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ADDITIONAL BIOACTIVE ACETOGENINS, ANNOMUTACIN AND (2,4-*TRANS* AND *CIS*)-10*R*-ANNONACIN-A-ONES, FROM THE LEAVES OF *ANNONA MURICATA*

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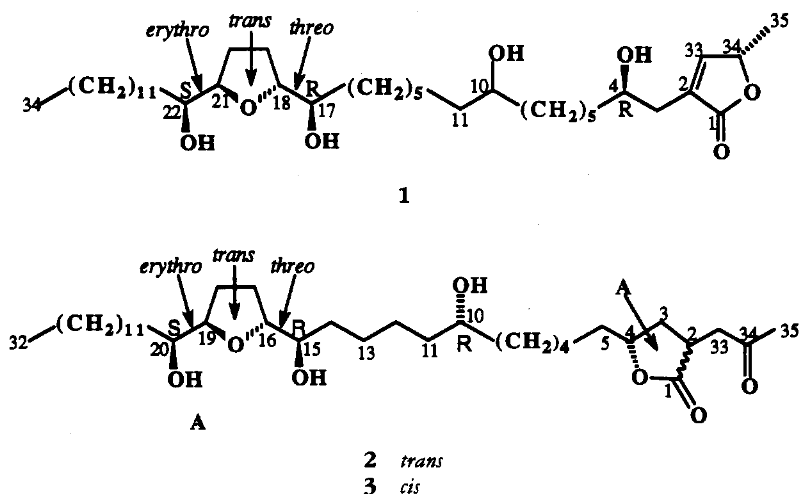
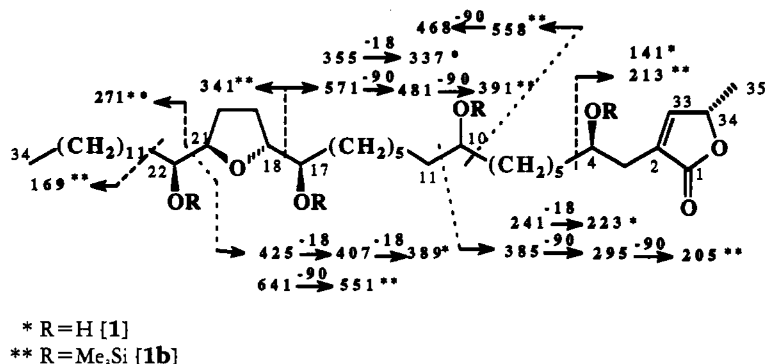
ABSTRACT.—In a continuation of our research on bioactive components from the leaves of *Annona muricata*, three novel monotetrahydrofuran Annonaceous acetogenins, namely, annomutacin [**1**], (2,4-*trans*)-10*R*-annonacin-A-one [**2**], and (2,4-*cis*)-10*R*-annonacin-A-one [**3**], have been identified. Their structures were deduced by ms, nmr, ir, and uv spectral and chemical methods, and the absolute configurations were determined by Mosher ester methodology. A known bioactive amide, *N*-*p*-coumaroyl tyramine, was also found. Compound **1** and the mixture of compounds **2** and **3** showed selective cytotoxicities against the human A-549 lung tumor cell line.

Annona muricata L. (Annonaceae) is a small tropical fruit tree whose seeds have yielded a number of cytotoxic and pesticidal monotetrahydrofuran (mono-THF) acetogenins (1–9). Previous studies with the leaves have resulted in the isolation of the novel mono-THF acetogenins, annomuricins A, B, and C (10,12), and muricatocins A, B, and C (11,12). In our continuing work, the leaves have now afforded the new bioactive mono-THF acetogenins, annomutacin [**1**] and a mixture of (2,4-*trans*)- 10*R*-annonacin-A-one [**2**] and (2,4-*cis*)-10*R*-annonacin-A-one [**3**]. The known bioactive amide, *N*-*p*-coumaroyl tyramine (13), was also isolated from the leaves.

