Lehualides A−D, Metabolites from a Hawaiian Sponge of the Genus *Plakortis*[⊥]

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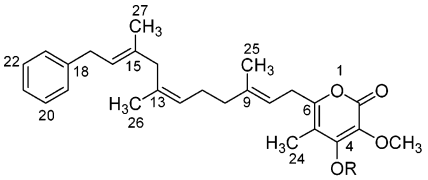
A collection of an undescribed marine sponge of the genus *Plakortis* yielded four new "polyketide-derived" metabolites, lehualides A−D (**1**−**4**). The structures of compounds **1**−**4** were elucidated by interpretation of spectral data. Compound **2** demonstrated cytotoxicity against an ovarian cancer cell line, while compound **4** was active against both ovarian cancer and leukemia cell lines.

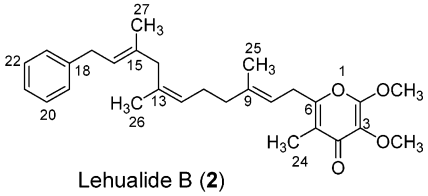
Marine sponges in the family Plakinadae are known to be rich sources of structurally unique and biologically active metabolites. Plakortin[1] and structurally related cycloperoxide ring-containing polyketides[1b−d] are commonly found in the extracts of *Plakortis*. These compounds have a broad range of biological activities such as antibacterial,[1b] antileishmanical,[1c] and cytotoxic.[1d,e] In addition, other recent examples include potent immunosuppressive agents, the plakosides,[2] and the activators of cardiac SR-Ca^{2+}-pumping ATPase, the plakortones.[3] In our continuing program to search for new bioactive compounds from marine organisms, it was found that the crude extract of an undescribed Hawaiian *Plakortis* sp. exhibited toxicity against brine shrimp. Bioassay-guided fractionation of the extract led to the isolation of four new metabolites, lehualides A−D (**1**−**4**). Herein, we report the details of the isolation, structure elucidation, and cytotoxicity of these new metabolites.

The sample of sponge was collected by hand using scuba from waters between Lehua Rock and Niihau Island, Hawaii, in July 2003. The freeze-dried sponge was repeatedly extracted with dichloromethane and 2-propanol (1:1). The crude extracts were combined, concentrated in vacuo, and partitioned between hexane and 80% aqueous MeOH. The aqueous MeOH layer was further extracted with dichloromethane. The dichloromethane-soluble materials were first chromatographed over silica gel vacuum liquid chromatography and followed by silica gel column chromatography. The brine shrimp toxic fractions were further purified by normal-phase HPLC to provide lehualides A (**1**, 26.4 mg), B (**2**, 28.7 mg), C (**3**, 10.6 mg), and D (**4**, 10.8 mg).

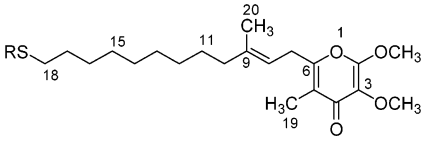
The HREIMS of lehualide A (**1**) revealed a molecular ion at *m/z* 422.2461, which was consistent with a molecular formula of $C_{27}H_{34}O_4$ and required 11 degrees of unsaturation. The ^{1}H NMR signals of **1** resonating in the aromatic region (δ 7.15 (3H) and 7.26 (2H)) and the presence of three aromatic signals [δ 141.7 (1 C), 128.3 (4 CH), and 125.7 (1CH)] in the ^{13}C NMR spectrum indicated the presence of a phenyl ring. Further analysis of the ^{13}C NMR spectrum of **1** showed that 10 out of the remaining 20 carbons



Lehualide A (**1**) R = H
Lehualide A acetate (**5**) R = Ac



Lehualide B (**2**)



Lehualide C (**3**) R = Ac
Lehualide D (**4**) R = H

resonated in the olefinic region and one was a carbonyl carbon (δ 160.2). Thus, 10 out of 11 degrees of unsaturation are now accounted for and lehualide A (**1**) must contain one additional ring.

