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Novel aromatics bearing 4-*O*-methylglucose unit isolated from the oriental crude drug *Bombyx Batryticatus*

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Abstract—Oriental crude drug, *Bombyx Batryticatus*, is dried silkworm larva, *Bombyx mori* L., which are dead and stiffened due to a *Beauveria bassiana* infection. In traditional Japanese, Korean, and Chinese medicine, it is employed as analgesic and anticonvulsant. We investigated the constituents of *Bombyx Batryticatus* and isolated four novel aromatics bearing 4-*O*-methylglucose moiety, BB-1, 2, 3, and 4 (**1**–**4**). It is speculated that these compounds are produced by an interaction between plants, insects, and microorganisms.

The oriental crude drug, *Bombyx Batryticatus*, is dried silkworm larva, *Bombyx mori* L., which are dead and stiffened due to a *Beauveria bassiana* infection. In traditional Japanese, Korean, and Chinese medicine, it is employed as analgesic and anticonvulsant for treating headaches, toothaches, tonsillitis, and convulsions.[1] The chemical constituents of the crude drug, *Bombyx Batryticatus*, have not been reported except for ammonium oxalate in the white powder on the body surface.[1] On the other hand, cyclodepsipeptides such as bassianolide[2] and beauvericin[3] were isolated when cultivating entomopathogenic fungi, *Beauveria* sp., from the cultivated mycelium as an insecticidal substance and a toxic substance to brine shrimps and mosquito larvae, respectively. Beauveriolide I, II, and III showed insecticidal activities[4] and inhibitory effects on lipid droplet formation.[5] Tenellin, bassianin,[6] pyridovericin, and pyridomacrolidin[7] were also isolated from the cultivated *Beauveria* sp. In our study on biologically active substances from crude drugs produced by the interactions of insects and microorganisms, we focused on the chemical constituents of *Bombyx Batryticatus* and isolated a novel flavan, BB-1 (**1**) as the acetate (**1a**) and a novel 2-arylbenzofuran, BB-2 (**2**). Two novel β-carboline-type alkaloids, BB-3, and BB-4 (**3** and **4**) were also purified as a mixture of their acetates (**3a** and **4a**).

Bombyx Batryticatus (20.0 kg) was extracted with methanol to give an extract (1.59 kg), which was then partitioned between ethyl acetate and water to yield ethyl acetate solubles (690 g). The ethyl acetate solubles were repeatedly separated by silica gel and ODS column chromatography to afford **2** (5.0 mg) and the mixture of **1**, **3**, and **4**. It was difficult to isolate pure compounds from this mixture using conventional column chromatography and HPLC due to similar polarities. Its ^{1}H NMR spectrum indicated that the compounds in the mixture did not bear an acetyl group. Thus, compounds **1**, **3**, and **4** were separated as their acetyl derivatives (**1a**, **3a**, and **4a**) obtained by reacting the mixture with acetic anhydride and pyridine.

Compound **1a**[8] has 1,2,3,4-tetra- and 1,2,4-trisubstituted benzene rings. Its ^{1}H NMR and ^{1}H–^{1}H COSY spectra showed a hydrogen sequence of H-2–H-4. The resonance positions of the signals mentioned above, along with a long range C–H coupling between C-2 and H-6′, indicated that **1a** is in the flavan class. The ^{1}H NMR spectrum exhibited signals due to a methoxyl and a 2-oxyethyl group, and these two groups bind to C-4′ and C-8, respectively, from C–H couplings as follows: C-4′–OCH_3, C-7–H-1″, C-8a–H-1″, and C-8–H-2″ (Fig. 1). In addition, ^{1}H NMR of **1a** indicated the presence of