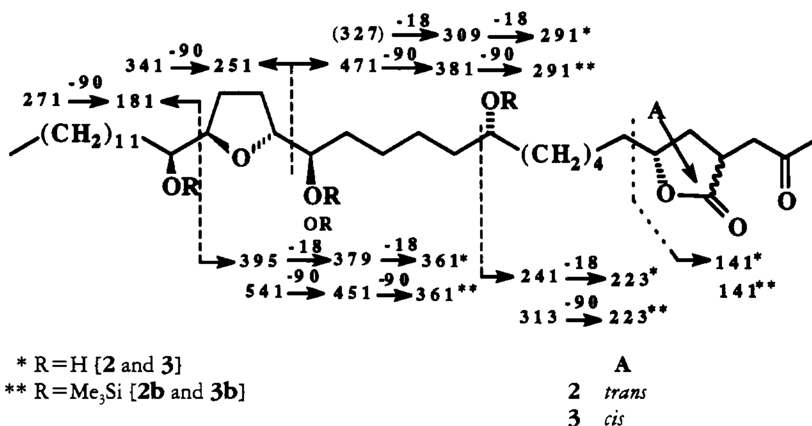
As described previously (10), the dried, powdered, leaves of *A. muricata*, obtained from plantation trees growing in Java, were extracted with 95% EtOH; the residue of the extract (F001) was partitioned through a standard extraction scheme (see Experimental), and the





* R=H [**1**]
** R=Me_3Si [**1b**]

FIGURE 1. Diagnostic eims fragment ions (m/z) of annomutacin [**1**] and its penta-TMSi derivative [**1b**].



* R=H [**2** and **3**]
** R=Me_3Si [**2b** and **3b**]

	A
2	*trans*
3	*cis*

FIGURE 2. Diagnostic eims fragment ions (m/z) of **2** and **3** and their tri-TMSi derivatives [**2b** and **3b**].

partition residues were evaluated for toxicity with a test for lethality to brine shrimp larvae (BST) (14,15). The most active fraction (F005, BST LC_{50} 0.17 µg/ml) was subjected to cc over Si gel eluted by gradients of hexane/EtOAc and EtOAc/MeOH. The fractions were analyzed by tlc and evaluated in the BST assay (10). A pool of active fractions was further subjected to repeated cc and hplc to yield compound **1** and a mixture of **2** and **3**. The known bioactive amide, *N-p*-coumaroyl tyramine, was isolated from another pool of active fractions using similar methods.

Annomutacin [**1**] and the mixture of (2,4-*trans*)-[**2**] and (2,4-*cis*)-10*R*-annonacin-A-ones [**3**] were obtained as colorless waxes. The ms (Figures 1 and 2) and nmr spectra indicated that **1–3** are mono-THF ring acetogenins (4–6). The hrfabms gave $[MH]^+$ ions at m/z 625.5010 (calcd 625.5043) and 597.4706 (calcd 597.4730), consistent with molecular formulas of $C_{37}H_{68}O_7$ [**1**] and $C_{35}H_{64}O_7$ [**2** and **3**], respectively. Compounds **1–3** showed a broad OH stretching absorption in the ir spectrum at 3250–3550 cm^{-1}. Four and three successive losses of H_2O (m/z 18), respectively, from the $[MH]^+$ from **1** and the mixture of **2** and **3** in the cims, at m/z 607, 589, 571, and 553 [**1**] and m/z 579, 561, and 543 (**2** and **3**), indicated the existence of four OH groups in **1** and three OH groups in **2** and **3**; these were confirmed by the formation of the tetra-acetate [**1a**] and the tetra-trimethylsilyl (TMSi) ether [**1b**] for **1**

and the tri-acetates [**2a** and **3a**] and the tri-trimethylsilyl (TMSi) ethers [**2b** and **3b**] for **2** and **3**. Compound **1a** gave four singlet proton peaks at δ 2.03, 2.04, 2.05, and 2.08, and compounds **2a** and **3a** gave three singlet proton peaks at δ 2.04, 2.05, and 2.08 (Table 1). The positions of the OH groups in **1** were assigned at C-4, C-10, C-17, and C-22, and in **2** and **3** at C-10, C-15, and C-20, by careful analysis of the fragments in the eims spectra of the TMSi derivatives [**1b** and **2b**, **3b**] at *m/z* 641, 571, 385, 271, and 213 for **1b** (Figure 1) and at *m/z* 541, 471, 313, and 271 for **2b** and **3b** (Figure 2). The placement of the mono-THF ring in **1** was determined to be at C-18/C-21 by the diagnostic fragments at *m/z* 641, 571, and 271, and, in **2** and **3**, to be at C-16/C-19 by fragments at *m/z* 541, 471, and 271 (TMSi-eims). The ir spectrum showed a strong absorption at 1765 cm^{-1}

TABLE 1. ^{1}H-Nmr Spectral Data for **1–3** and **1a–3a** and ^{13}C-Nmr Data for **1** and the mixture of **2** and **3** ($CDCl_3$, δ).

