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New steroids from *Clerodendrum colebrookianum*

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Abstract

Five new steroids, colebrin A–E (**1**–**5**) were isolated from the aerial parts of *Clerodendrum colebrookianum*. The structures of the new compounds were elucidated on the basis of spectral evidence.

Keywords: *Clerodendrum colebrookianum*; Steroids; Colebrin A–E

1. Introduction

Clerodendrum colebrookianum Walp. (Verbenaceae) is distributed widely in the south and south-east Asia. It mainly grows in the moist and waste place of the western and southern regions of Yunnan province, China, up to an altitude of 2100 m [1]. It has been used as a folk medicine in China for expelling toxin by cooling, cooling blood to induce diuresis and purging heat [2], and also used as a remedy for hypertension in India [3]. Some research groups have reported the chemical investigation of this plant [3–8]. In continuation of our studies on the bioactive

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constituents of the genus *Clerodendrum*, the examination of the steroid constituents of the title species led to the isolation of five new steroids, named colebrin A–E (**1**–**5**), together with three known compounds, clerosterol (**6**), β-sitosterol and daucosterol. The present paper deals with the isolation and structural elucidation of the new compounds.

2. Results and discussion

The new steroids **1**–**5**, were isolated from the EtOH extract of *C. colebrookianum* aerial parts.

Colebrin A (**1**) has a molecular formula of $C_{30}H_{48}O_3$, which was deduced by positive HRFABMS (obsd 457.3771, calcd 457.3682) together with ^{13}C-NMR and DEPT spectra. The IR spectrum showed absorption bands (3411 br, 3072, 1722, 1668, 1645 and 887 cm^{-1}), which correspond to free hydroxyl groups, carboxylic group and olefinic bonds. The presence of these groups was also confirmed by ^{13}C-NMR spectrum data (Table 1). By direct comparison of ^{13}C- and ^{1}H-NMR data (see Table 1 and Section 3) of **1** with those of clerosterol (**6**) [5], **1** had the same carbon skeleton and configuration at C-24 (i.e. 24 S/β) as **6**. A methylene at δ 31.90 in **6** was replaced by one new oxymethine at δ 68.82 and a formyloxy group [δ 160.8 (*d*), δ 8.04 (1H, *s*)] appeared in the NMR spectra of **1**. The new oxymethine was readily assigned to the C-7 position from the two-dimensional NMR spectra of **1**. The correlations between H-7 (δ 5.06) with carbon (δ 160.8) of formyloxy, and proton (δ 8.04) of formyloxy with C-7 (δ 68.82) could be clearly observed in the HMBC spectrum of **1** (Table 2). Moreover, the splitting signals of H-6 [δ 5.56 (1H, *d*, *J* 5.3 Hz)] and H-7 [δ 5.06 (1H, *t*, *J* 5.3 Hz)] in the ^{1}H-NMR spectrum of **1** disclosed that the formyloxy group is in α-orientation. The conclusion was also supported by comparing the ^{1}H-NMR spectral data of **1** with those of 24-methylene-5-cholestene-3β,7α-diol and 24-methylene-5-cholestene-3β,7β-diol [9]. Consequently, the structure of colebrin A was established to be 7α-formyloxy-clerosterol (**1**) [10].

Colebrin B (**2**) was obtained as colorless wax and had a molecular formula $C_{29}H_{50}O_3$ deduced from the positive HRFABMS (obsd $[M\text{-}H_2O + 1]^+$ 429.3696 calcd 429.3733) and the ^{13}C-NMR data. It contained five methyl groups (including one primary methyl group, one secondary methyl group and three tertiary methyl groups), 12 methylenes (including one terminal methylene), eight methines (including two oxymethines) and four quaternary carbons judging from its ^{1}H- and ^{13}C-NMR spectra (see Table 1 and Section 3). Analysis of ^{1}H- and ^{13}C-NMR spectral data revealed that **2** resembled clerosterol (**6**). The differences between **2** and **6** were that the carbon signals at δ 140.7 (*s*, C-5), 121.7 (*d*, C-6) arising from $\Delta^{5,6}$-double bond and a methylene at δ 31.9 (*t*, C-7) in **6** were replaced by those at δ 88.8 (*s*, C-5), 34.6 (*t*, C-6) and oxymethine at δ 68.4 (*d*, C-7) in the ^{13}C-NMR spectrum of **2**, respectively. It implied that compound **2** possessed two more hydroxyl groups than **6**, as confirmed by its FABMS. On the basis of its two-dimensional NMR spectra (Table 2), two additional hydroxyl groups were connected to

