

Novel Bibenzyl Derivatives from the Tubers of *Bletilla striata*

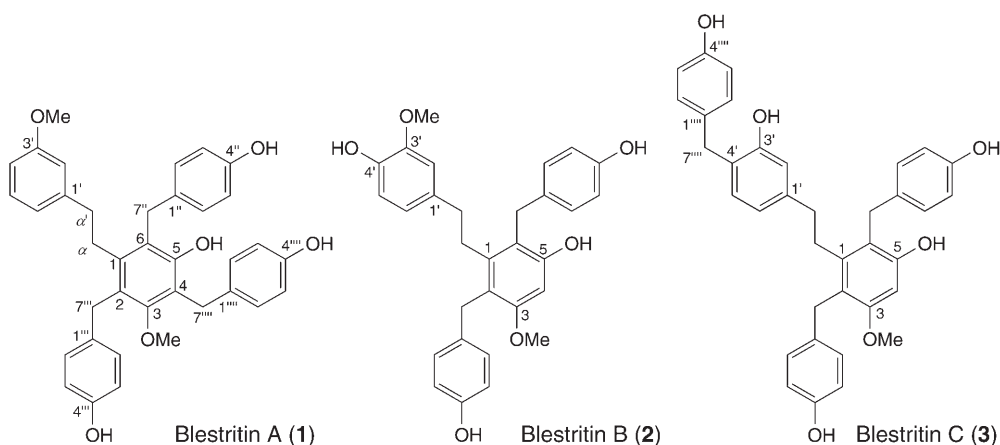
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Three novel bibenzyl derivatives, blestritins A–C (**1–3**), along with 18 known constituents, were isolated from the tubers of *Bletilla striata* (Orchidaceae), a traditional Chinese medicine used for the treatment of tuberculosis and haemorrhage of the stomach and lungs. Their structures were identified on the basis of spectroscopic analyses.

Introduction. – *Bletilla striata* (THUNB.) REICHB. F. (Orchidaceae) is mainly distributed in East Asia, and its tubers are used as a Chinese traditional medicine for the treatment of tuberculosis and haemorrhage of the stomach or lung [1]. Previous phytochemical studies on *Bletilla* species have led to the isolation of phenanthrene derivatives [2–11], bibenzyls [2][4], flavonoids and phenolic compounds [5], and cyanidin glycosides [12]. As part of our ongoing chemical study on *Bletilla striata*, three novel bibenzyl derivatives, blestritins A–C (**1–3**), were isolated from the tubers of *B. striata*, together with 18 known constituents. We report herein the isolation and structural elucidation of these compounds.



Results and Discussion. – Compound **1** was obtained as a white amorphous powder. Its molecular formula was established as $C_{37}H_{36}O_6$ by HR-ESI-MS, giving a quasimolecular ion (m/z 599.2408 ($[M+Na]^+$)), and by NMR analysis. The IR spectrum showed absorptions at 3405 (OH), 2937 (CH_2), and 1596 and 1511 (aromatic)

cm^{-1} . The UV spectrum with a maximum at 281 nm was in agreement with a bibenzyl (=1,1'-(ethane-1,2-diyl)bis[benzene]) derivative [2]. The structure of **1** was deduced from the ^1H - and ^{13}C -NMR (*Table*), ^1H , ^1H -COSY and HMBC (*Figure*), and ROESY data as 2,4,6-tris(4-hydroxybenzyl)-3,3'-dimethoxybibenzyl-5-ol¹⁾; **1** is a new compound and was assigned the trivial name blestritin A.