Positions	^{1}H nmr (500 MHz)				^{13}C nmr	
	1	**2,3**	**1a**	**2a,3a**	**1**	**2,3**
1	—	—			174.40	178.66 [**2**] 178.68 [**3**]
2	—	3.09 m		3.09 m	131.10	44.25 [**2**] 44.79 [**3**]
3a.....	2.40 m	2.59 ddd	2.53 m	2.59 dddd	33.40	25–37
3b	2.52 m	1.47 m	2.56 m	1.47 m		
4	3.84 m	4.55 [**2**] 4.39 [**3**] dddd	5.11 dq	4.55 [**2a**] 4.39 [**3a**] dddd	69.93	79.30 [**2**] 78.85 [**3**]
5–9 ...	1.27–1.52 m	1.27–1.62 m	1.27–1.57 m	1.27–1.62 m	25–38	25–38
10	3.60 m	3.59 m	4.87 m	4.83 m	71.86	71.78
11	1.37 m	1.41 m	1.57 m	1.41 m	37.29	37.59
12	1.27 m	1.27 m	1.27 m	1.27 m	25.51	25.45
13–14 .	1.27 m	1.27–1.62 m	1.27 m	1.27–1.62 m	22–34	25–38
15	1.27 m	3.40 m	1.27 m	4.83 m	25.95	74.27
16	1.37 m	3.81 m	1.57 m	3.96 m	33.23	83.16
17	3.42 m	1.99 m, 1.63 m	4.87 dt	1.95, 1.72 m	74.27	25–38
18	3.80 m	1.92 m, 1.57 m	3.97 m	1.95, 1.72 m	82.15	
19	1.67–1.97 m	3.87 m	1.65–1.90 m	3.96 m	28.57	82.13
20	1.67–1.97 m	3.81 m	1.65–1.90 m	4.91 m	25.27	71.54
21	3.88 m	1.37 m	3.97 m	1.37 m	82.45	25–38
22	3.84 m	1.27 m	4.87 dt	1.27 m	71.59	25–38
23–31 .	1.27–1.37 m	1.29–1.62 m	1.25–1.57 m	1.29–1.62 m	22–32	25–38
32	1.27 m	0.88 t	1.27 m	0.88 t	31.90	14.11
33a....	1.27 m	3.04 dd	1.27 m	3.04 dd	22.66	25–37
33b ...		2.66 dd		2.66 dd		
34	0.88 t	—	0.88 t	—	14.09	205.58 [**2**] 205.78 [**3**]
35	7.21 d	2.17 s	7.09 d	2.19 s	151.78	22.7
36	5.09 dq	—	5.02 dq	—	77.94	—
37	1.41 d	—	1.42 d	—	19.10	—
OAc-4 .	—	—	2.03 s	—	—	—
OAc-10	—	—	2.05 s	2.05 s	—	—
OAc-15	—	—	—	2.08 s	—	—
OAc-17	—	—	2.08 s	—	—	—
OAc-20	—	—	—	2.04 s	—	—
OAc-22	—	—	2.04 s	—	—	—

for the γ-lactone and at 1715 cm^{-1} for the ketone in **2** and **3**.

The mono-THF ring, with the usual OH groups on each side, was indicated, in **1** and the mixture of **2** and **3**, by ^{1}H-nmr chemical shifts (Table 1) at δ 3.42 (H-17), 3.80 (H-18), 3.88 (H-21), and 3.84 (H-22), for **1**, and δ 3.40 (H-15), 3.81 (H-16), 3.87 (H-19), and 3.81 (H-20), for **2** and **3**; and the ^{13}C-nmr signals (Table 1) at δ 74.27 (C-17), 82.15 (C-18), 82.45 (C-21), and 71.59 (C-22), for **1**, and at δ 74.27 (C-15), 83.16 (C-16), 82.13 (C-19), and 71.54 (C-20), for **2** and **3**, confirmed this functionality. The carbinol proton at C-10 resonated at δ 3.60, for **1**, and at δ 3.59, for **2** and **3**, and the corresponding ^{13}C-nmr signal at δ 71.86, for **1**, and at δ 71.78, for **2** and **3**. The nmr chemical shifts of H-4 and C-4 in **1** were located at δ 3.84 and 69.93 (Table 1), respectively.