Table 1
^{13}C-NMR spectral data of compounds **1**–**6** in $CDCl_3$ (100.6 MHz, δ in ppm from TMS)

C	1	2	3	4	5	6
1	36.7 *t*	37.5 *t*	37.3 *t*	36.9 *t*	36.9 *t*	37.2 *t*
2	31.2 *t*	31.9 *t*	31.4 *t*	31.9 *t*	31.9 *t*	31.6 *t*
3	71.1 *d*	67.6 *d*	79.8 *d*	79.2 *d*	79.2 *d*	71.8 *d*
4	41.8 *t*	35.8 *t*	38.9 *t*	38.6 *t*	38.6 *t*	42.3 *t*
5	148.6 *s*	88.8 *s*	140.4 *s*	144.8 *s*	145.2*s*	140.7 *s*
6	119.4 *d*	34.6 *t*	121.9 *d*	121.9 *d*	122.3*d*	121.7 *d*
7	68.8 *d*	68.4 *d*	31.9 *t*	63.4 *d*	86.3 *d*	31.9 *t*
8	35.4 *d*	30.3 *d*	31.9 *d*	34.7 *d*	34.7 *d*	31.9 *d*
9	43.1 *d*	45.8 *d*	50.1 *d*	42.4 *d*	48.8 *d*	50.1 *d*
10	37.3 *s*	39.6 *s*	36.6 *s*	36.6 *s*	36.7 *s*	36.5 *s*
11	20.7 *t*	21.2 *t*	21.1 *t*	21.1 *t*	21.1 *t*	21.0 *t*
12	39.0 *t*	40.1 *t*	39.8 *t*	39.2 *t*	39.6 *t*	39.7 *t*
13	42.2 *s*	42.8 *s*	42.3 *s*	42.1 *s*	42.9 *s*	42.3 *s*
14	49.5 *d*	56.3 *d*	56.8 *d*	49.0 *d*	56.1 *d*	56.8 *d*
15	28.2 *t*	28.1 *t*	28.1 *t*	28.2 *t*	28.2 *t*	28.1 *t*
16	29.4 *t*	29.3 *t*	29.4 *t*	29.3 *t*	29.3 *t*	29.4 *t*
17	55.8 *d*	56.3 *d*	56.2 *d*	55.9 *d*	55.7 *d*	56.1 *d*
18	11.4 *q*	11.6 *q*	11.8 *q*	11.8 *q*	11.8 *q*	11.8 *q*
19	18.6 *q*	18.7 *q*	19.3 *q*	19.0 *q*	18.7 *q*	19.3 *q*
20	35.7 *d*	35.5 *d*	35.5 *d*	35.8 *d*	35.5 *d*	35.5 *d*
21	18.1 *q*	17.9 *q*	18.6 *q*	18.9 *q*	18.7 *q*	18.6 *q*
22	33.6 *t*	33.7 *t*	33.6 *t*	33.4 *t*	33.7 *t*	33.7 *t*
23	23.9 *t*	22.7 *t*	24.3 *t*	24.9 *t*	24.9 *t*	24.3 *t*
24	50.0 *d*	49.5 *d*	49.4 *d*	49.5 *d*	49.5 *d*	49.5 *d*
25	147.5 *s*	147.6 *s*	147.4 *s*	147.6 *s*	147.6 *s*	147.5 *s*
26	111.3 *t*	111.3 *t*	111.3 *t*	111.6 *t*	111.3 *t*	111.3 *t*
27	17.8 *q*	17.3 *q*	17.8 *q*	17.8 *q*	17.9 *q*	17.8 *q*
28	26.5 *t*	26.5 *t*	26.5 *t*	26.5 *t*	26.5 *t*	26.5 *t*
29	11.9 *q*	12.0 *q*	11.9 *q*	11.9 *q*	12.0 *q*	12.0 *q*
Formyl	160.8 *d*					
1′			101.3 *d*	101.5 *d*	101.5 *d*	
2′			70.6 *d*	70.3 *d*	70.3 *d*	
3′			76.3 *d*	76.2 *d*	76.3 *d*	
4′			73.2 *d*	73.5 *d*	73.6 *d*	
5′			73.6 *d*	73.9 *d*	73.9 *d*	
6′			63.8 *t*	63.4 *t*	63.4 *t*	
1″			174.0 *s*	174.3 *s*	174.3 *s*	
2″			34.3 *t*	34.2 *t*	34.3 *t*	
3″			30.8 *t*	31.8 *t*	31.9 *t*	
4″–14″			29.7 *t*	29.7 *t*	29.7 *t*	
15″			24.9 *t*	24.9 *t*	24.9 *t*	
16″			22.6 *t*	22.7 *t*	22.7 *t*	
17″			14.0 *q*	14.0 *q*	14.0 *q*	