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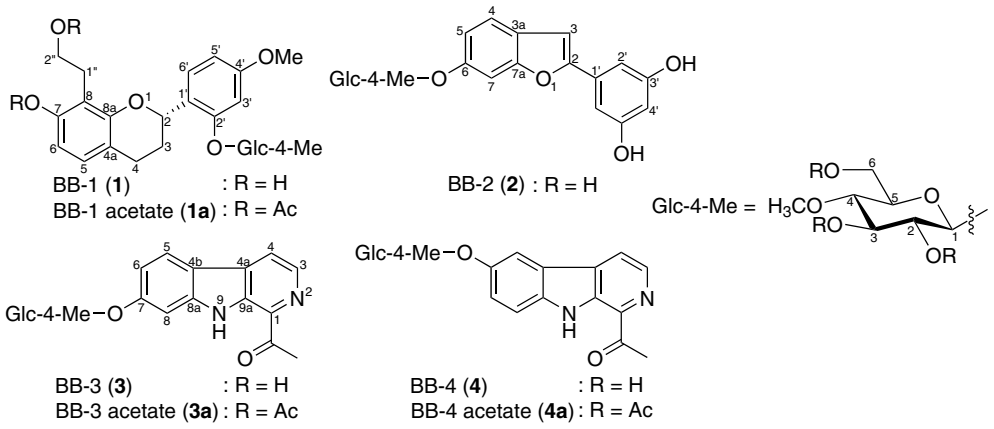


a 2,3,6-triacetylated 4-*O*-methylglucose unit and the long range C–H coupling at C-2′–GlcH-1 implied that the unit was bound to C-2′. Thus, it was concluded that **1a** is a pentaacetylated derivative of **1** (Fig. 1). Treatment of **1a** with sodium methoxide in methanol afforded **1**.[9] Acid treatment of a few milligrams of **1** with hydrogen chloride in methanol failed to hydrolyze **1** to an aglycone and 4-*O*-methylglucose since the reaction afforded unseparable products. A comparative study of the CD spectrum of **1** [λ_{max} 276 nm ($\Delta\varepsilon$ −1.47)] to those of flavans such as (2*S*)-flavan,[10] (2*S*)-5-methoxyflavan-7-ol,[11] and (2*R*)-flavan-7-ol[11] (λ_{max} 276 nm ($\Delta\varepsilon$ −0.43), 278 nm ($\Delta\varepsilon$ −0.59), and 281 nm ($\Delta\varepsilon$ +0.64), respectively) concluded that **1** has a 2*S*-configuration. The 4-*O*-methylglucose unit of **1** was suggested to be D-sugar from the fact that *B. bassiana* can specifically produce 4-*O*-methyl-β-D-glucose-conjugated derivatives of the substrates.[12]

Compound **2**[13] showed the presence of 1,2,4-trisubstituted and 1,3,5-trisubstituted benzene rings. In addition, the aromatic hydrogen signal was observed at δ 6.96, which was coupled with the signal at δ 7.28. The data is consistent with that of moracin M (**5**), a benzofuran derivative isolated from mulberry heartwoods, *Morus alba*.[14] The ^{1}H and ^{13}C NMR, and ^{1}H–^{1}H COSY spectra indicates that like **1**, **2** bears a 4-*O*-methyl-β-glucose unit in the molecule. HMBC spectrum of **2** showed a cross peak between C-6–GluH-1, indicating that the glucose unit was linked to C-6 (Table 1).

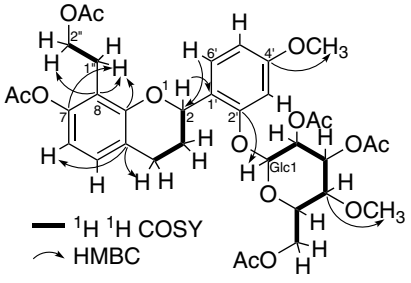
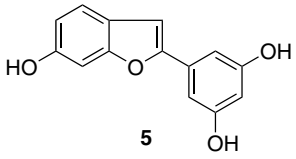


Figure 1. Structure elucidation of BB-1 acetate (**1a**).