Inspection of the ^{1}H−^{1}H COSY NMR spectrum of **1** allowed us to establish three spin systems: C-7 to C-8, C-10 to C-12, and C-16 to C-17 (Figure 1). The three-bond HMBC correlations from C-9 (δ 138.4) to H_2-7 (δ 3.19) and H_2-11 (δ 2.11) readily placed C-9 between C-8 (δ 117.4) and C-10 (δ 39.7). The C-9 olefinic quaternary carbon was shown to possess a methyl group subtituent based on the observed HMBC correlations between C-9 and a vinyl methyl group at δ 1.68 (H_3-25). Similarly, C-13, a quaternary olefinic carbon resonating at δ 133.5, was located between C-12 (δ 126.0) and an isolated methylene carbon, C-14 (δ 41.8), due to HMBC correlations from it to H_2-11 (δ 2.11) and H_2-14 (δ 2.73). The placement of the vinyl methyl group C-26 (δ 23.3) on C-13 was possible through HMBC correlations from C-12 and C-13 to H_3-26 (δ 1.59). The HMBC cross-peaks shown from C-15 (δ 134.0) to H_2-14 and H_2-17 (δ 3.36) clearly indicated that C-15 is adjacent to C-14 and C-16 (δ124.0). In addition, a vinyl methyl group (C-27; δ 15.9) was also found to be attached to C-15, due to the HMBC correlation shown from C-15 to H_3-27 (δ 1.63).

[⊥] Dedicated to the late Dr. Noriko Sata for her major contribution to this work.

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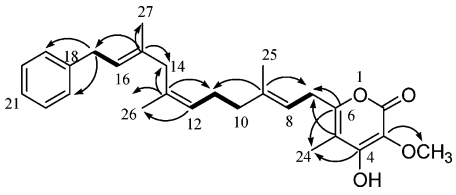

Figure 1. Spin systems deduced by the COSY spectrum (bold linkages) and key HMBC correlations (C→H arrows) of lehualide A (**1**).

Comparison of the molecular formula of lehualide A (**1**) with the assembled carbon framework required the elucidation of a $C_7H_7O_4$ subunit. The strong absorption at 1720 cm^{-1} in the IR spectrum and absorbance in the UV spectrum (λ_{max} = 242 and 292 nm) of compound **1** are in accordance with an extended α-pyrone chromophore.[4] The carbon signals observed at δ 160.2 (C-2), 124.9 (C-3), 157.8 (C-4), 105.6 (C-5), and 157.1 (C-6) in the ^{13}C NMR spectrum of **1** further confirmed the presence of an α-pyrone ring. Since all of the carbons of the α-pyrone ring are nonprotonated, as indicated by the HSQC and DEPT spectra, the α-pyrone ring must be fully substituted. A vinyl methyl group at C-24 (δ 9.28) was placed on C-5 due to the observed HMBC correlations from the polarized olefinic carbons (C-4 and C-6) to H_3-24 (δ 1.95). Oxygen atoms were placed on C-3 and C-4 of **1** since both carbons resonated at relatively high field. One of these oxygen atoms belonged to a methoxy group, which was placed at C-3 on the basis of a HMBC correlation shown from C-3 to the methoxy protons (δ 3.90). Thus, the remaining hydroxyl group is located on C-4. This placement was assured by an observed upfield shift of the C-4 carbon signal by 7.1 ppm in the ^{13}C NMR spectrum of lehualide A acetate (**5**). The HMBC cross-peaks shown from C-5 and C-6 to H_2-7 linked the 4-hydroxyl-3-methoxy-5-methyl-α-pyrone to the C-7 methylene group. Finally, the HMBC correlation shown from C-17 to H-19/H-23 attached the phenyl moiety to C-17, the methylene carbon of the olefinic chain, concluding the structure of lehualide A (**1**).

The geometry of the double bonds in the structure of lehualide A was primarily accomplished by interpretation of NOESY spectral data. The Δ^8 double bond was assigned as *E* stereochemistry on the basis of H_2-7/H_3-25 and H-8/H_2-10 NOESY correlations. The observed H_2-11/H_2-14 and H-12/H_3-26 NOESY cross-peaks supported the *Z* geometry of the Δ^{12} double bond. Finally, the H_2-17/H_3-27 NOESY correlation established the *E* stereochemistry of the Δ^{15} double bond.