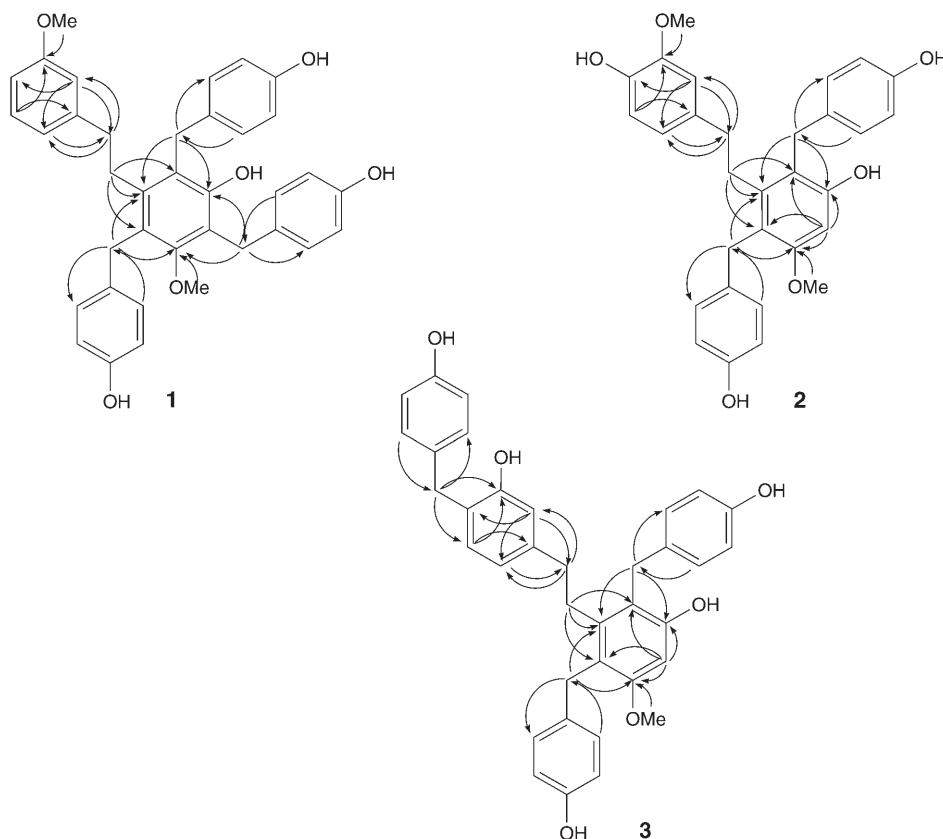


Figure. Key ^1H , ^{13}C long-range correlation signals ($^1\text{H} \rightarrow ^{13}\text{C}$) in the HMBC spectra of **1–3**

The ^1H -NMR displayed six *d* or *m* at $\delta(\text{H})$ 6.89 (*d*, $J = 8.6$ Hz, 2 H), 6.60–6.64 (*m*, 2 H), 6.83 (*d*, $J = 8.4$ Hz, 2 H), 6.59–6.63 (*m*, 2 H), 7.02 (*d*, $J = 8.5$ Hz, 2 H), and 6.63–6.66 (*m*, 2 H) due to three *AABB* systems characteristic of 4-substituted benzyl groups, and three *s* at $\delta(\text{H})$ 4.02 (2 H), 3.93 (2 H), and 4.04 (2 H) due to three benzyl CH_2 groups. Four aliphatic protons due to bibenzyl CH_2 groups appeared as a pair of *m* at $\delta(\text{H})$ 2.30–2.40 (2 H) and 2.61–2.71 (2 H), and two MeO at $\delta(\text{H})$ 3.45 (*s*) and 3.67 (*s*) and four aromatic protons at $\delta(\text{H})$ 6.40 (*br. s*, 1 H), 6.63–6.67 (*m*, 1 H), 7.08 (*t*, $J = 7.6$ Hz, 1 H), and 6.57 (*d*, $J = 7.6$ Hz, 1 H), assigned to H–C(2'), H–C(4'), H–C(5'), and H–C(6') by the ^1H , ^1H -COSY cross-peaks and coupling patterns, were also observed in the ^1H -NMR spectrum. The ^{13}C -NMR spectrum showed 37 C-signals assigned by DEPT experiments to two Me, five CH_2 , and sixteen CH groups, and

¹⁾ Trivial atom numbering; for systematic names, see *Exper. Part*.

Table. ^1H - and ^{13}C -NMR Data (400 and 100 MHz, resp., CD_3OD) of **1–3**). δ in ppm, J in Hz.

