Sesquiterpene Esters of Aristolochic Acid from the Root and Stem of *Aristolochia heterophylla*

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Three novel sesquiterpene esters of aristolochic acid, aristoloterpenate-II (**2**), -III (**3**), and-IV (**4**), together with known aristoloterpenate-I (**1**), were isolated and characterized from the root and stem of *Aristolochia heterophylla*. Their structures were elucidated by spectroscopic methods. The absolute configuration of these compounds at C-4′ was determined as *R* by circular dichroic studies. These compounds showed cytotoxicity against hepatoma G2, 2, 2, 15 cells.

Aristolochia heterophylla Hemsl (*Aristolochia shimada*) is a perennial shrub distributed in thickets and forests in mainland China and Taiwan. [1] The fruit and root have been used in traditional Chinese medicine as an expectorant, antitussive, analgesic, antiasthmatic, and also for the treatment of snakebite and lung inflammation.[2] As a result of our continuing search for novel bioactive natural products, we have isolated three new sesquiterpene esters of aristolochic acid, aristoloterpenate-II (**2**), -III (**3**) and -IV(**4**), as well as a known compound, aristoloterpenate-I (**1**), from the root and stem of *A. heterophylla*. We report herein the structure elucidation of these compounds, including their absolute configuration at C-4′ and cytotoxicity.

Results and Discussion

Aristoloterpenate-I (**1**) was obtained as optically active yellowish needles, mp 247−249 °C. Its molecular formula was determined as $C_{32}H_{31}NO_8$ by HRMS. The presence of an aristolochic acid moiety in the molecule was suggested by the UV absorption at 226, 239, 250, 268, 287, 322, and 393 nm and by IR bands at 1706, 1532, and 1342 cm^{-1}.[3] According to the above data, ^1H and ^{13}C NMR, and NOESY spectra, aristolochic acid-I (**5**) [4] was characterized as a partial moiety of compound **1**. On the other hand, the presence of a sesquiterpene moiety in the molecule was inferred by the appearance of 32 carbon signals of **1** in the ^{13}C NMR spectrum, which showed 15 carbon signals more than **5** together with the complex signals in the aliphatic region in the ^1H NMR spectrum. The sesquiterpene moiety was similar to that of manshurolide (**6**),[5] which was also isolated from the same plant. Results of a HMBC experiment (Table 3) showed that the planar structure of **1** was the same as aristoloterpenate-I. The absolute configuration at C-4′ was proposed as *S*, [6] according to the stereochemistry of manshurolide (**6**). However, the specific rotation or CD spectrum was not reported in the literature. Therefore, the absolute configuration at C-4′ of **1** is not clear. Nakanishi et al. have determined the absolute configuration of C-5 in 5-hydroxyfloridenol (**7**) as *S* from a positive Cotton effect at 242 nm of benzoate of **7** using the relationship between the allyl group and benzoate chirality.[7] The CD curve of aristoloterpenate-I (**1**) displayed a negative Cotton effect at 250 nm due to the aryl carboxylate chromophore. Therefore, the absolute configuration at C-4′ was determined as *R* not *S*, as reported in the literature. [6] The double bond stereochemistry at $\Delta^{2',3'}$ $\Delta^{6',7'}$ and $\Delta^{10',11'}$ were elucidated as *Z*, *E*, and *E* forms, respectively, by the NOESY experiment (Figure 1).

Aristoloterpenate-II (**2**) was isolated as optically active yellowish needles, mp 241−243 °C, and the molecular formula was determined as $C_{31}H_{29}NO_7$ by HRMS. The UV absorptions at 226, 241, 250, 267, 283, 321, and 392 nm revealed the presence of an aristolochic acid derivative, [3] which is similar to that of aristoloterpenate-I (**1**). The fragment ion peak at *m*/*z* 311 $[M-C_{15}H_{20}O]^+$ also attested that **2** was a sesquiterpene ester of aristolochic acid. The difference in the ^1H NMR spectrum of **2** from that of **1** was the four mutually coupled aromatic signals at δ 9.13 (d, J = 8.4 Hz), 8.00 (d, J = 8.4 Hz), 7.79 (t, J = 8.4 Hz), and 7.70 (t, J = 8.4 Hz), instead of three mutually coupled aromatic signals and one methoxy signal in **1** (Table 1). Therefore, the aristolochic acid moiety of **2** was determined as aristolochic acid-II (**8**). [4] According to the above results, the structure of aristoloterpenate-II could be assigned as **2**. The stereochemistry of **2** was established by the NOESY experiment (Figure 1). The absolute configuration at C-4′ of **2** was also determined as *R* by the negative Cotton effect at 253 nm in the CD spectrum.