HREIMS of lehualide B (**2**) gave an $[M]^+$ peak at 436.2640, yielding a molecular formula of $C_{28}H_{36}O_4$, with 11 degrees of unsaturation. The ^1H NMR of compound **2** was strikingly similar to that of **1** except for an additional methoxy group. The observed carbon signals at δ 159.7 (C-2), 128.2 (C-3), 176.6 (C-4), 119.7 (C-5), and 156.6 (C-6) in the ^{13}C NMR spectrum of **2** are consistent with the presence of a γ-pyrone moiety.[5] This was further supported by a strong absorption band at 1655 cm^{-1} in the IR spectrum of **2**.[5] The two *O*Me groups were assigned to C-2 and C-3 of the γ-pyrone ring due to the observed HMBC cross-peaks shown from C-2 to δ 3.97 (*O*Me-1) and from C-3 to δ 3.78 (*O*Me-2). The placement of the vinyl methyl group (C-24) on C-5 was based on HMBC correlations from both conjugated carbonyl carbon C-4 and the polarized olefinic carbon C-6 to H_3-24 (δ 1.97). Further analysis of 1D and 2D NMR spectra of **2** confirmed that the remainder of its chemical structure as well as its double-bond geometries were identical to those of lehualide A (**1**).

Lehualide C (**3**) was assigned the molecular formula $C_{23}H_{36}O_5S$ by HREIMS, requiring six degrees of unsaturation. Analysis of the ^1H NMR spectrum of compound **3** (Table 2) revealed, in contrast to lehualides A and B, the presence of only one vinyl proton at δ 5.17 (H-8) and the absence of phenyl proton signals. The observed resonances at δ 159.8 (C-2), 127.4 (C-3), 176.7 (C-4), 119.8 (C-5), and 156.8 (C-6) in the ^{13}C NMR spectrum (Table 2) clearly indicated that compound **3** possesses a γ-pyrone moiety, which accounted for four out of six degrees of unsaturation. The substitution pattern on the γ-pyrone ring of **3** was shown to be identical to that of **2** through interpretation of the HMBC spectral data. Of eight olefinic carbons shown in the ^{13}C NMR spectrum of **3**, five were assigned to the γ-pyrone system. Thus, compound **3** must contain a double bond [δ 116.4 (C-8) and 139.6 (C-9)] and a carbonyl carbon (δ 196.1), which also accounted for the last two degrees of unsaturation. As it was for compounds **1** and **2**, the HMBC correlations shown from both C-8 and C-9 to H_2-7 (δ 3.27) linked the double bond to the isolated methylene carbon (C-7). The cross-peaks shown from C-5 and C-6 to H_2-7 in the HMBC spectrum led to the attachment of the γ-pyrone ring to C-7. The C-9 olefinic quaternary carbon was shown to possess a methyl group substituent through an observed HMBC correlation shown between it and a vinyl methyl group at δ 1.70 (H_3-20). The carbonyl carbon was assigned to an acetyl group due to its HMBC correlation with a methyl proton at δ 2.32 in the HMBC spectrum.

Comparison of the above assigned partial structure with the molecular formula, the final assembling of the carbon framework of compound **3**, requires the assignment of a saturated hydrocarbon chain with an attached sulfur atom ($C_9H_{18}S$). The assignment of each nonoverlapped carbon and proton signal was accomplished by analysis of the 1D and 2D NMR data. The HMBC correlation shown from C-10 (δ 39.5) to H_3-20 allowed us to join the C-10 end of the hydrocarbon chain to the double bond. Therefore, the other end of the hydrocarbon chain must bear a thioester group. This conclusion was supported by the diagnostic chemical shift of C-18 (δ 29.1)[6] and the HMBC correlation shown from the carbonyl carbon to the methylene protons at δ 2.85 (H_2-18), completing the structure of lehualide C (**3**).

The ^1H and ^{13}C NMR spectra (Table 2) of lehualide D (**4**) were found to be strikingly similar to those of lehualide C (**3**) except for the absence of the acetyl group. This was supported by the HREIMS, which revealed the molecular formula of **4** to be $C_{21}H_{34}O_4S$. Detailed analyses of the 1D and 2D NMR spectra further confirmed the structure of lehualide D (**4**) to be a thiol analogue of lehualide C (**3**).