C-5 and C-7. The configurations at C-3, C-5 and C-7 of **2** were confirmed by comparison with 5α-cholestane-3α,7α,12α,24-tetrol [11]. Therefore, compound **2** was identified as (24S/β)-stigmast-25-ene-3β,5α,7α-triol [10].

Table 2
HMBC results of compounds **1–5** ($CDCl_3$)

H	C				
	1	2	3	4	5
3α	5	5	5, 1′	5, 1′	5, 1′
4		2, 10			
6	4, 8, 10		4, 8, 10	4, 8, 10	4, 8, 10
7		5, 9, 14			
7α					5
7β	5, 9, formyl			5	
18	12, 14, 17	12, 14, 17	12, 14, 17	12, 14, 17	12, 14, 17
19	1, 5, 9	1, 5, 9	1, 5, 9	1, 5, 9	1, 5, 9
21	17, 22	17, 22	17, 22	17, 22	17, 22
26	24, 27	24, 27	24, 27	24, 27	24, 27
27	24, 26	24, 26	24, 26	24, 26	24, 26
29	24	24	24	24	24
Formyl	7				
1′			3, 3′	3, 3′	3, 3′
6′			4′, 1″	4′, 1″	4′, 1″

Colebrin C **(3)** was obtained as colorless wax and had the molecular formula $C_{52}H_{90}O_7$ that was deduced from the positive HRFABMS (obsd 827.6681, calcd 827.6765) and NMR data. Its IR spectrum displayed the presence of hydroxyl (3393 cm^{-1}, br), carboxyl (1739 cm^{-1}, sh) and olefinic (1646, 1467 and 888 cm^{-1}) bands. The NMR spectra of **3** are very similar to those of clerosterol 3β-*O*-β-D-glucopyranoside [5], except for the NMR signals for margaroyl group. According to the HMBC and $^1H-^1H$ COSY experiments, the three pairs of significant $^1H-^{13}C$ long-range correlations (Table 2) between H-1′ and C-3, H-3 and C-1′, and H-6′ and C-1″ in **3** were clearly observed. These facts demonstrated that the glucosyl unit is attached at the C-3 position and the margaroyl group is connected to the C-6′ position of the glucosyl moiety. From all the evidence mentioned above, colebrin C was elucidated as clerosterol 3β-*O*-β-D-(6′-*O*-margaroyl)-glucopyranoside **(3)**.

Colebrin D **(4)**, colorless wax, has molecular formula $C_{52}H_{90}O_8$ determined by positive HRFABMS (obsd 843.6617, calcd 843.6657), together with its ^{13}C-NMR and DEPT spectra (Table 1). Its IR and NMR spectra resembled those of **3**. However, compound **4** had one more hydroxyl group than **3,** since one methylene at δ 31.9 (*t*, C-7) in **3** was replaced by a new oxymethine at δ 63.4 (*d*, C-7) in **4**. On the basis of the two-dimensional NMR spectra, H-6 and H-7 were assigned to δ 5.83 (1H, *br d*, *J* 5.0 Hz) and 4.09 (1H, *t*, *J* 5.0 Hz), respectively, which are similar to those of compound **1**. Thus, 7-OH should be located in the α-orientation. By further comparing the ^{13}C-NMR data of **4** with those of **3**, the signals of C-9 and C-14 in **4** were upfield shifted from δ 50.1 and 56.8 to 42.4 (−7.7 ppm) and 49.0 (−7.8 ppm) due to the stronger γ-gauche shielding effect among the 7α-OH with

C-9 and C-14, respectively. Accordingly, colebrin D **(4)** was deduced as 7α-hydroxyclerosterol 3β-*O*-β-D-(6′-*O*-margaroyl)–glucopyranoside.