Aristoloterpenate-III (**3**), optically active yellowish needles, showed a quasi molecular ion peak at *m*/*z* 558.2127 corresponding to $C_{32}H_{32}NO_8$ $[M + 1]^+$ in the HRFABMS. The UV and IR data of **3** were closely related to those of compound **1**, which indicated that it also has the aristolochic acid moiety. The ^1H and ^{13}C NMR data of **3** suggested that this compound possesses an aristolochic acid-I and manshurolide moiety as in **1**. The fragment ion peak at *m*/*z* 341 suggested the presence of the common structural unit of aristolochic acid-I. The sesquiterpene moiety of **3** was similar to that of **1** from the COSY, HMQC, and HMBC experiments (Table 3). The difference of configuration at $\Delta^{2',3'}$ between **1** and **3** was suggested by the NOESY experiment (Figure 1). The configuration of **3** at $\Delta^{2',3'}$ was determined as the *E* form by the clear NOE correlation between the aldehyde (δ 9.51) and H-3′(δ 6.36). Therefore, the stereochemistry of **3** at three double bonds was established as all *E* forms. According to the model for compound **3** and the appearance of a negative Cotton effect

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Table 1. ^{1}H NMR Spectra of Compound **1**, **2**, **3**, **4**, and **6** ($CDCl_3$)

compound	1	2	3	4	6
2	7.87 (s)	7.73 (s)	7.70 (s)	7.70 (s)	
5	8.58 (d, 8.8 Hz)	9.13 (d, 8.4 Hz)	8.68 (dd, 8.5, 3.6 Hz)	9.13 (d, 8.4 Hz)	
6	7.64 (t, 8.8 Hz)	7.79 (t, 8.4 Hz)	7.71 (t, 8.5 Hz)	7.81 (td, 8.4, 2.4 Hz)	
7	7.04 (d, 8.8 Hz)	7.70 (t, 8.4 Hz)	7.10 (dd, 8.5, 3.6 Hz)	7.71 (td, 8.4, 2.4 Hz)	
8		8.00 (d, 8.4 Hz)		7.99 (dd, 8.4, 2.4 Hz)	
9	8.79 (s)	8.35 (s)	8.84 (s)	8.34 (s)	
OCH_2O	6.34 (s)	6.40 (s)	6.36 (s)	6.40 (s)	
OCH_3	4.03 (s)		4.06 (s)		
1′	10.25 (s)	10.25 (s)	9.51 (s)	9.52 (s)	
3′	6.08 (d, 11.2 Hz)	6.08 (d, 10.8 Hz)	6.36 (d, 10.6 Hz)	6.36 (d, 9.6 Hz)	6.81 (s)
4′	6.37 (dd, 11.2, 4.6 Hz)	6.37 (m)	5.70 (td, 10.6, 3.6 Hz)	5.72 (td, 9.6, 3.4 Hz)	5.10 (m)
5′ a	2.72 (br d, 12.2 Hz)	2.73 (br d, 11.4 Hz)	2.80 (dd, 10.6, 3.6 Hz)	2.81 (dd, 12.8, 3.4 Hz)	2.42 (d, 14.0 Hz)
5′ b	2.39 (br d, 12.2 Hz)	2.40 (br d, 11.4 Hz)	2.43 (t, 10.6 Hz)	2.43 (dd, 12.8, 9.6 Hz)	2.65 (dd, 14.0, 5.6 Hz)
7′	5.04 (br d, 11.2 Hz)	5.05 (br d, 11.2 Hz)	5.10 (t, 7.0 Hz)	5.11 (t, 7.6 Hz)	4.75 (dd, 12.4, 1.2 Hz)
8′ a	2.30 (br t,11.2 Hz)	2.32 (br t, 11.2 Hz)	2.14 (t, 7.0 Hz)	2.15 (t, 7.6 Hz)	1.93 (m)
8′ b	1.98 (1 Hbr t, 11.2 Hz)	2.00 (br t, 11.2 Hz)			2.32 (ddd, 11.2, 4.0, 1.2 Hz)
9′ a	2.20 (m)	2.18 (m)	2.05 (m)	2.06 (m)	1.95 (m)
9′ b	2.02 (m)	2.03 (m)			2.17 (m)
11′	4.81 (br d, 8.4 Hz)	4.82 (br d, 8.4 Hz)	4.85 (t, 7.8 Hz)	4.85 (t, 7.6 Hz)	4.72 (dd, 12.4, 1.2 Hz)
12′ a	2.42 (m)	2.42 (m)	2.33 (m)	2.32 (m)	2.08 (m)
12′ b	2.07 (m)	2.06 (m)	2.21 (m)	2.22 (m)	2.57 (m)
13′ a	2.84 (dt, 13.0, 4.0 Hz)	2.85 (br d, 12.4 Hz)	2.63 (dd, 9.8, 5.1 Hz)	2.64 (dd, 9.7, 4.9 Hz)	2.28 (m)
13′ b	1.80 (td, 13.0, 4.0 Hz)	1.80 (br t, 12.4 Hz)	2.40 (t, 9.8 Hz)	2.40 (t, 9.7 Hz)	2.54 (m)
14′	1.61 (s)	1.61 (s)	1.68 (s)	1.69 (s)	1.58 (s)
15′	1.34 (s)	1.34 (s)	1.40 (s)	1.41 (s)	1.43 (s)

