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Microbial Transformation of Baccatin VI and 1β-Hydroxy Baccatin I by *Aspergillus niger*

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Abstract—The biotransformation of baccatin VI (**1**) and 1β-hydroxybaccatin I (**2**) with the filamentous fungus *Aspergillus niger* produced four new taxane diterpenoids taxumairol S_1 (**3**), taxumairol T_1 (**4**) and taxumairol S (**5**), taxumairol T (**6**), respectively. 1β-Dehydroxybaccatin VI (**7**) remained unreacted under the same condition.

The antitumor agent paclitaxel (Taxol), which was first found in the bark of *Taxus brevifolia*, is the most effective chemotherapeutic anticancer agent from natural sources.[1,2] The most promising options for large scale production of Taxol are likely cell culture[3] and semisynthesis.[4] The biotransformation of Taxol and other taxoids is an important area of study in clinical pharmacology to get new compounds of pharmaceutical and agricultural potential.[5] The biotransformation of Taxol and other taxoids offers potential pathways to the preparation of chemically inaccessible metabolites. Unusual structures can be obtained, which would not be accessible by chemical reactions.[6] The main disadvantage of this route is that the products are not predictable and the yields are low. *Taxomyces andreanae,* an endophytic fungus originally isolated from the bark of *T. brevifolia*, was able to produce paclitaxel in culture media.[7] Filamentous fungi, for example *Cunninghamella echinulata, Cunninghamella elegans, Absidia coerula, Microsphaeropsis onychiuri, Mucor* sp., *Alternaria alternata* and plant cells were known to carry out regio- and stereoselective hydroxylation, deacetylation, rearrangement, hydrolysis and epimerization of different taxoid compounds.[8–14] These novel findings stimulates us to investigate the biotransformation of some taxoids using the fungus *Aspergillus niger*.

The filamentous fungi, *A. niger* 31130, purchased from Food Industry Research & Development Institute, Hsinchu, Taiwan, was used for the biotransformation. The strain was maintained in 1 L malt agar I (Blakeslee's formula) supplemented with 100 mg/L of substrates (**1**, **2** and **7**, which were isolated from *Taxus maire* Lemee & Levl.).[15,16] After rotatory shaking with 170 rpm and 2-inch stroke at 25 °C for 2 days, the cell beads grew to be about 5 mm diameter. The fungus culture continued to grow for an additional 3 days at 90 rpm and was harvested for solvent extraction. The fermentation broth was extracted with EtOAc. The EtOAc layer was dried with anhyd. Na_2SO_4 and evaporated under vacuum to give the crude residue, which on column chromatography (silica gel) followed by PTLC using *n*-hexane–CH_2Cl_2–MeOH (7:7:1) as eluent afforded **3**–**6**. The substrate **1** gave taxumairols S_1 (**3**, yield 12%) and T_1 (**4**, yield 7.8%). 1β-Hydroxybaccatin I (**2**) gave an unseparable mixture of taxumairols S and T (**5** and **6**, yield 12.6%), while the substrate **7** remained unaffected under the same condition.

Taxumairol S_1 (**3**), $[\alpha]_D^{25}$ −30° (CH_2Cl_2), had a molecular formula of $C_{37}H_{46}O_{15}$ as deduced by a combination of FABMS and ^1H and ^{13}C NMR spectroscopy. The ^1H NMR data (Table 1) of **3** showed a broad singlet at δ 5.24 (H-5) and a pair of AB quartet at δ 3.60, 3.48 (H-20, J = 12 Hz) in addition to an AX spin system at δ 6.06 and 6.31 (H-10 and -9). The ^1H and ^{13}C NMR (Table 2) spectra also exhibited four methyl, five acetyl and benzoyl signals together with characteristic quarternary carbons (δ 69.3, C-1; δ 74.2, C-4; δ 75.4, C-15), and the methylene carbon (δ 62.0, C-20). Detailed analysis of the ^1H, ^{13}C NMR, COSY and HSQC spectra revealed that 3 is a 5/7/6 taxene with an opened oxetane