Colebrin E **(5)** was determined to have a molecular formula of $C_{52}H_{90}O_8$ by ^{13}C-NMR (including DEPT) spectrum and its positive HRFABMS (obsd 843.6617, calcd 843.6655). The spectral data of IR, MS and NMR were quite similar to those of **4**, except for the ^{1}H-NMR signals for H-6 and H-7, which appeared at δ 5.59 (1H, *br s*) and 4.07 (1H, *br d*, *J* 8.6 Hz) in **5,** respectively. On the basis of the NMR spectra, **5** was to be a 7-OH epimer of **4**, namely, 7-OH of **5** was in the β-orientation. This conclusion was supported by comparing the signals of C-9 and C-14 in **5** with those of **4**, which were downfield shifted from δ 42.4 and 49.0 to 48.8 (+6.4 ppm) and 56.1 (+7.1 ppm), respectively, due to the stronger γ-gauche shielding effect disappeared among the 7β-OH with C-9 and C-14 in **5**. Therefore, colebrin E **(5)** was characterized as 7β-hydroxyclerosterol 3β-*O*-β-D-(6′-*O*-margaroyl)-glucopyranoside.

3. Experimental

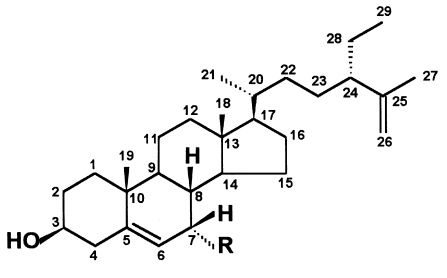
3.1. Plant material

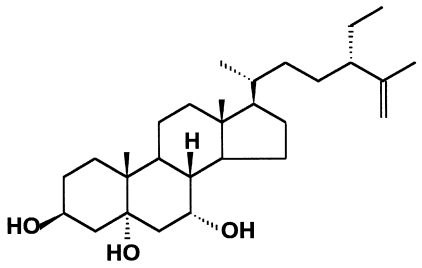
C. colebrookianum aerial parts were collected in Xishuangbanna of Yunnan province, China, in September 1996 and identified as *Clerodendrum colebrookianum* Walp. by Professor Rui-Zheng Fang at Kunming Institute of Botany, Chinese Academy of Science, where a voucher specimen (KIB 96-09-18, Fang) is deposited.

3.2. Extraction and isolation

The air-dried and powdered aerial parts (6.0 kg) were extracted with 95% EtOH (3 × 20 l) under reflux to give a crude extract (619 g). The extract was suspended in water and then successively partitioned with petrol (60–90°C), EtOAc and *n*-BuOH to afford petrol, EtOAc and *n*-BuOH residues (272.0, 98.0 and 206.4 g, respectively). The petrol residue was subjected to CC over Si-gel eluting with petrol/chloroform (1:0, 9:1, 3:1 and 0:1), chloroform/acetone (9:1, 3:1 and 0:1) to give fractions I–VII. Fr. III (20.0 g) and IV (8.0 g) were CC on Si-gel developing with petrol/chloroform to afford compound **6** (3.5 g) and β-sitosterol (120 mg), respectively. Fr. V (17.5 g), VI (8.0 g) and VII (20.0 g) were subjected to CC and medium pressure column on Si-gel with petrol/acetone, chloroform/acetone and chloroform/MeOH repeatedly to yield compounds **1** (44 mg), **2** (10 mg), **3** (893 mg), **4** (12 mg), **5** (14 mg) and daucosterol (35 mg), respectively.