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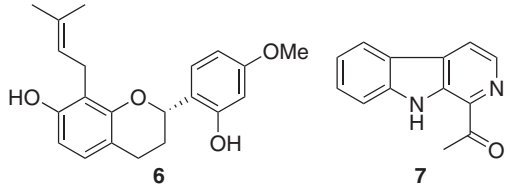
A HPLC method using reversed- and normal-phase ODS column was unsuccessful in separating **3a** and **4a**. Thus, their structures were analyzed using spectral data of the mixture.[15] ^{1}H and ^{13}C NMR spectra (Table 2) of the mixture displayed approximately a 2:1 ratio of **3a** and **4a** and suggested structural similarities. ^{1}H NMR spectrum showed aromatic hydrogen signals for the major compound **3a** assignable to a 1,2,4-trisubstituted benzene ring. Other aromatic hydrogen signals at δ 8.04 and 8.50 (d each, $J = 5.0$ Hz) implied the presence of a pyridine moiety in the molecule. Moreover, ^{1}H NMR signals for a methyl ketone and a triacetylated 4-*O*-methylglucose were observed in the aliphatic hydrogen region. This NMR data along with the ion peak at *m*/*z*: 530 (M^+) ($C_{26}H_{28}N_2O_{10}$) in FAB-MS indicated that **3a** was a 4-*O*-methyl-β-glucose derivative of arenarine D, β-carboline-type alkaloid isolated from *Arenaria kansuensis*[16] and the C–H correlation substantiated the structure of **3a** (Fig. 2). Compound **4a** showed ^{1}H and ^{13}C NMR signals due to the same partial structures as those of **3a**. The distinct difference of **4a** and **3a** was the resonance positions of the aromatic hydrogen signals of 1,2,4-trisubstituted benzene ring, which suggested that **4a** is a regional isomer of the hydroxy group at C-6. Since natural products **3** and **4** did not bear an acetyl group as mentioned above, their structures were concluded to be the deacetylated ones of **3a** and **4a**.



6 **7**

Table 1. ^{1}H and ^{13}C NMR spectral data of **1a**, **1**, and **2**

	BB-1 acetate (**1a**)[a,b,d]		BB-1 (**1**)[a,c,d]		BB-2 (**2**)[a,c,d]	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	72.8	5.10 (1H, dd, 9.9, 2.2)	73.6	5.48 (1H, dd, 9.8, 2.3)	157.2	
3	28.7	1.79 (1H, m) 2.22 (1H, m)	30.3	1.83 (1H, m) 2.17 (1H, m)	102.0	6.96 (1H, d, 0.9)
3a					125.4	
4	25.1	2.69 (1H, ddd, 19.2, 5.5, 3.3) 2.93 (1H, ddd, 19.2, 10.0, 3.3)	25.8	2.59 (1H, dd, 15.7, 4.3) 2.67 (1H, dd, 15.7, 4.3)	121.9	7.44 (1H, d, 8.5)
4a	119.7		114.5			
5	128.0	6.96 (1H, d, 8.4)	128.2	6.73 (1H, d, 8.2)	114.8	7.02 (1H, dd, 8.5, 2.1)
6	113.9	6.57 (1H, d, 8.4)	108.5	6.33 (1H, d, 8.2)	157.1	
7	148.2		155.5		100.6	7.28 (1H, dd, 2.1, 0.9)
7a					156.5	
8	117.5		113.5			
8a	154.0		155.2			
1′	123.8		125.5		133.4	
2′	153.9		156.2		104.1	6.77 (2H, d, 2.2)
3′	103.1	6.69 (1H, d, 2.4)	103.3	6.80 (1H, d, 2.4)	159.9	
4′	160.0		161.3		103.8	6.26 (1H, d, 2.2)
5′	107.5	6.65 (1H, dd, 8.5, 2.4)	108.8	6.62 (1H, dd, 8.6, 2.4)		
6′	126.9	7.35 (1H, d, 8.5)	128.0	7.32 (1H, d, 8.6)		
1″	23.8	2.87 (2H, t, 7.1)	27.7	2.89 (2H, m)		
2″	63.2	4.13 (1H, dt, 10.5, 7.1) 4.20 (1H, dt, 10.5, 7.1)	62.7	3.65 (2H, m)		
4′OMe	55.5	3.79 (3H, s)	55.9	3.78 (3H, s)		

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[b] Dissolved in $CDCl_3$.
[c] Dissolved in CD_3OD.
[d] Signals of acetoxyl groups and 4-*O*-methyl glucose moiety are omitted. See Refs. 8, 9 and 13.

Microbial biotransformations of various organic compounds using *Beauveria* sp. introduced a hydroxy group into the substrate to yield the corresponding alcohol derivative. Oxidation of double bond with *Beauveria* sp. produced an epoxide or diol.[17] Reduction of the carbonyl group was also reported in the biotransformation.[18] Furthermore, β-4-*O*-methylglucosylation with *Beauveria bassiana* has been more commonly observed in the biotransformation of substrates containing a phenolic hydroxy group.[12] These reports suggested that prenylflavan (**6**)[19], a constituent of mulberry leaves that the silkworm eats, would be a source of compound **1**.