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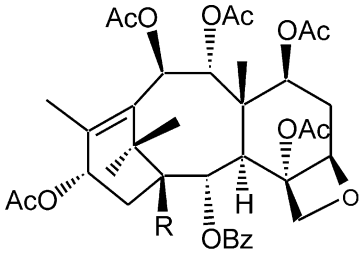
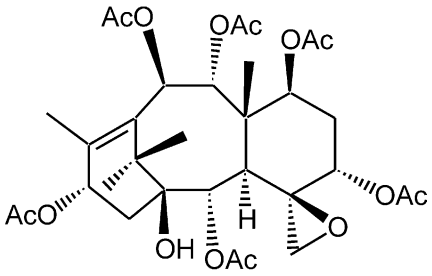
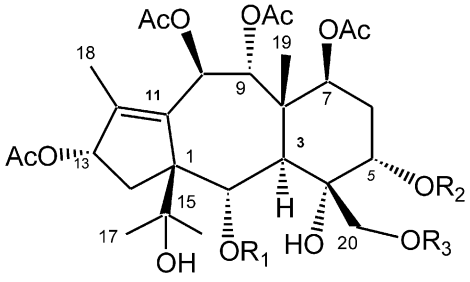


1, R = OH
7, R = H



2



3, R_1 = Bz, R_2 = Ac, R_3 = H
4, R_1 = Bz, R_2 = H, R_3 = Ac
5, R_1 = Ac, R_2 = H, R_3 = Ac
6, R_1 = Ac, R_2 = Ac, R_3 = H

ring.[17,18] The 11(1→15)-*abeo*-taxane skeleton bearing a dimethyl carbinol group at C-1 was confirmed from the observation of adjacent carbons of C-1 and C-15, as revealed by the cross-peaks from both Me-16 (δ 1.18) and Me-17 (δ 1.13) to C-1 and C-15 in the HMBC spectrum. The HMBC correlations of C-3 (δ 43.8)/H-2 (δ 6.27), H-5 and H-20, and C-4/H-3 (δ 3.05) and H-5, as well as C-20/H-5 also indicated that the benzoyl group was kept at C-2 and the oxetane D-ring was opened.

Taxumairol T_1 (**4**), an isomer of **3**, had a composition of $C_{37}H_{46}O_{15}$ as derived from FABMS and DEPT spectra ($[\alpha]_D$ −7.4°, CH_2Cl_2). Its IR and UV absorption bands indicated the presence of hydroxyl, acetyl and benzoyl groups. This finding was supported by the ^1H and ^{13}C