Previous chemical studies of sponges in the family Plakinadae have resulted in simple polyketide-derived compounds[1d] and numerous structurally modified polyketides with cyclic peroxides,[1] peroxylactones,[7] and peroxyketals.[8] Lehualides A (**1**) and B (**2**) are structurally similar to other phenyl-containing compounds isolated from *Plakortis* sp.[1a] and *P. halichondrioides*.[9] However, the lehualides appear to be the first set of polyketide metabolites from *Plakortis* sp. to possess α- and γ-pyrone moieties. Interestingly, lehualides C (**3**) and D (**4**) represent the only metabolites from the sponge of this genus to contain the thioester and thiol functionalities.

Lehualide A (**1**) showed mild brine shrimp toxicity (LD_{50} 50 μg/mL), while lehualides B (**2**), C (**3**), and D (**4**) were moderately toxic to brine shrimp (LD_{50} 15 μg/mL). Lehualide B (**2**) showed moderate cytotoxicity in vitro against an ovarian cancer cell line (IGROV-ET) with a GI_{50} value of

Table 1. NMR Spectral Data of Lehualides A (**1**) and B (**2**) Recorded in $CDCl_3$

	lehualide A (**1**)			lehualide B (**2**)	
C/H no.	δ_C	δ_H (*J* in Hz)	HMBC[a]	δ_C	δ_H (*J* in Hz)
2	160.2 qC			159.7 qC	
3	124.9 qC			128.2 qC	
4	157.8 qC			176.6 qC	
5	105.6 qC			119.7 qC	
6	157.1 qC			156.6 qC	
7	30.2 CH_2	3.19 d (6.9)	C: 5, 6, 8, 9	29.9 CH_2	3.25 d (7.2)
8	117.4 CH	5.18 br t (6.9)	C: 6, 7, 10, 25	116.7 CH	5.18 br t (7.2)
9	138.4 qC			139.0 qC	
10	39.7 CH_2	2.05 t (6.9)	C: 8, 9, 11, 12, 25	39.5 CH_2	2.01 t (7.2)
11	26.4 CH_2	2.11 t (6.9)	C: 9, 10, 12, 13	26.2 CH_2	2.11 t (7.2)
12	126.0 CH	5.19 m	C: 11, 14, 26	125.6 CH	5.18 m
13	133.5 qC			133.4 qC	
14	41.8 CH_2	2.73 br s	C: 12, 13,15, 16, 26, 27	41.7 CH_2	2.72 br s
15	134.0 qC			133.7 qC	
16	124.0 CH	5.36 tq (7.5, 1.0)	C: 14, 17, 18, 27	124.0 CH	5.34 br t (7.5)
17	34.2 CH_2	3.36 d (7.5)	C: 15, 16, 18, 19, 23	34.1 CH_2	3.35 d (7.5)
18	141.7 qC			141.5 qC	
19	128.3 CH	7.15 m	C: 17, 21, 23	128.2 CH	7.13 m
20	128.3 CH	7.26 m	C: 18, 22	128.2 CH	7.25 m
21	125.7 CH	7.15 m	C: 19, 23	125.6 CH	7.13 m
22	128.3 CH	7.26 m	C: 18, 20	128.2 CH	7.25 m
23	128.3 CH	7.15 m	C: 17, 21, 19	128.2 CH	7.13 m
24	9.3 CH_3	1.95 br s	C: 4, 5, 6	9.6 CH_3	1.97 s
25	16.4 CH_3	1.68 br s	C: 8, 9, 10	16.2 CH_3	1.69 br s
26	23.3 CH_3	1.59 br s	C: 12, 13, 14	23.2 CH_3	1.58 br s
27	15.9 CH_3	1.63 d (1.0)	C: 14, 15, 16	15.8 CH_3	1.62 br s
*O*Me-1	59.8 CH_3	3.90 s	C: 3	56.1 CH_3	3.97 s
*O*Me-2				60.2 CH_3	3.78 s
OH		6.70 br s			

[a] Correlations were observed after optimization for $^nJ_{CH} = 7$ Hz. Protons showing long-range correlation with indicated carbon.