	1		2		3	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
C(1)		141.2 (s)		143.2 (s)		143.2 (s)
C(2)		126.2 (s)		121.8 (s)		120.7 (s)
C(3)		158.3 (s)		158.7 (s)		158.8 (s)
C(4) or H–C(4)		121.7 (s)	6.48 (s)	98.4 (d)	6.47 (s)	98.4 (d)
C(5)		154.6 (s)		156.2 (s)		156.4 (s)
C(6)		125.5 (s)		120.7 (s)		120.0 (s)
$\text{CH}_2(\alpha)$	2.61–2.71 (m)	33.9 (t)	2.60–2.70 (m)	34.0 (t)	2.62–2.71 (m)	33.8 (t)
C(1')		145.5 (s)		135.6 (s)		143.2 (s)
H–C(2')	6.40 (br. s)	115.0 (d)	6.38 (d, $J=1.8$)	113.3 (d)	6.52 (d, $J=1.6$)	116.1 (d)
C(3')		161.5 (s)		149.0 (s)		156.4 (s)
H–C(4') or C(4')	6.63–6.67 (m)	113.2 (d)		145.8 (s)		127.6 (s)
H–C(5')	7.08 (t, $J=7.6$)	130.7 (d)	6.60–6.65 (m)	116.3 (d)	6.79 (d, $J=7.6$)	131.7 (d)
H–C(6')	6.57 (d, $J=7.6$)	122.1 (d)	6.44 (dd, $J=8.0, 1.8$)	121.8 (d)	6.38 (dd, $J=7.6, 1.6$)	120.9 (d)
$\text{CH}_2(\alpha')$	2.30–2.40 (m)	38.2 (t)	2.16–2.26 (m)	37.5 (t)	2.16–2.26 (m)	37.9 (t)
C(1'')		133.9 (s)		134.7 (s)		134.9 (s)
H–C(2'',6'')	6.89 (d, $J=8.6$)	130.5 (d)	6.94 (d, $J=8.4$)	130.4 (d)	6.92 (d, $J=8.6$)	130.6 (d)
H–(3'',5'')	6.60–6.64 (m)	116.5 (d)	6.60–6.64 (m)	116.2 (d)	6.59–6.63 (m)	116.4 (d)
C(4'')		156.8 (s)		156.3 (s)		156.4 (s)
$\text{CH}_2(7'')$	4.02 (s)	32.5 (t)	3.91 (s)	31.5 (t)	3.90 (s)	31.7 (t)
C(1''')		134.7 (s)		134.8 (s)		134.9 (s)
H–(2''',6''')	6.83 (d, $J=8.4$)	130.4 (d)	6.85 (d, $J=8.8$)	130.2 (d)	6.84 (d, $J=8.7$)	130.4 (d)
H–(3''',5''')	6.59–6.63 (m)	116.5 (d)	6.58–6.62 (m)	116.2 (d)	6.56–6.59 (m)	116.4 (d)
C(4''')		156.8 (s)		156.3 (s)		156.4 (s)
$\text{CH}_2(7''')$	3.93 (s)	32.4 (t)	3.89 (s)	31.4 (t)	3.89 (s)	31.5 (t)
C(1''''')		133.9 (s)				134.2 (s)
H–C(2''',6''''')	7.02 (d, $J=8.5$)	130.7 (d)			6.97 (d, $J=8.3$)	131.3 (d)
H–C(3''',5''''')	6.63–6.66 (m)	116.5 (d)			6.63–6.66 (m)	116.4 (d)
C(4''''')		156.8 (s)				156.7 (s)
$\text{CH}_2(7''''')$	4.04 (s)	30.6 (t)			3.79 (s)	35.9 (t)
MeO–C(3)	3.45 (s)	62.7 (q)	3.76 (s)	56.3 (q)	3.78 (s)	56.4 (q)
MeO–C(3')	3.67 (s)	56.1 (q)	3.78 (s)	56.7 (q)		

fourteen quaternary C-atoms. According to the ^1H - and ^{13}C -NMR data and the molecular formula, the basic structure of **1** was characterized as a bibenzyl derivative with three 4-substituted benzyl, one OH and two MeO groups. In the ROESY plot, the NOE correlations H–C(α)/H–C(7'') and H–C(7'''), MeO ($\delta(\text{H})$ 3.67)/H–C(2') and H–C(4'), and MeO ($\delta(\text{H})$ 3.45)/H–C(7''') and H–C(7''''') were observed, indicating the location of three 4-substituted benzyl groups at C(2), C(4), and C(6), and of the MeO groups at C(3') and C(3), which were further supported by ^{13}C , ^1H long-range correlation signals in its HMBC plot (Figure).