These structural units were further confirmed by COSY and single-relayed COSY data in which the proton coupling correlations from H-3↔H-4, and H-10↔(H-8, H-9 and H-11), and (H-17 and H-22)↔(H-15, H-16, H-18, H-19, and H-20, H-21, H-23, and H-24), in **1**, and H-10↔(H-8, H-9, H-11), and (H-15 and H-20)↔(H-13, H-14, H-16, H-17, and H-18, H-19, H-21, and H-22), in **2** and **3** (Table 1) could be clearly seen. The assignments of the relative stereochemistry around the mono-THF ring of **1**, **1a**, **2**, **2a**, and **3**, **3a** were determined using the methodology of Hoye and coworkers (16,17) and Born *et al.* (18), as well as by comparison with several acetogenins having the erythro configuration at C-19 to C-20, for example, annonacin A (19), (2,4-*cis* and *trans*)-annonacin-A-one (20), and jetein (21). In addition, the recent paper by Fujimoto *et al.* (22) describes model mono-THF analogues, with flanking hydroxyls, having all possible relative stereochemistries; and **1** and the mixture of **2** and **3** match with the threo-trans-erythro model. The OH-substituted CH centers, at C-17 and C-22 in **1**, and at C-15 and C-20 in **2** and **3**, flanking the ring region (C-18 to C-21 and C-16 to C-19), exhibited very similar chemical shifts in the ^{1}H- and ^{13}C-nmr spectra as with the above models (Table 1). The proton signals for H-17 at δ 3.42 and H-22 at δ 3.84, in **1**, and for H-15 at δ 3.39 and H-20 at δ 3.87, in **2** and **3**, were shifted downfield in **1a** to δ 4.87 for H-17 and δ 4.87 for H-22, and in **2a** and **3a** to δ 4.83 for H-15 and δ 4.89 for H-20. The stereochemistry of C-17/C-18 and C-21/C-22 in **1**, and C-15/C-16 and C-19/C-20 in **2** and **3**, was thus concluded to be threo and erythro for **1–3**, and the stereochemistry is trans for the THF ring in each compound (16,17).

Annomutacin [**1**] is analogous to annomontacin (23) which is threo-trans-threo at C-17 to C-22. The OH-substituted CH center at C-22 is the only difference between these two compounds. The carbinol protons at C-22 in annomontacin and annomutacin [**1**] resonated at δ 3.42 and 3.84, and the corresponding ^{13}C-nmr signals were at δ 74.32 and 71.59, respectively. Thus, the stereochemistry of C-21/C-22 in annomutacin [**1**] was concluded to be erythro. The other spectral data of annomontacin (23) and annomutacin [**1**] are identical.

The ^{1}H- and ^{13}C-nmr spectral data of the mixture of (2,4-*trans* and *cis*)-10*R*-annonacin-A-one [**2** and **3**] were essentially identical with those of the mixture of (2,4-*cis* and *trans*)-annonacin-A-one isolated from *Asimina triloba* (20), and the shape of the ^{1}H-nmr signal at δ 3.40 (C-10) of both of these two pairs of compounds was their only difference. The peak at δ 3.40 was split as a pseudo-quartet in the mixture of **2** and **3**, but appeared as a multiplet in (2,4-*cis* and *trans*)-annonacin-A-one. The differences between these compounds were, thus, believed to be due to different absolute configurations of the carbinol centers at C-10. The absolute stereochemistry at C-10 of (2,4-*cis* and *trans*)-annonacin-A-one has not been reported (20), although

the absolute stereochemistry of isoannonacin is C-10*R* (25). Examination in the ^{1}H-nmr spectrum of the H-10 resonance of isoannonacin showed a signal identical to those of H-10 in the mixture of **2** and **3** and supported our suspicion that **2** and **3** must be C-10*R*.

Rieser *et al.* have reported the determination of the absolute configuration of stereogenic carbinol centers in several Annonaceous acetogenins using advanced Mosher ester methodology (6,24). Thus, the per-(*S*)- and (*R*)-methoxytrifluoromethyl phenylacetic acid (MTPA) esters (Mosher esters) of **1** and the mixture of **2** and **3** were prepared and numbered **1s**,**1r**; **2s**,**2r**; and **3s**,**3r**, respectively. COSY ^{1}H-nmr analysis of these derivatives was then performed. The ^{1}H-nmr chemical shift data of **1s** and **1r** showed that the absolute configuration at C-4 of **1** is *R* (Table 2). This result is identical to all acetogenins examined so far that possess an OH group at C-4.