Table 2. NMR Spectral Data of Lehualides C (**3**) and D (**4**) Recorded in $CDCl_3$

	lehualide C (**3**)		lehualide D (**4**)	
C/H no.	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	159.8 qC		159.8 qC	
3	127.4 qC		127.4 qC	
4	176.7 qC		176.8 qC	
5	119.8 qC		119.8 qC	
6	156.8 qC		156.9 qC	
7	30.0 CH_2	3.27 d (7.2)	30.0 CH_2	3.27 d (6.9)
8	116.4 CH	5.17 t (7.2, 1.2)	116.4 CH	5.17 t (6.9, 1.0)
9	139.6 qC		139.6 qC	
10	39.5 CH_2	2.00 t (7.2)	39.5 CH_2	2.05 t (7.1)
11	27.8 CH_2	1.33 quint (7.2)	27.8 CH_2	1.34 quint (7.1)
12	29.4 CH_2	1.25 m	29.4 CH_2	1.24 m
13	29.4 CH_2	1.25 m	29.4 CH_2	1.24 m
14	29.4 CH_2	1.25 m	29.4 CH_2	1.24 m
15	29.4 CH_2	1.25 m	29.4 CH_2	1.24 m
16	29.4 CH_2	1.25 m	29.4 CH_2	1.24 m
17	29.5 CH_2	1.59 m	29.2 CH_2	1.66 m
18	29.1 CH_2	2.85 t (7.2)	39.0 CH_2	2.66 t 7.5
19	9.7 CH_3	1.95 s	9.7 CH_3	1.95 s
20	16.2 CH_3	1.70 d (1.2)	16.2 CH_2	1.69 d 1.0
*O*Me-1	56.3 CH_3	3.90 s	56.3 CH_3	3.99 s
*O*Me-2	60.3 CH_3	3.79 s	60.3 CH_3	3.78 s
*S*Ac	196.1 CH_3	2.32 s		
	30.6 CH_3			

0.83 μM. Lehualide D (**4**) exhibited moderate cytotoxicity to ovarian (IGROV-ET) and leukemia (K562) cell lines with GI_{50} values of 0.73 and 0.23 μM, respectively. Interestingly, lehualides A (**1**) and C (**3**) did not show significant levels of cytotoxicity against any of the cancer cell lines.

Experimental Section

General Experimental Procedures. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 FTIR. The ^1H and ^{13}C NMR spectra were recorded on 300 and 500 MHz NMR spectrometers. ^1H chemical shifts are referenced to the residual $CDCl_3$ signal (δ 7.26), and ^{13}C chemical shifts are referenced to the $CDCl_3$ solvent peak (δ 77.0). Low- and high-resolution EIMS were recorded on a VG-70SE mass spectrometer. Silica gel plates were used for analytical thin-layer chromatography. Chromatographic silica gel (200−425 mesh) was used for normal flash and column chromatography. All solvents were distilled from glass prior to use.

Animal Material. The sponge was collected from the vertical walls of large caves at depths of 10−20 m between Lehua Rock and the north shore of Niihau Island, Hawaii. The sponge forms small oval encrustations about 1 cm thick; the surface is covered in short thick nodules about 2−4 mm high and wide. The texture is corky and leathery. In life the sponge is dark chocolate brown, some specimens being somewhat lighter in color. The sponge has diod spicules in one to two size categories, about 180−250 μm in length and occasional triods 110 μm in total length. The sponge is an undescribed species of *Plakortis* (order Homosclerophorida, family Plakinidae) closest to *P. ceylonica* (Dendy, 1905) in the large size of the diods. A voucher specimen has been deposited in the Natural History Museum, London (BMNH 2003.9.25.2).

Extraction of *Plakortis* and Isolation of Lehualides A−D (1−4). A freeze-dried sample of sponges (58.0 g dry weight) was immersed in dichloromethane/2-propanol (1:1) at room temperature. The combined extracts were concentrated in vacuo. The crude extract was partitioned between hexane and MeOH/H_2O (80:20). The aqueous MeOH layer was subsequently extracted with dichloromethane. The dichloromethane layer (4.11 g) was subjected to silica gel vacuum liquid chromatography using stepwise gradient elution (hexane/EtOAc/CH_2Cl_2/MeOH). The fraction eluted with 100% hexane was further fractionated by silica gel gravity column chromatography [hexane/EtOAc (2:1)] to provide a total of 11 fractions. Normal-phase HPLC [Phenomenex Sphereclone silica, 10 μm, 250 × 21.2 mm, gradient elution from hexane/2-propanol (95:5) to 100% 2-propanol in 50 min] of the brine shrimp toxic fractions (fractions 8−11) provided four new compounds,

lehualides A (**1**, 26.4 mg), B (**2**, 28.7 mg), C (**3**, 10.6 mg), and D (**4**, 10.8 mg).