Colebrin A (**1**). $C_{30}H_{48}O_3$, colorless wax, $[\alpha]_D^{20}$ −117° (*c* 0.112, MeOH); IR bands (film): 3411 (br), 3072, 2935, 2870, 1722, 1668, 1645, 1461, 1377, 1179, 1059, 951, 887 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.04 (1H, *s*, –OCOH), 5.56 (1H, *d*, *J* 5.3 Hz, H-6), 5.06 (1H, *t*, *J* 5.3 Hz, H-7β), 4.70 (1H, *br d*, *J* 2.5 Hz, H-26a), 4.61 (1H, *br d*, *J* 2.5 Hz, H-26b), 3.56 (1H, *m*, H-3α), 1.54 (3H, *s*, H-27), 0.98 (3H, *s*, H-19), 0.88

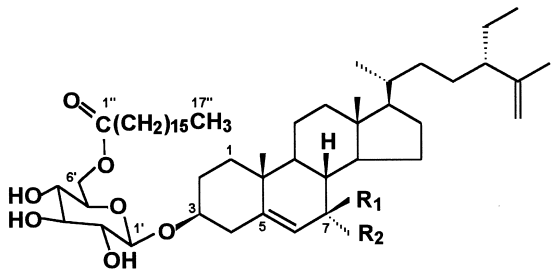




1 R = OCH (O)

6 R = H

2



	R_1	R_2
3	H	H
4	H	OH
5	OH	H

(3H, *d*, *J* 6.5 Hz, H-21), 0.77 (3H, *t*, *J* 7.4 Hz, H-29), 0.65 (3H, *s*, H-18); positive FABMS *m/z* (rel. int.): 457 $[M + 1]^+$ (8), 439 $[457\text{-}H_2O]^+$ (6), 411 (81), 393 (63), 255 (14), 159 (38), 145 (28); positive HRFABMS *m/z*: 457.3771 (calcd 457.3682); ^{13}C-NMR data (see Table 1).

Colebrin B (**2**). $C_{29}H_{50}O_3$, colorless wax, $[\alpha]_D^{20}$ 0° (*c* 0.212, MeOH); IR bands (film): 3432 (br), 2928, 2855, 1460, 1377, 1271, 890 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.70 (1H, *br d*, *J* 2.3 Hz, H-26a), 4.64 (1H, *br s*, H-7α), 4.61 (1H, *br d*, *J* 2.3 Hz, H-26b), 3.65 (1H, *m*, H-3α), 2.88 (2H, *dd*, *J* 12.1, 5.1 Hz, H-4), 1.54 (3H, *s*, H-27), 1.18 (3H, *s*, H-19), 0.88 (3H, *d*, *J* 6.4 Hz, H-21), 0.77 (3H, *t*, *J* 7.4 Hz, H-29), 0.65 (3H, *s*, H-18); positive FABMS *m/z* (rel. int.): 429 $[M\text{-}H_2O + 1]^+$(5), 411 $[M\text{-}2H_2O + 1]^+$ (22), 393 (7), 255 (6), 159 (8), 145 (11); positive HRFABMS *m/z*: $[M\text{-}H_2O + 1]^+$ 429.3696 (calcd 429.3733); ^{13}C-NMR data (see Table 1).

Colebrin C (**3**). $C_{52}H_{90}O_7$, colorless wax, $[\alpha]_D^{25}$ −48.1° (*c* 0.243, $CHCl_3$); IR bands (film): 3393 (br), 2927, 2854, 1739, 1674, 1646, 1467, 1377, 1175, 1082, 1021, 888,

722 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.34 (1H, *br d*, *J* 5.2 Hz, H-6), 4.70 (1H, *br d*, *J* 2.2 Hz, H-26a), 4.61 (1H, *br d*, *J* 2.2 Hz, H-26b), 4.43 (1H, *dd*, *J* 12.0, 5.0 Hz, H-6′a), 4.35 (1H, *d*, *J* 7.7 Hz, H-1′), 4.25 (1H, *dd*, *J* 12.0, 1.8 Hz, H-6′b), 3.55 (2H, *m*, H-3′ and 4′), 3.45 (1H, *m*, H-3α), 3.35 (2H, *m*, H-2′ and 5′), 2.32 (2H, *t*, *J* 7.6 Hz, H-2″), 1.54 (3H, *s*, H-27), 1.16–1.32 (28H, *m*, H-3″–16″), 1.01 (3H, *s*, H-19), 0.88 (3H, *d*, *J* 6.5 Hz, H-21), 0.86 (3H, *t*, *J* 7.2 Hz, H-17″), 0.77 (3H, *t*, *J* 7.4 Hz, H-29), 0.65 (3H, *s*, H-18); positive FABMS *m/z* (rel. int.): 827 $[M + 1]^+$ (4), 411 (6), 395 (41), 393 (33), 269 (5), 255 (15), 239 (18), 161 (35), 146 (36), 145 (39); positive HRFABMS *m/z*: 827.6681 (calcd 827.6765); ^{13}C-NMR data (see Table 1).