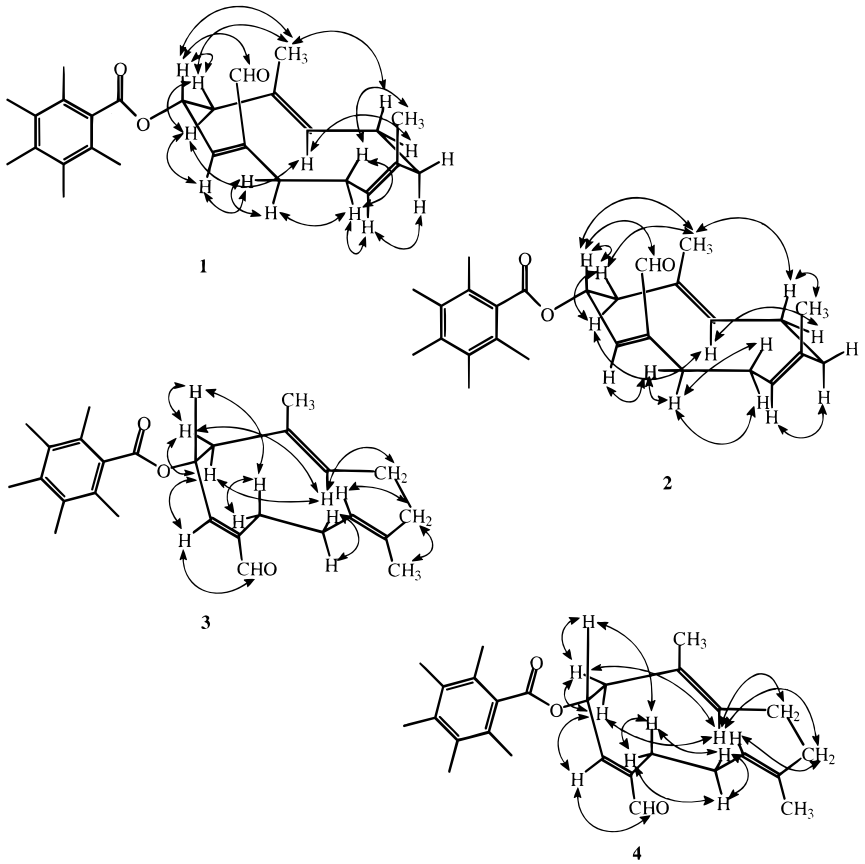
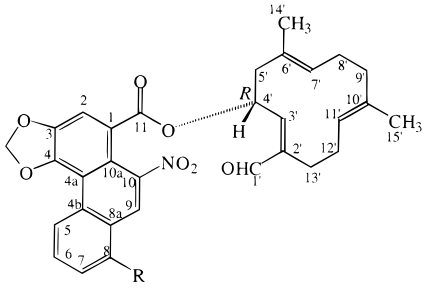


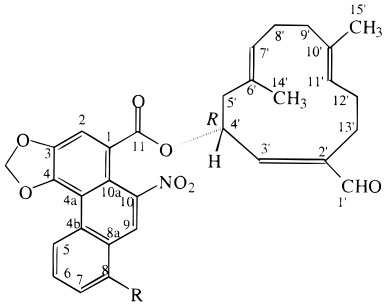
Figure 1. NOESY correlations for aristoloterpenate-I(**1**), -II(**2**), -III(**3**), and -IV(**4**)

at 238 nm in the CD spectrum, we suggest that the structure of **3** was conformer **A** or **B**. However, the structure was determined as **A** by the NOE correlation of 8.2% between the H-4′ (δ 5.70) and H-13′ (δ 2.40). The above summarized evidence indicated that the absolute stereochemistry at C-4′ was *R*.