Table 1. ^1H δ (multiplicity, *J* in Hz)[a] NMR data for compounds **3–6**

	3	**4**	**5**	**6**
2	6.27 (d, 7.1)	6.36 (d, 7.3)	5.93 (d, 6.7)	6.02 (d, 6.5)
3	3.05 (d, 7.1)	3.15 (d, 7.3)	2.48 (d, 6.7)	2.48 (d, 6.5)
5	5.24 (br s)	3.80 (br s)	3.75 (br s)	4.96 (br s)
6	2.00 (m)	1.80 (m)	2.17 (m)	2.17 (m)
		2.08 (m)		
7	5.47 (t, 7.0)	5.57 (dd, 10.8, 4.6)	5.46 (dd, 4.5, 11.3)	5.35 (dd, 4.3, 11)
9	6.06 (d, 10.6)	6.01 (d, 10.5)	6.10 (d, 10.8)	6.10 (d, 10.8)
10	6.31 (d, 10.6)	6.33 (d, 10.5)	6.18 (d, 10.8)	6.18 (d, 10.8)
13	5.65 (t, 7.5)	5.64 (d, 7.1)	5.55 (t, 6.7)	5.55 (t, 6.7)
14	2.50 (m)	2.54 (m)	1.75 (m)	1.75 (m)
			2.39 (m)	2.39 (m)
16	1.18 (s)	1.16 (s)	1.17 (s)	1.10 (s)
17	1.13 (s)	1.08 (s)	1.25 (s)	1.25 (s)
18	1.91 (s)	1.95 (s)	1.85 (s)	1.87 (s)
19	1.26 (s)	1.28 (s)	1.49 (s)	1.52 (s)
20	3.6 (d, 12.0)	4.20 (d, 12.5)	4.82 (d, 11.2)	4.07 (d, 11.8)
	3.48 (d, 12.0)	4.14 (d, 12.5)	4.17 (d, 11.2)	3.23 (d, 11.8)
OAc-2			1.97 (s)[b]	1.96 (s)[b]
OAc-5	2.10 (s)[b]			1.98 (s)[b]
OAc-7	2.10 (s)[b]	2.07 (s)[b]	2.02 (s)[b]	2.00 (s)[b]
OAc-9	2.04 (s)[b]	2.03 (s)[b]	2.07 (s)[b]	2.01 (s)[b]
OAc-10	2.00 (s)[b]	2.00 (s)[b]	2.10 (s)[b]	2.09 (s)[b]
OAc-13	1.97 (s)[b]	1.96 (s)[b]	2.17 (s)[b]	2.16 (s)[b]
OAc-20		2.12 (s)[b]	1.95 (s)[b]	
$OCOC_6H_5$				
o	7.99 (d, 7.4)	8.01 (d, 7.5)		
m	7.59 (t, 7.4)	7.61 (t, 7.5)		
p	7.45 (t, 7.4)	7.51 (t, 7.5)		

[a]Measured at 300 MHz in $CDCl_3$.
[b]Data interchangeable.

Table 2. ^{13}C δ (multiplicity)[a] NMR data for compounds **3–6**

	3	4	5	6
1	69.3 (s)	69.3 (s)	68.2 (s)	68.1 (s)
2	68.5 (d)	70.5 (d)	70.5 (d)	68.7 (d)
3	43.8 (d)	44.1 (d)	42.6 (d)	42.5 (d)
4	74.2 (s)	74.2 (s)	75.5 (s)	75.1 (s)
5	71.3 (d)	70.4 (d)	69.7 (d)	73.0 (d)
6	31.0 (t)	31.1 (t)	31.0 (t)	31.0 (t)
7	67.5 (d)	67.8 (d)	68.2 (d)	68.1 (d)
8	44.7 (s)	44.6 (s)	44.9 (s)	44.5 (s)
9	76.2 (d)	76.5 (d)	77.3 (d)	76.8 (d)
10	69.8 (d)	68.6 (d)	67.7 (d)	67.7 (d)
11	136.4 (s)	136.7 (s)	138.2 (s)	137.2 (s)
12	146.9 (s)	147.0 (s)	145.9 (s)	145.1 (s)
13	79.5 (d)	79.7 (d)	79.4 (d)	80.0 (d)
14	36.6 (t)	36.6 (t)	37.8 (t)	37.7 (t)
15	75.4 (s)	75.4 (s)	75.7 (s)	76.0 (s)
16	25.4 (q)	25.8 (q)	27.6 (q)	27.6 (q)
17	27.5 (q)	27.9 (q)	29.7 (q)	29.3 (q)
18	11.5 (q)	12.0 (q)	12.0 (q)	11.8 (q)
19	14.5 (q)	14.4 (q)	14.2 (q)	14.9 (q)
20	62.0 (t)	63.9 (t)	68.1 (t)	64.3 (t)
OAc-2			167.5 (s)	167.8 (s)[b]
			20.58 (q)	20.5 (q)[b]
OAc-5	169.2 (s)[b]			168.0 (s)[b]
	20.5 (q)[b]			20.7 (q)[b]
OAc-7	169.2 (s)[b]	169.2 (s)[b]	169.5 (s)[b]	169.4 (s)[b]
	20.5 (q)[b]	20.5 (q)[b]	21.18 (q)[b]	20.9 (q)[b]
OAc-9	169.7 (s)[b]	170.3 (s)[b]	169.7 (s)[b]	170.1 (s)[b]
	20.7 (q)[b]	20.7 (q)[b]	21.54 (q)[b]	20.96 (q)[b]
OAc-10	167.5 (s)[b]	167.7 (s)[b]	169.6 (s)[b]	170.4 (s)[b]
	20.1 (q)[b]	20.2 (q)[b]	21.63 (q)[b]	21.03 (q)[b]
OAc-13	170.2 (s)[b]	171.1 (s)[b]	169.0 (s)[b]	170.6 (s)[b]
	21.4 (q)[b]	21.5 (q)[b]	21.92 (q)[b]	21.09 (q)[b]
OAc-20		169.7 (s)[b]	172.0 (s)[b]	
		20.0 (q)[b]	22.07 (q)[b]	
$OCOC_6H_5$	166.0	166.4 (s)		
i	129.6 (s)	130.2 (s)		
o	129.3 (d)	129.7 (s)		
m	128.6 (d)	128.9 (d)		
p	133.5 (d)	133.7 (d)		