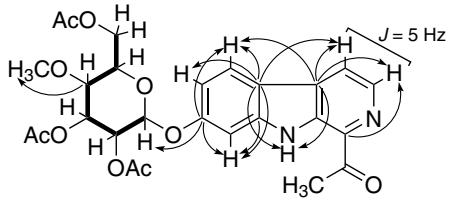


Figure 2. Structure elucidation of BB-3 acetate (**3a**).

Table 2. ^{1}H and ^{13}C NMR Spectral Data of **3a** and **4a**

	BB-3 acetate (**3a**)[a,b]		BB-4 acetate (**4a**)[a,b]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	135.8[c]		137.5	
3	138.5	8.50 (1H, d, 5.0)	137.9	8.51 (1H, d, 4.8)
4	118.4	8.04 (1H, d, 5.0)	119.0	8.09 (1H, d, 4.8)
4a	131.2		136.3[c]	
4b	116.3		136.0[c]	
5	122.8	8.00 (1H, d, 8.6)	110.5	7.74 (1H, d, 2.4)
6	111.5	6.98 (1H, dd, 8.6, 2.1)	151.5	
7	158.3		120.8	7.28 (1H, dd, 8.8, 2.4)
8	99.6	7.15 (1H, d, 2.1)	112.5	7.47 (1H, d, 8.8)
8a	142.2		131.2	
9		10.23 (1H, br.s)		10.23 (1H, br.s)
9a	135.8[c]		135.8[c]	
1-*C*OCH$_3$	203.2		203.1	
1-CO*CH*$_3$	25.9	2.87 (3H, s)	25.9	2.87 (3H, s)

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$.
[b] Signals of acetoxyl groups and 4-*O*-methyl glucose moiety are omitted. See Ref. 15.
[c] Assignments are interchangeable.

The prenyl side chain of **6** would be transformed to epoxide or diol, which is oxidatively cleaved to give an aldehyde. Reduction of the aldehyde and 4-*O*-methyl-β-glucosylation would afford **1**. 2-Arylbenzofuran (**5**)[14] of mulberry heartwoods is thought to be the original constituent of **2**. Compounds **3** and **4** might be produced by hydroxylation of 1-acetyl-β-carboline (**7**)[20] followed by 4-*O*-methyl-β-glucosylation. Compounds **5** and **7** have yet to be isolated from mulberry leaves, while there are the facts that silkworms feed only mulberry leaves and *B. bassiana* is an entomopathogenic fungus. We can thus presume that compounds **5** and **7** initially from mulberry leaves were transformed to compounds **2**, **3**, and **4** while preparing the crude drug, *Bombyx Batryticatus*.

