Anomalous Microbial Transformations on the Taxane Ring of 10-DAB by a Strain of the Fungus *Curvularia lunata*: Transbenzoylation, Transacetylation, and Opening of the Oxetane Ring

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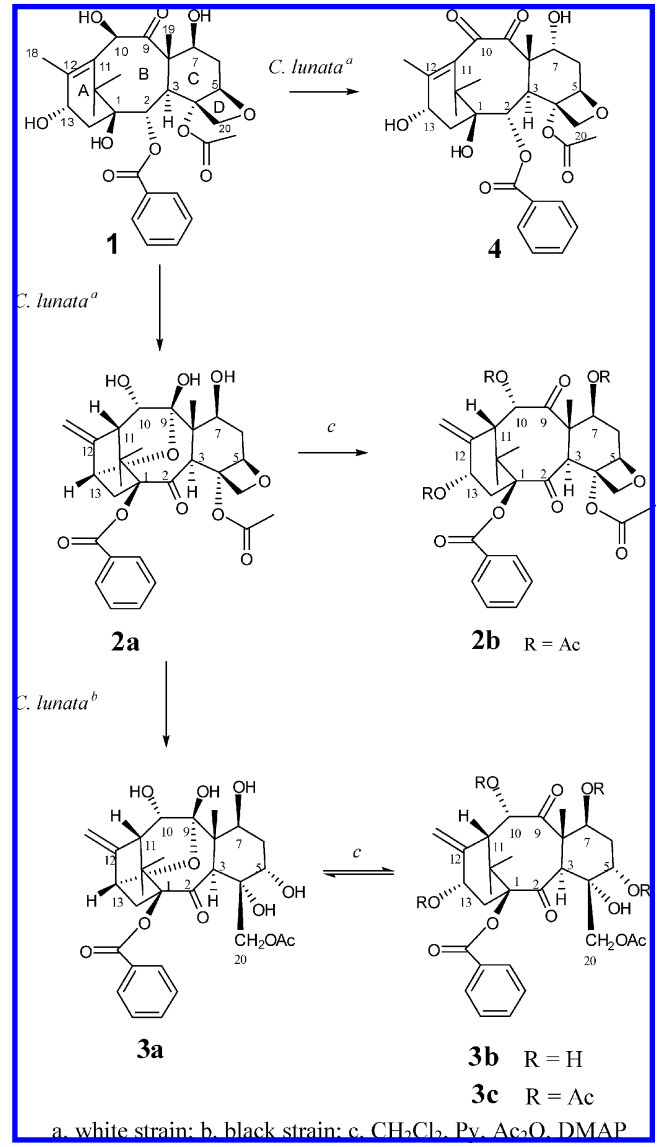
The fermentation of 10-deacetylbaccatin III (10-DAB) (**1**) with *Curvularia lunata* afforded the taxane hemiacetals **2a** and **3**, characterized by extensive structural modification, including C-2 to C-1 transbenzoylation, oxidation of the C-2 hydroxyl, formation of a C-9/C13 hemiacetal, epimerization at C-10, and migration of the endocyclic double bond to an exocyclic location. In compound **3**, an acetate-assisted opening of the oxetane ring was also observed.

Since the discovery of Taxol, a potent antitumor drug, great progress has been made in understanding its biological activity and chemistry.[1] Structural modifications of naturally occurring bioactive substances by microbial transformations, especially in a way impossible by conventional chemical methods, are an important area of natural product chemistry. However, few investigations have been conducted on biotransformations of taxanes by filamentous fungi,[2] although interesting results were obtained with *Absidia coerulae* and different strains of *Cunninghamella echinulata*. In the realm of taxane derivatives, these fungi are capable of performing site-specific hydroxylations, oxidations, and reductions and can accomplish skeletal rearrangements.[3] It is clear that microbial enzymatic systems may be useful tools to mimic some important steps of taxoid biosynthesis, including the extensive oxidation of the taxane core, shedding light on some aspects of the process by which these compounds are produced in *Taxus* spp.[3] Unpredictability of the nature of the compound obtained is the main disadvantage of the use of microorganisms.