[a]Measured at 75 MHz in $CDCl_3$.
[b]Data interchangeable.

NMR spectral data of **4**. The characteristic resonances such as peaks for four methyls, five acetyls, and the quarternary carbons (δ 69.3, C-1; δ 75.4, C-15) suggested that **4** is a 5/7/6 taxane with an opened oxetane ring skeleton. Analysis of ^1H and ^{13}C NMR spectra of **4** (Tables 1 and 2) indicated that they were very similar to those of **3**. Comparison of ^1H NMR data with those of **3** revealed that the only difference between them was that the signal of H-5 is shifted upfield to δ 3.80, while signals of H-20 are shifted downfield to δ 4.20 and 4.14. The assignment of the acetyl at C-20 was further confirmed by HMBC experiment (Fig. 1).

Taxumairol S (**5**) had a composition of $C_{32}H_{44}O_{15}$ as deduced from FABMS and DEPT spectra. The ^1H and ^{13}C NMR data of **5** were very similar to those of **4** suggesting that compound **5** is a close analogue of **4**. Characteristic peaks included four methyls, six acetyls, six oxygenated methine protons, two quarternary oxygenated carbons at δ 75.5 (C-4) and δ 75.7 (C-15). However, the benzoyl signals in **4** disappeared in **5**. Instead, an extra acetyl group (δ 1.97s, 20.6, 167.5) at C-2 was observed in **5**.

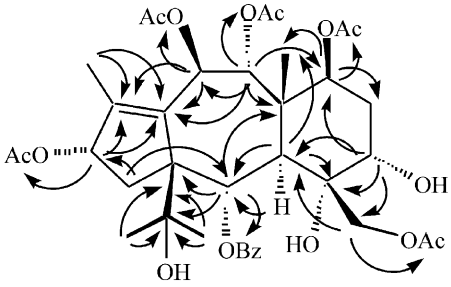


Figure 1. HMBC correlation of **4**.

Taxumairol T (**6**) is an isomer of **5**. The ^1H and ^{13}C NMR data of **6** (Tables 1 and 2) showed four typical methyl singlets, six acetyl singlets, a doublet at δ 2.48 (H-3), a broad singlet at δ 4.96 (H-5), an oxygenated methine signal of H-5 at δ 4.96 and the oxygenated methylene signals of H-20 at δ 4.07 and 3.23 ($J = 11.8$ Hz). Detailed analysis of the 2D-NMR spectra (COSY, HMQC and HMBC) verified the structure of **6**, which bears a dimethyl carbinol group at C-1 in the skeleton of 11(1→15)-*abeo*-taxane. The above findings determined the acetoxyl group to be at C-5 and the hydroxyl group at C-20 in taxumairol T (**6**).