Preparation of Lehualide A Acetate (5). Lehualide A (**1**, 4.1 mg) was treated with acetic anhydride (100 μL) and pyridine (100 μL) and stirred at room temperature for 16 h to yield 2.2 mg of lehualide A acetate (**5**).

Lehualide A (1): clear oil; UV (MeOH) λ_{max} (log ϵ) 242 (3.77), 292 (3.63) nm; IR (neat) ν_{max} 3300, 2927, 1720, 1687, 1650, 1439, 1093 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS (M^+) *m/z* 422.2461 (calcd for $C_{27}H_{34}O_4$, Δ −0.4 mmu).

Lehualide B (2): clear oil; UV max (MeOH) λ_{max} (log ϵ) 292 (3.92) nm; IR (neat) ν_{max} 2927, 1655, 1453, 1377, 1331, 1265, 1154, 1029 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HREIMS (M^+) *m/z* 436.2640 (calcd for $C_{28}H_{36}O_4$, Δ −2.6 mmu).

Lehualide C (3): clear oil; UV (MeOH) λ_{max} nm (log ϵ) 240 (3.92) nm; IR ν_{max} (film) 2927, 1690, 1657, 1465, 1335, 1156 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2; HREIMS (M^+) *m/z* 424.2266 (calcd for $C_{23}H_{36}O_5S$, Δ 1.7 mmu).

Lehualide D (4): clear oil; UV (MeOH) λ_{max} (log ϵ) 252 (3.92) nm; IR ν_{max} (film) 2925, 1659, 1464, 1378, 1333, 1266, 1156, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2; HREIMS (M^+) *m/z* 382.2190 (calcd for $C_{21}H_{34}O_4S$, Δ −1.2 mmu).

Lehualide A acetate (5): clear oil; ^{1}H NMR (300 MHz, $CDCl_3$) 3.20 (d, 2H, J = 6.6 Hz, H-7), 5.18 (brt, 1H, J = 6.6 Hz, H-8), 2.00 (m, 2H, H-10), 2.11 (m, 2H, H-11), 5.20 (br t, 1H, J = 6.3 Hz, H-12), 2.74 (br s, 2H, H-14), 5.36 (tq, 1H, J = 7.2, 1.2 Hz, H-16), 3.37 (d, 2H, J = 7.2 Hz, H-17), 7.16 (m, 3H, H-19, 21, 23), 7.27 (m, 2H, H-20, 22), 1.85 (s, 3H, H-24), 1.68 (s, 3H, H-25), 1.59 (s, 3H, H-26), 1.64 (s, 3H, H-27), 3.87 (s, 3H, *O*Me), 2.34 (s, 3H, *O*Ac); ^{13}C NMR (300 MHz, $CDCl_3$) 160.5 s (C-2), 134.0 s (C-3), 150.7 s (C-4), 107.8 s (C-5), 155.6 s (C-6), 30.2 t (C-7), 117.2 d (C-8), 138.6 s (C-9), 39.7 t (C-10), 26.3 t (C-11), 125.9 d (C-12), 133.3 s (C-13), 41.8 t (C-14), 134.0 s (C-15), 124.0 d (C-16), 34.2 t (C-17), 141.7 s (C-18), 128.3 d (C-19, 20, 22, 23), 125.6 d (C-21), 10.0 q (C-24), 16.4 q (C-25), 23.3 q (C-26), 15.9 q (C-27), 59.2 q (OMe), 20.4 q (OAc), 166.8 s (OAc); positive ion EIMS (M^+) *m/z* 464.2590 (calcd for $C_{29}H_{36}O_5$, Δ −2.7 mmu).

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