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References and Notes

1. Namba, T. In: *Coloured Illustrations of Wakan-Yaku*; Hoikusha: Osaka, 1980; Vol. II; pp 325–327.
2. Hamill, R. L.; Higgens, C. E.; Boaz, H. E.; Gorman, M. *Tetrahedron Lett.* **1969**, 4255–4258.
3. Suzuki, A.; Kanaoka, M.; Isogai, A.; Murakoshi, S.; Ichinoe, M.; Tamura, S. *Tetrahedron Lett.* **1977**, 2167–2170.
4. Mochizuki, K.; Ohmori, K.; Tamura, H.; Shizuri, Y.; Nishiyama, S.; Miyoshi, E.; Yamamura, S. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 3041–3046.
5. (a) Namatame, I.; Tomoda, H.; Si, S.; Yamaguchi, Y.; Masuma, R.; Omura, S. *J. Antibiot.* **1999**, *5*(2), 1–6; (b) Namatame, I.; Tomoda, H.; Tabata, N.; Si, S.; Omura, S. *J. Antibiot.* **1999**, *52*, 7–12.
6. (a) McInnes, A. G.; Smith, D. G.; Wat, C.-K.; Vining, L. C.; Wright, J. L. C. *J.Chem. Soc., Chem. Commun.* **1974**, *8*, 281–282; (b) McInnes, A. G.; Smith, D. G.; Walter, J. A.; Vining, L. C.; Wright, J. L. C. *J. Chem. Soc., Chem. Commun.* **1974**, *8*, 282–284.
7. Takahashi, S.; Uchida, K.; Kakinuma, N.; Hashimoto, R.; Yanagisawa, T.; Nakagawa, A. *J. Antibiot.* **1998**, *51*, 1051–1054.
8. Data for **1a**: yellowish oil; $[\alpha]_D^{25}$ −65.7 (*c* 0.207, chloroform); EIMS *m/z* 702 $[M]^+$, 400, 303, 243 (base), 201, 43; HREIMS *m/z* 702.2498 (702.2521 calcd for $C_{35}H_{42}O_{15}$). NMR data of the aromatic ring are shown in Table 1, and other signals are listed below. ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.5, 169.7, 169.6, 169.5, 99.4 (Glc1), 77.9 (Glc4), 74.6 (Glc3), 73.2 (Glc5), 71.5 (Glc2), 67.4 (Glc4O*Me*), 62.9 (Glc6), 21.1, 21.0, 20.9, 20.7, 20.6. ^1H NMR (400 MHz, $CDCl_3$) δ 5.24 (1H, dd, *J* = 9.6, 8.8 Hz, Glc3), 5.17 (1H, dd, *J* = 9.6, 7.7 Hz, Glc2), 5.00 (1H, d, *J* = 7.7 Hz, Glc1), 4.44 (1H, dd, *J* = 12.0, 2.2 Hz, Glc6), 4.25 (1H, dd, *J* = 12.0, 6.3 Hz, Glc6), 3.72 (1H, ddd, *J* = 9.9, 6.3, 2.2 Hz, Glc5), 3.44 (3H, s, Glc4O*Me*), 3.41 (1H, dd, *J* = 9.9, 8.8 Hz, Glc4), 2.31 (3H, s), 2.11 (3H, s), 2.07 (3H, s), 1.97 (3H, s), 1.90 (3H, s).
9. Data for **1**: yellowish oil; $[\alpha]_D^{25}$ −48.1 (*c* 0.387, methanol); EIMS *m/z* 492 $[M]^+$, 474, 316, 298, 150 (base), 137; HREIMS *m/z* 492.1969 (492.1994 calcd for $C_{25}H_{32}O_{10}$). NMR data of the aromatic ring are shown in Table 1, and other signals are listed below. ^{13}C NMR (100 MHz, CD_3OD) δ 102.8 (Glc1), 80.7 (Glc4), 78.2 (Glc3), 77.3 (Glc5), 75.1 (Glc2), 62.2 (Glc6), 60.8 (Glc4O*Me*). ^1H NMR (400 MHz, CD_3OD) δ 4.86 (1H, d, *J* = 7.7 Hz, Glc1), 3.85 (1H, dd, *J* = 12.0, 2.3 Hz, Glc6), 3.70 (1H, dd, *J* = 12.0, 5.0 Hz, Glc6), 3.57 (3H, s, Glc4O*Me*), 3.55 (1H, dd, *J* = 9.4, 9.2 Hz, Glc3), 3.44 (1H, dd, *J* = 9.4, 7.7 Hz, Glc2), 3.43 (1H, ddd, *J* = 9.8, 5.0, 2.3 Hz, Glc5), 3.17 (1H, dd, *J* = 9.8, 9.2 Hz, Glc4).
10. Antus, S.; Kurtan, T.; Juhasz, L.; Kiss, L.; Hollosi, M.; Majer, Zs. *Chirality* **2001**, *13*, 493–506.
11. Cardillo, G.; Merlini, L.; Nasini, G.; Salvadori, P. *J. Chem. Soc. C* **1971**, 3967–3970.