Colebrin D (**4**). $C_{52}H_{90}O_8$, colorless wax, $[\alpha]_D^{24}$ −21.3° (*c* 0.200, $CHCl_3$); IR bands (film): 3421 (br), 2926, 2854, 1736, 1465, 1377, 1174, 1082, 1021, 889, 722 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.83 (1H, *br d*, *J* 5.0 Hz, H-6), 4.70 (1H, *br d*, *J* 2.0 Hz, H-26a), 4.61 (1H, *br d*, *J* 2.0 Hz, H-26b), 4.34 (1H, *d*, *J* 7.7 Hz, H-1′), 4.30 (2H, *br s*, H-6′), 4.09 (1H, *t*, *J* 5.0 Hz, H-7β), 3.56 (2H, *m*, H-3′ and 4′), 3.45 (1H, *m*, H-3α), 3.36 (2H, *m*, H-2′ and 5′), 2.34 (2H, *t*, *J* 7.5 Hz, H-2″), 1.54 (3H, *s*, H-27), 1.14-1.34 (28H, *m*, H-3″–16″), 1.01 (3H, *s*, H-19), 0.88 (3H, *d*, *J* 6.5 Hz, H-21), 0.80 (3H, *t*, *J* 7.2 Hz, H-17″), 0.77 (3H, *t*, *J* 7.4 Hz, H-29), 0.66 (3H, *s*, H-18); positive FABMS *m/z* (rel. int.): 842 $[M]^+$ (5), 427 (36), 409 (100), 393 (64), 269 (12), 255 (10), 239 (20), 161 (28), 159 (30), 145 (32); positive HRFABMS *m/z*: 843.6617 (calcd 843.6657); ^{13}C-NMR data (see Table 1).

Colebrin E (**5**). $C_{52}H_{90}O_8$, colorless wax, $[\alpha]_D^{24}$ −42.2° (*c* 0.450, $CHCl_3$); IR bands (film): 3421 (br), 2927, 2854, 1738, 1466, 1377, 1175, 1081, 1020, 888, 722 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.59 (1H, *br s*, H-6), 4.70 (1H, *br d*, *J* 2.0 Hz, H-26a), 4.61 (1H, *br d*, *J* 2.0 Hz, H-26b), 4.36 (1H, *d*, *J* 7.4 Hz, H-1′), 4.31 (2H, *br s,* H-6′), 4.07 (1H, *br d*, *J* 8.6 Hz, H-7α), 3.53 (2H, *m*, H-3′ and 4′), 3.47 (1H, *m*, H-3α), 3.36 (2H, *m*, H-2′ and 5′), 2.32 (2H, *t*, *J* 7.6 Hz, H-2″), 1.55 (3H, *s*, H-27), 1.14–1.37 (28H, *m*, H-3″–16″), 0.98 (3H, *s*, H-19), 0.88 (3H, *d*, *J* 6.5 Hz, H-21), 0.83 (3H, *t*, *J* 7.3 Hz, H-17″), 0.77 (3H, *t*, *J* 7.3 Hz, H-29), 0.63 (3H, *s*, H-18); positive FABMS *m/z* (rel. int.): 842 $[M]^+$ (8), 427 (38), 409 (100), 393 (60), 269 (16), 255 (10), 239 (25), 161 (28), 159 (30), 145 (32); positive HRFABMS *m/z*: 843.6617 (calcd 843.6655); ^{13}C-NMR data (see Table 1).

Acknowledgements

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