH layer (ca. 100 g dry residue), and a hexane layer. The MeOH residue was repeatedly chromatographed over silica gel columns and chromatotron separations, directed by BST activity, using gradients of C_6H_6-EtOAc-MeOH, hexane-EtOAc, and $CHCl_3$-MeOH, and gave the oils of **1** (12 mg) and **3** (4 mg) as one-spot materials on TLC.

Gonioheptolide A (1). Colorless oil, $[\alpha]^{25}_D$ + 5.0 (c 0.5 in $CHCl_3$); UV λ_{max} (MeOH): 210 nm (log ε 3.6), 258 nm (log ε 2.2); IR ν_{max} (film) cm^{-1}: 3405 (s), 2920, 1726 (s), 1658, 1441, 1199, 1124, 1081, 1036, 910, 760, and 700; CIMS (isobutane) m/z (%): 283 (MH^+, 41), 265 (MH^+ - H_2O, 2), 251 (MH^+ - CH_3OH, 100), 247 (MH+ - 2 H_2O, 58), 233 (251 - H_2O, 33), 229 (MH+ - 3 H_2O, 24), 215 (251 - 2 H_2O, 13), 197 (251 - 3 H_2O, 2), 163 (84), 145 (50), 115 (11), and 91 (17); HRCIMS (isobutane) m/z: 283.1176 for $C_{14}H_{19}O_6$ (MH^+, calcd 283.1182) and 251.0914 for $C_{13}H_{15}O_5$ (calcd 251.0919); EIMS m/z (%): 264 (3), 251 (15), 233 (8), 215 (6), 163 (7), 144 (15), 133 (21), 125 (23), 120 (33), 117 (12), 107 (61), 105 (30), 97 (31), 91 (100), 79 (50), 77(67), 65 (27), and 55 (65); ^1H NMR (see Table 1); ^{13}C NMR (see Table 1); COSY (500 MHz, in $CDCl_3$, nt 16, ni 128, 2 K x 2 K).

Gonioheptolide A triacetate (2). **1** (4 mg) was acetylated (Ac_2O-pyridine; 24 h; room temp.), and the mixture was partitioned between water and $CHCl_3$. The $CHCl_3$ extract on concentration and silica gel micro-column chromatography afforded **2**, ca. 4 mg oil. $[\alpha]^{25}_D$ + 29.0 (c 0.2 in $CDCl_3$) CIMS (isobutane) m/z (%): 409 (MH^+, 53), 377 (MH^+ - CH_3OH, 8), 349 (MH^+ - AcOH, 100), 335 (377 - 42, 13), 289 (MH^+ - 2 AcOH, 14), 275 (335 - AcOH, 14), 229 (MH+ - 3 AcOH, 18), and 215 (335 - 2 AcOH, 16); ^1H NMR (see Table 1); COSY (500 MHz, in $CDCl_3$, nt 16, ni 128, 2 K x 2 K); HMQC[27] (500 MHz, in $CDCl_3$, nt 16, ni 512, j 140, dm 'nny', mbond 'n', d1 2, d2 0, null 0, presaturation not used, spin not used, 2 K x 2 K, by a Varian VXR-500S inverse-geometry probe); HMBC[27] (500 MHz, in $CDCl_3$, taumb 0.05 sec, dm 'nnn', mbond 'y', the same as HMQC for other parameters) (results see Figure 1A); NOESY (500 MHz, in $CDCl_3$, nt 16, ni 292, mix 0.4 sec, dm 'nnnn', 2 K x 2 K) (results see Figure 1B).

Gonioheptolide B (3). Colorless oil, $[\alpha]^{25}_D$ + 17.3 (c 0.2 in $CHCl_3$); UV λ_{max} (MeOH): 209 nm (log ε 3.6), 257 nm (log ε 2.3); IR ν_{max} (film) cm^{-1}: 3416 (s), 2925, 1725 (s), 1496, 1453, 1287, 1187, 1041, 912, 760, and 700; CIMS (isobutane) m/z (%): 297 (MH^+, 57), 279 (MH^+ - H_2O, 7), 261 (MH^+ - 2 H_2O, 43), 251 (MH^+ - EtOH, 77), 243 (MH+ - 3 H_2O, 17), 233 (251 - H_2O, 100), 215 (251 - 2 H_2O, 36), 197 (251 - 3 H_2O, 2), 177 (57), 159 (32), 137 (17), 131 (15), 119 (19), 115 (43), 107 (14), and 91 (31); HRCIMS (isobutane) m/z: 297.1335 for $C_{15}H_{21}O_6$ (MH^+, calcd 297.1338); EIMS m/z (%): 171 (2), 162 (3), 149 (2), 145 (3), 144 (3), 133 (10), 131 (7), 125 (6), 120 (10), 119 (2), 115 (10), 107 (23), 105 (19), 97 (10), 91 (30), 79 (25), 77 (36), 65 (10), and 55 (41); ^1H and ^{13}C NMR (see Table 1); COSY (500 MHz, in $CDCl_3$, nt 16, ni 128, 2 K x 2 K).

Gonioheptolide B triacetate (4). Acetylation of **3** (2 mg) by the same procedure as with **1** gave **4**, ca. 1.5 mg oil. $[\alpha]^{25}_D$ + 28.0 (c 0.15 in $CDCl_3$); CIMS (isobutane) m/z (%): 423 (MH^+ - AcOH, 63), 377 (MH^+ - EtOH, 9), 363 (MH^+ - AcOH, 100), 335 (363 - CO, 12), 303 (MH^+ - 2 AcOH, 20), 275 (335 - AcOH, 7), 261 (8), 257 (377 - 2 AcOH, 1), 243 (MH^+ - 3 AcOH, 10), and 215 (243 - CO, 7); ^1H NMR (see Table 1); COSY (500 MHz, in $CDCl_3$, nt 16, ni 128, 2 K x 2 K); NOESY (500 MHz, in $CDCl_3$, nt 16, ni 292, mix 0.4 sec, dm 'nnnn', 2 K x 2 K).

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