12. (a) Olivo, H. F.; Peeples, T. L.; Rios, M.-Y.; Velazquez, F.; Kim, J.-W.; Narang, S. *J. Mol. Catal. B* **2003**, *21*, 97–105; (b) Gotor, V.; Quiros, M.; Liz, R.; Frigola, J.; Fernandez, R. *Tetrahedron* **1997**, *53*, 6421–6432; (c) Lacroix, I.; Biton, J.; Azerad, R. *Bioorg. Med. Chem.* **1997**, *5*, 1369–1380; (d) Vigne, B.; Archelas, A.; Furstoss, R. *Tetrahedron* **1991**, *47*, 1447–1458; (e) Vigne, B.; Archelas, A.; Fourneron, J. D.; Furstoss, R. *Tetrahedron* **1986**, *42*, 2451–2456.
13. Data for **2**: pale yellow oil; $[\alpha]_D^{25}$ −45.8 (*c* 0.511, methanol); EIMS *m/z* 418 $[M]^+$, 268, 242 (base), 228, 213; HREIMS *m/z* 418.1285 (418.1264 calcd for $C_{21}H_{22}O_9$); NMR data of the aromatic ring are shown in Table 1, and other signals are listed below. ^{13}C NMR (100 MHz, CD_3OD) δ 102.9 (Glc1), 80.7 (Glc4), 78.0 (Glc3), 77.2 (Glc5), 75.1 (Glc2), 62.1 (Glc6), 60.9 (Glc4O*Me*). ^1H NMR (400 MHz, CD_3OD) δ 4.91 (1H, d, *J* = 7.7 Hz, Glc1), 3.88 (1H, dd, *J* = 12.0, 2.1 Hz, Glc6), 3.72 (1H, dd, *J* = 12.0, 4.8 Hz, Glc6), 3.59 (3H, s, Glc4O*Me*), 3.59 (1H, dd, *J* = 9.3, 8.9 Hz, Glc3), 3.48 (1H, dd, *J* = 9.3, 7.7 Hz, Glc2), 3.46 (1H, ddd, *J* = 9.7, 4.8, 2.1 Hz, Glc5), 3.21 (1H, dd, *J* = 9.7, 8.9 Hz, Glc4).
14. Deshpande, V. H.; Srinivasan, R.; Rao, A. V. Rama *Indian J. Chem.* **1975**, *13*, 453–457.
15. Data for mixture of **3a** and **4a**: yellowish oil; FABMS *m/z* 530 $[M]^+$. NMR data of the aromatic ring are shown in Table 2, and other signals are listed below. **3a**: ^{13}C NMR (100 MHz, $CDCl_3$) δ 99.2 (Glc1), 77.7 (Glc4), 74.9 (Glc3), 73.3 (Glc5), 71.7 (Glc2), 62.8 (Glc6), 60.5 (Glc4O*Me*). ^1H NMR (400 MHz, $CDCl_3$) δ 5.28 (1H, dd, *J* = 8.9, 8.1 Hz, Glc3), 5.21 (1H, dd, *J* = 8.1, 7.3 Hz, Glc2), 5.16 (1H, d, *J* = 7.3 Hz, Glc1), 4.46 (1H, dd, *J* = 12.0, 2.3 Hz, Glc6), 4.31 (1H, dd, *J* = 12.0, 5.4 Hz, Glc6), 3.75 (1H, ddd, *J* = 9.5, 5.4, 2.3 Hz, Glc5), 3.49 (1H, dd, *J* = 9.5, 8.9 Hz, Glc4), 3.46 (3H, s, Glc4O*Me*). **4a**: ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.4 (Glc1), 77.7 (Glc4), 74.9 (Glc3), 73.3 (Glc5), 71.9 (Glc2), 62.8 (Glc6), 60.5 (Glc4O*Me*). ^1H NMR (400 MHz, $CDCl_3$) δ 5.26 (1H, t, *J* = 9.2 Hz, Glc3), 5.19 (1H, dd, *J* = 9.2, 7.6 Hz, Glc2), 5.07 (1H, d, *J* = 7.6 Hz, Glc1), 4.46 (1H, dd, *J* = 12.1, 2.2 Hz, Glc6), 4.30 (1H, dd, *J* = 12.1, 5.2 Hz, Glc6), 3.70 (1H, ddd, *J* = 9.8, 5.2, 2.2 Hz, Glc5), 3.48 (1H, dd, *J* = 9.8, 9.2 Hz, Glc4), 3.46 (3H, s, Glc4O*Me*).
16. Wu, F. E.; Koike, K.; Nikaido, T.; Sakamoto, Y.; Ohmoto, T.; Ikeda, K. *Chem. Pharm. Bull.* **1989**, *37*, 1808–1809.
17. Johnson, R. A.; Herr, M. E.; Murray, H. C.; Chidester, C. G.; Han, F. J. *J. Org. Chem.* **1992**, *57*, 7209–7212.
18. Sundby, E.; Azerad, V.; Anthonsen, T. *Biotechnol. Lett.* **1998**, *20*, 337–340.
19. Doi, K.; Kojima, T.; Makino, M.; Kimura, Y.; Fujimoto, Y. *Chem. Pharm. Bull.* **2001**, *49*, 151–153.
20. Zhou, T.-S.; Ye, W.-C.; Wang, Z.-T.; Che, C.-T.; Zhou, R.-H.; Xu, G.-J.; Xu, L.-S. *Phytochemistry* **1998**, *49*, 1807–1809.