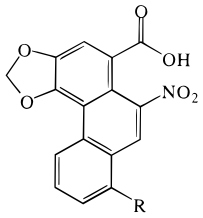
The HRFABMS mass established the quasi molecular formula for aristoloterpenate IV(**4**) as $C_{31}H_{30}NO_7$. By comparison of UV, IR, ^{1}H NMR, and NOESY spectra with those of **2** and **3**, the sesquiterpene moiety of **4** was determined to be the same as **3**. On the other hand, the ^{1}H NMR spectrum of **4** (Table 1) contained four mutually coupled protons at δ 9.13 (1H, d, J = 8.4 Hz), 7.99 (1H, dd, J = 8.4, 2.4 Hz), 7.81 (1H, td, J = 8.4, 2.4 Hz), and 7.71 (1H, td, J = 8.4, 2.4 Hz) in ring C, indicating that the aristolochic acid moiety was aristolochic acid-II (**8**), which is the same as in **2**. In addition, according to the CD spectrum and NOESY experiment (Figure 1), the stereochemistry of **4** was the same as **3**. Therefore, the structure of aristoloterpenate-IV was assigned as **4**. The presence of



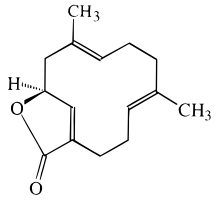
1. R=OCH_3
2. R=H



3. R=OCH_3
4. R=H



5. R=OCH_3
8. R=H



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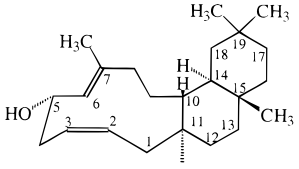

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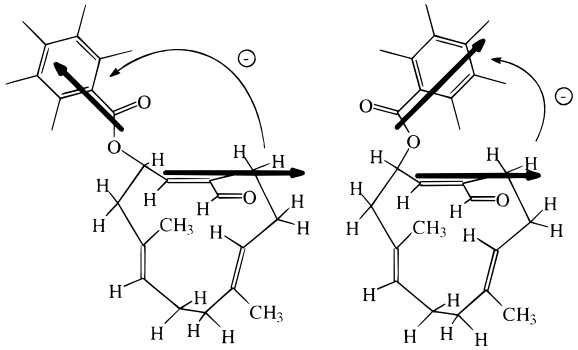

3A **3B**

these compounds represents the second example of the occurrence of the sesquiterpene esters of aristolochic acid from natural sources.

Compounds **1**−**4** were subjected to cytotoxicity evaluation. The IC_{50} values of compounds **1**−**4** against hepatoma G2, 2, 2, 15 cells were 4.83, 8.23, 5.44, and 7.53 μM, respectively.

Table 2. ^{13}C NMR Spectra of Compound **1**, **3**, and **6**

compound	**1**	**3**	**6**
1	123.2 (s)	123.3 (s)	
2	112.7 (d)	112.8 (d)	
3	145.8 (s)	145.9 (s)	
4	146.6 (s)	146.6 (s)	
4a	118.3 (s)	118.4 (s)	
4b	130.8 (s)	130.8 (s)	
5	118.9 (d)	119.1 (d)	
6	130.9 (d)	131.0 (d)	
7	107.8 (d)	107.9 (d)	
8	156.7 (s)	156.9 (s)	
8a	119.9 (s)	120.1 (s)	
9	121.2 (d)	121.3 (d)	
10	145.5 (s)	145.6 (s)	
10a	118.1 (s)	118.2 (s)	
11	166.0 (s)	165.8 (s)	
OCH_2O	102.4 (t)	102.4 (t)	
OCH_3	55.9 (q)	55.9 (q)	
1′	191.2 (d)	195.9 (d)	173.8 (s)
2′	141.7 (s)	145.8 (s)	133.1 (s)
3′	144.4 (d)	148.9 (d)	150.6 (d)
4′	67.6 (d)	71.6 (d)	80.9 (d)
5′	44.9 (t)	43.6 (t)	40.7 (t)
6′	128.5 (s)	129.1 (s)	128.7 (s)
7′	130.6 (d)	129.5 (d)	130.3 (d)
8′	25.0 (t)	24.9 (t)	25.2 (t)
9′	39.6 (t)	38.4 (t)	39.0 (t)
10′	134.9 (s)	133.6 (s)	135.5 (s)
11′	125.2 (d)	125.5 (d)	125.1 (d)
12′	25.9 (t)	25.1 (t)	24.5 (t)
13′	31.7 (t)	25.6 (t)	25.6 (t)
14′	15.9 (q)	18.6 (q)	18.9 (q)
15′	14.9 (q)	15.4 (q)	14.9 (q)