Incubation of **1** and **2** with the same medium under the same condition but without the fungus has resulted in no conversion of **1** and **2**. It was concluded that compounds **3–6** are new metabolites derived from microbial transformation. A series of taxumairols have been previously isolated and characterized.[18–21] Accordingly, compounds **3–6** were designated as taxumairols S_1, T_1, S and T, respectively.

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References and Notes

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2. Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. In *Progress in the Chemistry of Organic Natural Products*; Herg, W., Kirby, G. W., Tamm, C., Eds.; Springer: New York, 1993; Vol. 62, p 1.
3. Klein, L. L.; Li, L.; Maring, C. J.; Yeung, C. M.; Thomas, S. A.; Grampovnik, D. J.; Plattner, J. J. *J. Med. Chem.* **1995**, *38*, 1482.
4. Nicolaou, K. C.; Dai, W. M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl* **1994**, *33*, 15.
5. Holland, H. L. *Organic Synthesis with Oxidative Enzymes*; V Chemie: Weinheim, 1992; p 95.
6. Sun, D. A.; Nikolakakis, A.; Sauriol, F.; Mamer, O. L; Zamia, L. O. *Bioorg. Med. Chem.* **2001**, *9*, 1985.
7. Stierle, A.; Strobel, G.; Stierle, D. *Science* **1993**, *260*, 214.
8. Zhang, J.; Zhang, L.; Wang, X.; Qiu, D.; Sun, D.; Gu, J.; Fang, Q. *J. Nat. Prod.* **1998**, *61*, 497.
9. Hu, S.; Sun, D. A.; Tian, X.; Fang, Q. *Tetrahedron Lett.* **1997**, *38*, 2721.

10. Hu, S.; Tian, X.; Zhu, W.; Fang, Q. *Tetrahedron* **1996**, *52*, 8739.
11. Hu, S.; Tian, X.; Zhu, W.; Fang, Q. *J. Nat. Prod.* **1996**, *59*, 1006.
12. Sun, D. A.; Sauriol, F.; Mamer, O.; Zamir, L. O. *Bioorg. Med. Chem.* **2001**, *9*, 793.
13. Sun, D. A.; Nikolakakis, A.; Sauriol, F.; Mamer, O.; Zamir, L. O. *Bioorg. Med. Chem.* **2001**, *9*, 1985.
14. Dai, J.; Cui, Y.; Zhu, W.; Guo, H.; Ye, M.; Hu, Q.; Zhang, D.; Zheng, J.; Guo, D. *Planta Med.* **2002**, *68*, 1113.
15. Shen, Y. C.; Tai, H. R.; Hsieh, P. W.; Chen, C. Y. *Chin. Pharm. J.* **1996**, *48*, 207.
16. Shen, Y. C.; Tai, H. R.; Chen, C. Y. *J. Nat. Prod.* **1996**, *59*, 173.
17. Baloglu, E.; Kingston, D. G. I. *J. Nat. Prod.* **1999**, *62*, 1448.
18. Shen, Y. C.; Prakash, C. V. S.; Chen, Y. J.; Hwang, J. F.; Kuo, Y. H.; Chen, C. Y. *J. Nat. Prod.* **2001**, *64*, 950.
19. Shen, Y. C.; Chang, Y. T.; Lin, Y. C.; Lin, C. L.; Kuo, Y. H.; Chen, C. Y. *Chem. Pharm. Bull.* **2002**, *50*, 781.
20. Shen, Y. C.; Chang, Y. T.; Wang, S. S.; Lin, Y. C.; Chen, C. Y. *Chem. Pharm. Bull.* **2002**, *50*, 1561.
21. Shen, Y. C.; Wang, S. S.; Pan, Y. L.; Lo, K. L.; Chakraborty, R.; Chien, C. T.; Kuo, Y. H.; Lin, Y. C. *J. Nat. Prod.* **2002**, *65*, 1848.