The Mosher ester data also suggested the absolute stereochemical assignments of the carbinol centers adjacent to the mono-THF ring as C-17*R* and C-22*S*, in **1** (Table 3), and as C-15*R* and C-20*S*, in **2** and **3** (Table 4). The determination of the absolute stereochemistry of the carbinol center at C-10 of **1** could not be achieved by direct application of the Mosher-ester method because the ^{1}H-

TABLE 2. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configuration at C-4 of the Tetra (*S*)- and (*R*)-MTPA Esters of **1**.

MTPA ester of	CH_2-5	H-4	CH_2-3		H-33	H-34	Me-35	Configuration
1s δ (*S*)	1.57	5.30	2.57	2.52	6.72	4.85	1.27	4*R*
1r δ (*R*)	1.56	5.36	2.65	2.56	6.95	4.89	1.29	
Δδ	+0.02	−0.06	−0.08	−0.04	−0.23	−0.04	−0.02	

TABLE 3. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configurations at C-17 and C-22 of the Tetra (*S*)- and (*R*)-MTPA Esters of **1**.

MTPA Ester of	CH_2-16	H-17	H-18	CH_2-19/20	H-21	H-22	CH_2-23	Configuration
1s	1.55	5.21	3.93	1.86	3.92	5.01	1.53	
δ (*S*)	1.48			1.65			1.45	
1r	1.50	5.26	3.98	1.83	3.74	4.96	1.56	
δ (*R*)	1.44			1.57			1.49	
Δδ	pos.	neg.	neg.	pos.	pos.	pos.	neg.	17*R*, 22*S*

TABLE 4. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configurations at C-15 and C-20 of the Tri (*S*)- and (*R*)-MTPA Esters of **2** and **3**.

MTPA Ester of	CH_2-14	H-15	H-16	CH_2-17/18	H-19	H-20	CH_2-21	Configuration
2s,**3s** δ (*S*)	1.53	5.21	3.90	1.86	3.91	5.06	1.51	
	1.46			1.65			1.43	
2r,**3r** δ (*R*)	1.48	5.26	3.97	1.83	3.73	5.01	1.54	
	1.42			1.57			1.47	
Δδ	pos.	neg.	neg.	pos.	pos.	pos.	neg.	15*R*, 20*S*

nmr chemical shifts at CH_2-8, CH_2-9, CH_2-11, and CH_2-12 in the MTPA esters (**1s**,**1r**) cannot be confidently assigned. However, in the mixture of **2** and **3**, the ^{1}H-nmr chemical shifts at CH-4 are affected by the MTPA ester at C-10 and can be assigned for each isomer (Table 5). Thus, the absolute configuration of the carbinol center at C-10 was easily deduced as 10*R* for **2** and **3** by analysis of the Mosher ester data. Consequently, **2** and **3** are, indeed, the ketolactones of an annonacin A-type of mono-THF acetogenin (26,27). We have named **2** and **3**, respectively, (2,4-*trans* and *cis*)-10*R*-annonacin-A-one; and we propose that the (2,4-*cis* and *trans*)-annonacin-A-ones previously reported (20) from *Asimina triloba* are C-10*S*.

Annomutacin [**1**] and the mixture of (2,4-*trans* and *cis*)-10*R*-annonacin-A-ones [**2** and **3**] were significantly bioactive in the BST; they were selectively cytotoxic (seven-day MTT assays) to the human A549 solid lung tumor cell line with **2** and **3** showing moderate activity against the breast cell line (MCF-7) (Table 6). All of the Annonaceous acetogenins act, at least in part, as potent inhibitors of complex I in mitochondria (28,29).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—As described previously (10).

PLANT MATERIAL.—As described previously (10).

EXTRACTION AND ISOLATION.—For the column chromatographic separation of F005, a total of 154 fractions was collected. The BST-active fractions Nos. 90–95 and Nos. 76–85 were further subjected to repeated flash chromatography to yield crude compounds of **1** and the mixture of **2** and **3**, respectively; each was then purified by hplc over Si gel, eluted with hexane-MeOH (90:1, flow rate 10 ml/min), to afford, as colorless waxes, **1** and a mixture of **2** and **3**. A bioactive amide was isolated from other fractions (Nos. 86–87), using similar methods, and was identified as *N-p*-coumaroyl tyramine (13).