Table 3. ^2J, ^3J Correlations of HMBC of Aristoloterpenate-I (**1**) and -III (**3**)

	HMBC correlated carbons	
H	**1**	**3**
2	C-4, C-10a, C-11	C-4, C-10a, C-11
5	C-4a, C-7, C-8a	C-4a, C-7, C-8a
6	C-7, C-8	C-8
7	C-8, C-8a	C-5, C-8
9	C-10, C-10a	C-10, C-10a
OCH_2O	C-3, C-4	C-4
OCH_3	C-8	C-8
1′	C-2′, C-13′	C-2′
3′	C-1′, C-5′, C-13′	C-1′
4′	C-11	
5′ a	C-3′, C-4′, C-6′, C-7′	
5′ b	C-3′, C-4′, C-6′, C-7′, C-14′	C-4′, C-14′
7′	C-5′, C-14′	C-5′, C-14′
8′ a	C-6′, C-7′, C-10′	
8′ b		
9′ a	C-5′, C-7′, C-10′	
9′ b	C-10′	
11′	C-9′, C-15′	
12′ a		
12′ b	C-11′	
13′ a	C-2′, C-3′, C-11′	
13′ b	C-1′, C-3′, C-12′	C-1′
14′	C-5′, C-6′, C-7′	C-5′, C-7′
15′	C-9′, C-10′, C-11′	C-9′, C-10′, C-11′

Experimental Section

General Experimental Procedures. Melting points (Yanagimoto apparatus) are uncorrected. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. UV spectra in MeOH solution were obtained on a Hitachi UV-3210 spectrophotometer. IR spectra of KBr disk were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. MS and HRMS

were measured on VG-70−250S spectrometer having a direct inlet system. ^{1}H NMR and ^{13}C NMR spectra were determined on Bruker AMX-400 and Varian Unity plus 400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard.

Plant Material. *Aristolochia heterophylla* Hemsl. collected from Tsueg Feng, Nantou Hsien, Taiwan, in May 1992, and verified by Prof. C.-S. Kuoh. A voucher specimen is deposited in the Herbarium of Cheng Kung University, Taiwan.

Extraction and Separation. The fresh stem and root (4.5 Kg) of *A. heterophylla* were extracted with 7 L MeOH for 10 times at room temperature and concentrated to give a deep brown syrup. The syrup was partitioned succesively between H_2O and $CHCl_3$, and then n-BuOH. The $CHCl_3$ layer was filtered to obtain a precipitate and the filtrate solution. The filtrate was dried over Na_2SO_4 and then concentrated under reduced to leave a brown syrup that was chromatographed directly on Si gel and eluted with a gradient of $CHCl_3$ and MeOH to afford seven fractions. Fraction 2 was rechomatographed on Si gel and eluted with *n*-hexane−EtOAc(19:1) to give aristoloterpenate-I (**1**) (15 mg), -II (**2**) (2 mg), -III (**3**) (4 mg), and -IV (**4**) (1 mg), successively.

Aristoloterpenate-I (1): yellow needles from Me_2CO; mp 247−249 °C; $[\alpha]_D$ −39.5° (*c* 0.015, $CHCl_3$); UV (MeOH)λ_{max} (log ϵ) 226 (4.50), 239 (4.40), 250 (4.36), 268 (4.21), 287 (3.98), 322 (3.99), and 393 (3.76) nm; IR (KBr) ν_{max} 2924, 2855, 1706, 1690, 1645, 1596, 1517, 1463, 1342, 1271, 1242, 1142, 1043, 952, 812, and 752 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m*/*z* 557 (M^+, 10%), 341 (52), 324 (11), 295 (68), 280 (9), 278 (9), 265 (7), 216 (8); HREIMS *m*/*z* 557.2045 (*anal* calcd for $C_{32}H_{31}NO_8$, 557.2050); CD (*c* 3.95 × 10^{-5}, $CHCl_3$) 237 ([θ] +5017), 241 (0), 250 (−12 590), 265 (−3698), 285 (0) nm.