Compound **2** was isolated as a white, amorphous powder with the molecular formula $\text{C}_{30}\text{H}_{30}\text{O}_6$ as established by HR-ESI-MS. The IR and UV spectra of **2** were similar to those of **1**. The structure of **2** was deduced as 2,6-bis(4-hydroxybenzyl)-3,3'-

dimethoxybibenzyl-4',5-diol¹), called blestritin B, from ¹H- and ¹³C-NMR (Table), ¹H,¹H-COSY and HMBC (Figure), and ROESY data.

The ¹H-NMR of **2** exhibited the resonances of two pairs of 4-substituted benzyl groups at δ (H) 6.94 (*d*, *J* = 8.4 Hz, 2 H), 6.60–6.64 (*m*, 2 H), 6.85 (*d*, *J* = 8.8 Hz, 2 H), and 6.58–6.62 (*m*, 2 H), two benzyl CH₂ groups at δ (H) 3.91 (*s*, 2 H) and 3.89 (*s*, 2 H), four aliphatic protons due to bibenzyl CH₂ groups at δ (H) 2.16–2.26 (*m*, 2 H) and 2.60–2.70 (*m*, 2 H), two MeO groups at δ (H) 3.76 (*s*) and 3.78 (*s*), an *ABX* system at δ (H) 6.38 (*d*, *J* = 1.8 Hz, 1 H), 6.44 (*dd*, *J* = 8.0, 1.8 Hz, 1 H), 6.60–6.65 (*m*, 1 H), and one *s* at 6.48 (*s*, 1 H). In the ¹³C-NMR and DEPT spectra, 30 C-signals belonging to two Me, four CH₂, and twelve CH groups, and twelve C-atoms were observed. These data revealed a bibenzyl skeleton with two 4-substituted benzyl, two OH, and two MeO groups. The aromatic protons appearing as an *ABX* system at δ (H) 6.38, 6.60–6.65, and 6.44 were assigned to H–C(2'), H–C(5'), and H–C(6'), and the *s* at δ (H) 6.48 to H–C(4), respectively, according to the ¹³C,¹H long-range correlation signals observed for H–C(α')/C(1'), C(2'), and C(6'), H–C(2')/C(4') and C(6'), H–C(5')/C(1') and C(3'), and H–C(4)/C(2), C(3), C(5), and C(6) in the HMBC plot (Fig.). In the ROESY plot of **2**, NOE correlations MeO (δ (H) 3.76)/H–C(4) and H–C(7''') and MeO (δ (H) 3.78)/H–C(2') were found, indicating the location of two MeO groups at C(3) and C(3').

To compound **3**, obtained as a white amorphous powder, the elemental formula C₃₆H₃₄O₆ was assigned as deduced from HR-ESI-MS and NMR data. The ¹H- and ¹³C-NMR spectra and molecular formula suggested that the structure of **3** was very similar to that of **2**, except for the appearance of another 4-substituted benzyl group and the absence of a MeO group in **3**. The additional benzyl group was located at C(4') according to the ¹³C,¹H long-range correlation signals at H–C(7''''')/C(3'), C(4'), and C(5') (Fig.), and also the NOE correlation H–C(7''''')/H–C(5'). The NOE correlations MeO/H–C(4) and H–C(7''''') suggested the location of the MeO group at C(3). Thus, compound **3** was determined as 2,4',6-tris(4-hydroxybenzyl)-3-methoxybibenzyl-3',5-diol¹), which has been given the trivial name blestritin C.