We began a microbial transformation program on 10-deacetylbaccatin III (10-DAB) (**1**) with microorganisms screened for their ability to hydroxylate or oxidize. We reported previously that the microbial transformation of **1** with *Trametes hirsuta* induced 13-oxidation, whereas incubation with a strain of *Curvularia lunata* resulted in the isolation of the 7-*epi*-10-DAB and 7-*epi*-10-oxo-10-DAB (**4**).[4] As part of our ongoing studies on microbial transformation of 10-DAB (**1**), we have extended the previous studies on the fermentation of **1** with *Curvularia lunata* by investigating the effect of increased incubation time. This led to the identification of two strains of *Curvularia lunata* (176-ICRM and 198-ICRM) capable of extensively modifying 10-DAB (**1**). In fact, from cultures of both fungi it was possible to obtain up to six products structurally similar to 10-DAB. Large-scale fermentations were then carried out in order to identify the minor compounds. In this way, it was possible to obtain the new taxoids (**2a** and **3**). Their structures were determined on the basis of ^{13}C and ^{1}H NMR experiments and chemical reactions.



Results and Discussion

Incubation of **1** with *C. lunata* gave the new compounds **2a** and **3** in low yields. Compound **2a**, a white solid, exhibited a molecular peak at *m/z* 543 $[MH]^+$, corresponding to the molecular formula $C_{29}H_{34}O_{10}$, with a decrease of two mass units compared to the parent compound **1**. Acetylation of compound **2a** afforded the triacetyl derivative **2b**. The structures of **2a** and **2b** were deduced through a complete proton and carbon NMR study. Their ^{1}H and ^{13}C NMR assignments are given in Tables 1 and 2, respectively, together with those of the parent compound **1** reported for the purpose of comparison. Long-range proton−carbon correlations and the most significant observed NOEs are reported in Tables 3 and 4 (Supporting Information), respectively. The proton−carbon multiple bond connectivities were acquired for compound **2a**, while the NOE

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Table 1. ^{1}H NMR Spectroscopic Data (δ_H (J in Hz) 500 MHz, $CDCl_3$) for **1**, **2a**,**b**, and **3a**−**c**

atom	**1**[a]	**2a**	**2b**[b]	**3a**[b]	**3b**[b]	**3c**[b]
H-2	5.65, dd (7.2, 1.2)					
H-3	4.04, d (7.2)	4.62, br, s	4.75, br, s	4.77, s	5.41, s	4.82, s
H-5	4.97, dddd, (9.6, 2.2, 1.1, 1.1)	4.91, dd, (8.5, 1.5)	5.01, dd, (9.8, 5.1)	3.97, dd, (3.7, 2.5)	4.14, dd, (4.1, 2.1)	5.71, dd, (4.2, 2.5)
H-6a	2.45, ddd, (14.3, 9.6, 6.9)	2.74, ddd, (15.1, 8.5, 8.0)	2.64, α, ddd, (14.5, 9.8, 6.1)	2.10, α, ddd, (14.4, 5.0, 3.7)	2.09, α, ddd, (14.3, 4.6, 4.1)	2.06, α, ddd, (14.4, 4.4, 4.2)
H-6b	1.83, ddd, (14.3, 11.1, 2.2)	1.94, ddd, (15.1, 9.0, 1.5)	2.05, β, ddd, (14.5, 12.5, 5.1)	1.82, β, ddd, (14.4, 12.0, 2.5)	1.74, β, ddd, (14.3, 12.9, 2.1)	1.93, β, ddd, (14.4, 12.9, 2.5)
H-7	4.35, ddd, (11.1, 7.4, 6.9)	4.93, d, (9.0, 8.0)	5.74, dd, (12.5, 6.1)	4.88, dd, (12.0, 5.0)	4.90, dd, (12.9, 4.6)	5.93, dd, (12.9, 4.4)
H-10	5.27, d, (2.3)	3.86, br, d, (4.0)	5.40, d, (1.8)	3.79, br, s	4.51, br, s	5.29, d, (1.7)
H-11		2.19, br, d, (1.5)	2.71, br, d, (1.8)	2.19, br, d, (1.8)	2.77, br, s	2.70, d, (1.7)
H-13	4.91, dddq, (9.2, 7.3, 4.7, 1.4)	4.69, ddd, (3.0, 2.5, 1.5)	5.86, d, (9.1)	4.72, dd, (3.2, 2.9)	4.71, br, d, (8.5)	6.02, dd, (9.5, 1.0)
H-14a	2.47, dd, (15.6, 7.3)	3.54, α, dd, (15.2, 3.0)	2.84, α, d, (17.2)	3.67, α, dd, (15.2, 3.2)	3.28, α, br, d, (16.3)	3.20, α, dd, (17.0, 1.0)
H-14b	2.33, ddd, (15.6, 9.2, 1.2)	1.96, β, dd, (15.2, 2.5)	2.34, β, dd, (17.2, 9.1)	2.06, β, dd, (15.2, 2.9)	2.25, β, dd, (16.3, 8.5)	2.39, β, dd, (17.0, 9.5)
CH_3-16	1.09, s	1.15, s	1.26, s	1.22, s	1.26, s	1.32, s
CH_3-17	1.11, s	1.32, s	1.41, s	1.37, s	1.43, s	1.42, s
CH_3-18	2.06, d, (1.4)					
H-18a		5.24, br, d, (2.0)	5.71, br, d, (2.0)	5.24, br, d, (1.4)	5.47, br, s	5.77, br. s
H-18b		5.08, br, d, (2.0)	5.51, br, d, (2.0)	5.08, br, d, (1.4)	5.39, br, s	5.39, br, s
CH_3-19	1.72, s	1.68, s	1.88, s	1.16, s	1.33, s	1.64, s
H-20a	4.18, dd, (8.1, 1.1)	5.14, d, (8.8)	5.54, β, br, d, (8.8)	5.05, d, (12.3)	4.48, d, (13.1)	4.51, d, (13.1)
H-20b	4.17, dd, (8.1, 1.1)	4.67, d, (8.8)	4.41, α, br, d, (8.8)	4.71, d, (12.3)	4.43, d, (13.1)	4.50, d, (13.1)
OH-1	3.40, s					
OBz	8.11, m, (2H)	7.88, m, (2H)	7.94, m, (2H)	7.94, m, (2H)	7.99, m, (2H)	7.97, m, (2H)
	7.65, m, (1H)	7.55, m, (1H)	7.57, m, (1H)	7.60(m), m, (1H)	7.62, m, (1H)	7.62, m, (1H)
	7.54, m, (2H)	7.42, m, (2H)	7.44, m, (2H)	7.44, m, (2H)	7.47, m, (2H)	7.47, m, (2H)
OR-4	2.26, s (OAc)	2.27, s (OAc)	2.32, s (OAc)[c]	*n.o.* (OH)	5.97, br, s (OH)	5.24, br, s, (OH)
OR-5				*n.o.*[d] (OH)	*n.o.* (OH)	2.16, s (OAc)[c]
OR-7	4.12, d, (7.4) (OH)	3.37, br, s (OH)	2.21, s (OAc)[c]	*n.o.* (OH)	*n.o.* (OH)	1.88, s (OAc)[c]
OH-9		8.00, br, s		*n.o.*		
OR-10	4.22, d, (2.3) (OH)	4.36, d, (4.0) (OH)	2.06, s (OAc)[c]	*n.o.* (OH)	*n.o.* (OH)	2.40, s, (OAc)
OR-13	4.53, d, (4.7) (OH)		1.88, s (OAc)[c]		*n.o.* (OH)	2.05, s (OAc)[c]
OAc-20				2.07, s	2.08, s	2.13, s