Aristoloterpenate-II (2): yellow needles from Me_2CO;. mp 241−243 °C; $[\alpha]_D$ −35.4° (*c* 0.016, $CHCl_3$); UV (MeOH)λ_{max} (log ϵ) 226 (4.57), 241 (4.46), 250 (4.41), 267 (4.27), 283 (4.08), 321 (4.05), and 392 (3.82) nm; IR (KBr) ν_{max} 2928, 2856, 1707, 1680, 1597, 1534, 1518, 1342, 1042, and 953 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; FABMS *m*/*z* 528 ([M + 1] $^+$, 3%), 527 (M^+, 2), 311 (14), 294 (80), 265 (28), 250 (12), 248 (9), 235 (3); HRFABMS ([M + 1] $^+$) *m*/*z* 528.2033 (*anal* calcd for $C_{31}H_{30}NO_7$, 528.2022), [M − $C_{15}H_{20}O$]$^+$ *m*/*z* 311.0436 (*anal* calcd for $C_{16}H_9NO_6$, 311.0429); CD (*c* 1.84 × 10^{-4}, $CHCl_3$) 229 ([θ] +2770), 237 (0), 243 (−5740), 253 (−9583), 282 (0) nm.

Aristoloterpenate-III (3): yellow needles from Me_2CO; mp 245−247 °C; $[\alpha]_D$ −86.0° (*c* 0.038, $CHCl_3$); UV (MeOH)λ_{max} (log ϵ) 225 (4.51), 251 (4.31), 267 (4.18), 284 (3.98), 321 (3.96), and 391 (3.74) nm; IR (KBr) ν_{max} 2922, 2856, 1705, 1695, 1598, 1529, 1452, 1382, 1338, 1263, 1230, 1137, 1043, 948, 806, and 756 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; EIMS *m*/*z* 557 (M^+, 3%), 341 (24), 324 (26), 309 (13), 295 (62), 293 (100), 278 (83), 265 (13), 250 (30), 216 (21), 187 (10), 164 (23), 137 (18), 105 (27), 91 (47), 79 (38), 67 (42); HRFABMS ([M + 1]$^+$) *m*/*z* 558.2127 (*anal* calcd for $C_{32}H_{32}NO_8$, 558.2128), [M − $C_{15}H_{20}O$]$^+$ *m*/*z* 341.0540 (*anal* calcd for $C_{17}H_{11}NO_7$, 341.0536); CD (*c* 4.77 × 10^{-5}, $CHCl_3$) 205 ([θ] +19 320), 212 (0), 218 (−8549), 221 (−10 260), 238 (−32 110), 254 (−27 400) nm.

Aristoloterpenate-IV (4): yellow needles from Me_2CO; mp 234−236 °C; $[\alpha]_D$ −88.3° (*c* 0.011, $CHCl_3$); UV (MeOH)λ_{max} (log ϵ) 210 (4.60), 219 (4.62), 243 (4.57), 251 (4.60), 266 (4.44), 298 (4.17) and 375 (3.74) nm; IR (KBr) ν_{max} 2924, 2855, 1710, 1694, 1596, 1531, 1519, 1454, 1388, 1351, 1267, 1232, 1118, 1041, 943, 794, and 758 cm^{-1};^{1}H NMR, Table 1; ^{13}C NMR, Table 2; FABMS *m*/*z* 528 ([M + 1] $^+$, 1%), 527 [M^+, 1], 311 (4), 294 (17), 265 (7), 250 (3), 248 (3), 235 (2); HRFABMS ([M + 1] $^+$) *m*/*z* 528.2028 (*anal* calcd for $C_{31}H_{30}NO_7$, 528.2022), [M − $C_{15}H_{20}O$]$^+$ *m*/*z* 311.0423(*anal* calcd for $C_{16}H_9NO_6$, 311.0429); CD (*c* 4.22 × 10^{-5}, $CHCl_3$) 209 ([θ] +21 760), 215 (0), 223 (−20 670), 229 (−18 210), 248 (−46 970) nm.

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