In addition to the three new compounds, 18 known ones were also isolated and characterized as 3'-*O*-methylbatatasin III (= 5-methoxy-3-[2-(3-methoxyphenyl)ethyl]phenol) [13], batatasin III (= 3-[2-(3-hydroxyphenyl)ethyl]-5-methoxyphenol) [13], 5,4'-dimethoxybibenzyl-3,3'-diol [14], bulbocol (= 4-[(4-hydroxyphenyl)methyl]-3-methoxy-5-[2-(3-methoxyphenyl)ethyl]phenol) [15], gymconopin D (= 2-[(4-hydroxyphenyl)methyl]-5-methoxy-3-[2-(3-methoxyphenyl)ethyl]phenol) [16], 2-(4-hydroxybenzyl)-3-methoxybibenzyl-3',5-diol [4], 2-(4-hydroxybenzyl)-5-methoxybibenzyl-3,3'-diol [4], bulbocodin (= 3-[2-{2-hydroxy-5-[4-hydroxyphenyl)methyl]phenyl}ethyl]-2,4-bis[(4-hydroxyphenyl)methyl]-5-methoxyphenol) [15], bulbocodin D (= 3-[2-(3-hydroxyphenyl)ethyl]-2,6-bis[(4-hydroxyphenyl)methyl]-5-methoxyphenol) [17], 2,6-bis(4-hydroxybenzyl)-5,3'-dimethoxybibenzyl-3-ol [2], 2',6'-bis(4-hydroxybenzyl)-5-methoxybibenzyl-3,3'-diol [2], 4-methoxyphenanthrene-2,7-diol [18], 3,4-dimethoxyphenanthrene-2,7-diol [18], 2,4-dimethoxyphenanthrene-3,7-diol [18], dactylorhin A (= [(2*R*)-2-(β -D-glucopyranosyloxy)-2-(2-methylpropyl)-1,4-dioxobutane-1,4-diyl]) bis-(oxymethylene-4,1-phenylene) bis[β -D-glucopyranoside] [19], dactylorhin E (= 4-[[[(2*R*)-2-(carboxymethyl)-2-(β -D-glucopyranosyloxy)-4-methyl-1-oxopentyl]oxy]methyl]phenyl β -D-glucopyranoside) [19], gymnoside I (= 4-[[[(2*R*)-2-(carboxymethyl)-2-hydroxy-4-methyl-1-oxopentyl]oxy]methyl]phenyl β -D-glucopyranoside) [20], and gymnoside II (= 4-[[[(3*R*)-3-carboxy-3-hydroxy-5-methyl-1-oxohexyl]oxy]methyl]phenyl β -D-glucopyranoside) [20]. Among them, 3'-*O*-methylbatatasin III, batatasin

III, 5,4'-dimethoxybibenzyl-3,3'-diol, gymconopin D, bulbocodin, bulbocodin D, 4-methoxyphenanthrene-2,7-diol, 3,4-dimethoxyphenanthrene-2,7-diol, 2,4-dimethoxyphenanthrene-3,7-diol, dactylorhin A, dactylorhin E, gymnoside I, and gymnoside II were found for the first time in this plant.

Experimental Part

General. Column chromatography (CC): silica gel H60 (Qingdao Haiyang Chemical Group Corporation, Qingdao, P. R. China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Prep. HPLC: Varian SD-1 instrument equipped with a RP-C₁₈ column (Merck NW25, 20 mm × 250 mm; 10 ml/min) and ProStar 320-UV-Vis detector (254 nm). TLC: HSG₂₅₄ silica gel plates (Yantai Chemical Industrial Institute, Yantai, P. R. China). UV Spectra: Beckman DU-7 spectrometer; λ (log ε) in nm. IR Spectra: Perkin-Elmer 577 spectrometer; ν̄ in cm⁻¹. NMR Spectra: Bruker AM-400 spectrometer; δ in ppm rel. to SiMe₄ as internal standard, J in Hz. HR-ESI-MS: Mariner spectrometer; in m/z.

Plant Material. The tubers of *Bletilla striata* were purchased from the Shanghai Yanghetang Herb Medicine Company in September, 2006, and identified by Prof. Jin-Gui Shen of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen was deposited in the Herbarium of the Shanghai Institute of Materia Medica (No. 20070330).