Annomutacin [**1**].—Colorless wax (7 mg); $[\alpha]^{22}D$ +60.0° (c=0.001, EtOH); hrfabms (glycerol) m/z $[MH]^+$ 625.5010 for $C_{37}H_{69}O_7$ (calcd 625.5043); cims (n-BuOH) m/z 625 (100), 607 (46), 589 (23), 571 (1), 553 (1), 425 (1), 407 (1), 389 (1), 355 (1), 337 (1), 241 (1), 223 (1), 199 (1), 141 (1); ^{1}H-nmr ($CDCl_3$, 500 MHz) data, see Table 1; ^{13}C-nmr ($CDCl_3$, 125 MHz) data, see Table 1; ir (film) ν max 3387 (br OH), 2921, 2851, 1743, 1466, 1320, 1073 cm^{-1}; uv λ max (MeOH) 212 nm (ϵ=9.2×10^3).

TABLE 5. ^{1}H-Nmr Chemical Shifts for the H-4 Signals of the Tri (*S*)- and (*R*)-MTPA Esters of **2** and **3**.

MTPA Ester of	H-4	MTPA Ester of	H-4	Configuration
2s δ (*S*)	4.53	**3s** δ (*S*)	4.37	
2r δ (*R*)	4.51	**3r** δ (*R*)	4.35	
Δδ	0.02	Δδ	0.02	4*R*, 10*S*

TABLE 6. Bioactivities of **1** and the Mixture of **2** and **3**.

Compounds	BST[a] LC_{50} (μg/ml)	A-549[b] ED_{50} (μg/ml)	MCF-7[c] ED_{50} (μg/ml)	HT-29[d] ED_{50} (μg/ml)
1[e]	3.91×10^{-2}	1.57×10^{-2}	>1.0	>1.0
2, **3**[e]	1.12×10^{-1}	1.74×10^{-1}	5.70×10^{-1}	>1.0
Adriamycin[e,f]	3.13×10^{-1}	3.56×10^{-3}	1.42×10^{-1}	3.01×10^{-2}

[a]Brine shrimp lethality test (14,15).
[b]Human lung carcinoma (30).
[c]Human breast carcinoma (31).
[d]Human colon adenocarcinoma (32).
[e]Same cytotoxicity runs; values in different runs were within one order of magnitude of each other.
[f]Positive control standard.

Tetra-acetate [**1a**].— Eims *m/z* 551 (5), 491 (9), 481 (2), 431 (11), 421 (1), 361 (2), 325 (3), 141 (6); ^{1}H-nmr ($CDCl_3$, 500 MHz) data, see Table 1.

*(2,4-*Trans *and* cis*)-10*R*-annonacin-A-ones* [**2** *and* **3**].—As a colorless wax, a mixture of both **2** and **3** (7 mg); $[\alpha]^{22}D$ +15.0° (c=0.002); hrfabms (glycerol) *m/z* $[MH]^+$ 597.4706 for $C_{35}H_{65}O_7$ (calcd 597.4730); cims *m/z* $[MH]^+$ 597 (44), 579 (43), 561 (100), 543 (25), 395 (2), 385 (1), 379 (3), 367 (2), 361 (3), 309 (6), 291 (4), 241 (9), 223 (2), 141 (2); eims *m/z* 395 (1), 385 (2), 379 (2), 367 (3), 361 (3), 327 (2), 309 (7), 291 (5), 241 (2), 223 (3), 141 (5); ^{1}H-nmr ($CDCl_3$, 500 MHz) data, see Table 1; ^{13}C-nmr ($CDCl_3$, 125 MHz) data, see Table 1; ir (film) ν max 3443 (br OH), 2920, 2851, 1765, 1715, 1465, 1356, 1166, 1075 cm^{-1}; uv λ max (MeOH) 208 nm (ϵ 2.5$\times$10^{3}).

Tri-acetates [**2a**,**3a**].—^{1}H-Nmr ($CDCl_3$, 500 MHz) data, see Table 1.

TMSi derivatives [**1b**, **2b**, *and* **3b**].—Eims of **1b** *m/z* 714 (10), 641 (16), 571 (100), 558 (1), 551 (36), 481 (63), 461 (11), 385 (95), 356 (36), 341 (39), 295 (18), 271 (77), 213 (46), 205 (12), 169 (36); eims of **2b** and **3b** *m/z* 812 (2), 601 (10), 541 (16), 511 (10), 499 (2), 471 (59), 409 (3), 381 (21), 361 (9), 341 (20), 319 (3), 313 (41), 291 (7), 271 (42), 251 (3), 213 (4), 181 (2), 141 (6).

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