Extraction and Isolation. Powdered air-dried tubers of *B. striata* (3.0 kg) were percolated with 95% EtOH (20.0 l) at r.t. The extract was concentrated, the residue suspended in H₂O (2.0 l) and then extracted successively with CHCl₃ (3 × 2.0 l), and BuOH (3 × 2.0 l), yielding a CHCl₃ extract (60.0 g) and a BuOH extract (25.3 g). The CHCl₃ extract (60.0 g) was subjected to CC (SiO₂, petroleum ether/acetone 10:1 → 1:1): *Fractions 1–7*. *Fr. 2* (820 mg) was resubjected to CC (Sephadex LH-20, EtOH): *3'-O-methylbatatasin III* (129 mg). *Fr. 4* (4.0 g) was separated by CC (Sephadex LH-20, EtOH): *Fr. 4.1* (1.5 g), *Fr. 4.2* (2.0 g), and *Fr. 4.3* (581 mg). *Fr. 4.2* (2.0 g) was separated by prep. HPLC (RP-18, MeOH/H₂O 4:6 → 10:0): *batatasin III* (592 mg), *4,5-dimethoxybibenzyl-3,3'-diol* (9 mg), *bulbocol* (125 mg), *gymconopin D* (122 mg), and *2-(4-hydroxybenzyl)-3-methoxybibenzyl-3',5'-diol* (10 mg). *Fr. 4.3* (581 mg) was subjected to prep. HPLC (RP-18, MeOH/H₂O 4:6 → 10:0): *4-methoxyphenanthrene-2,7-diol* (12 mg), *3,4-dimethoxyphenanthrene-2,7-diol* (14 mg), and *2,4-dimethoxyphenanthrene-3,7-diol* (8 mg). *Fr. 6* (4.1 g) was separated by prep. HPLC (RP-18, MeOH/H₂O 4:6 → 10:0): **1** (9 mg), **2** (7 mg), *2-(4-hydroxybenzyl)-5-methoxybibenzyl-3',3'-diol* (60 mg), *bulbocodin D* (61 mg), *2,6-bis(4-hydroxybenzyl)-3',5'-dimethoxybibenzyl-3-ol* (63 mg), and *2',6'-bis(4-hydroxybenzyl)-5-methoxybibenzyl-3,3'-diol* (152 mg). *Fr. 7* (6.1 g) was subjected to prep. HPLC (RP-18, MeOH/H₂O 4:6 → 10:0): **3** (5 mg) and *bulbocodin* (41 mg). The BuOH extract (25.3 g) was subjected to CC (SiO₂, CHCl₃/MeOH/H₂O 3:1:0.1 → 7:3:0.5): *gymnoside I* (420 mg), *gymnoside II* (165 mg), *dactylorhin E* (85 mg), and *dactylorhin A* (50 mg).

Blestitin A (= 2,4,6-Tris(4-hydroxybenzyl)-3,3'-dimethoxybibenzyl-5-ol = 2,4,6-Tris[4-(4-hydroxyphenyl)methyl]-3-methoxy-5-[2-(3-methoxyphenyl)ethyl]phenol; **1**): White amorphous powder. UV (MeOH): 281 (2.14). IR (KBr): 3405, 2937, 1612, 1596, 1511, 1438, 1384, 1236, 1170, 1085, 819. ¹H- and ¹³C-NMR: Table. HR-ESI-MS: 599.2408 ([M + Na]⁺, C₃₇H₃₆NaO₆⁺; calc. 599.2410).

Blestitin B (= 2,6-Bis(4-hydroxybenzyl)-3,3'-dimethoxybibenzyl-4',5'-diol = 3-[2-(4-Hydroxy-3-methoxyphenyl)ethyl]-2,4-bis[4-(4-hydroxyphenyl)methyl-5-methoxyphenol]; **2**): White amorphous powder. UV (MeOH): 281 (2.17). IR (KBr): 3413, 2919, 1614, 1511, 1459, 1384, 1236, 1105, 819. ¹H- and ¹³C-NMR: Table. HR-ESI-MS: 509.1924 ([M + Na]⁺, C₃₀H₃₀NaO₆⁺; calc. 509.1940).

Blestitin C (= 2,4',6'-Tris(4-hydroxybenzyl)-3-methoxybibenzyl-3',5'-diol = 3-[2-{3-hydroxy-4-[(4-hydroxyphenyl)methyl]phenyl}ethyl]-2,4-bis[4-(4-hydroxyphenyl)methyl]-5-methoxyphenol; **3**): White amorphous powder. UV (MeOH): 281 (2.27). IR (KBr): 3403, 2917, 1594, 1511, 1444, 1382, 1232, 1170, 1107, 815. ¹H- and ¹³C-NMR: Table. HR-ESI-MS: 585.2230 ([M + Na]⁺, C₃₆H₃₄NaO₆⁺; calc. 585.2253).

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Received October 8, 2008