[a] The data of the starting material 10-DAB (**1**) are inserted for a best comparison of the NMR data. [b] Where possible, the indication α or β of the nuclei orientation is reported. [c] Assignments may be interchanged. [d] Not observed.

Table 2. ^{13}C NMR Spectroscopic Data (δ_C, mult., 125 MHz, $CDCl_3$) of **1**, **2a**,**b**, and **3a**−**c**[a]

position	1	2a	2b	3a	3b	3c
1	79.0, qC	87.1, qC	87.3, qC	88.3, qC	89.3, qC	88.2, qC
2	75.2, CH	209.3, qC	205.6, qC	209.6, qC	207.7, qC	206.0, qC
3	47.0, CH	50.2, CH	47.2, CH	52.1, CH	49.3, CH	52.6, CH
4	81.0, qC	81.5, qC	82.1, qC	76.2, qC	74.9, qC	74.3, CH
5	84.2, CH	84.8, CH	82.7, CH	68.0, CH	68.9, CH	69.5, CH
6	37.2, CH_2	37.9, CH_2	32.6, CH_2	33.0, CH_2	30.3, CH_2	28.0, CH_2
7	72.2, CH	72.3, CH	70.2, CH	67.7, CH	65.3, CH	68.6, CH
8	57.9, qC	53.7, qC	56.6, qC	54.0, qC	57.2, qC	56.6, qC
9	211.9, qC	98.2, qC	202.3, qC	99.0, qC	215.5, qC	202.3, qC
10	75.0, CH	69.0, CH	72.0, CH	68.1, CH	70.4, CH	72.4, CH
11	135.2, qC	56.1, CH	58.0, CH	56.4, CH	61.9, CH	58.5, CH
12	142.3, qC	139.1, qC	136.7, qC	138.9, qC	142.7, qC	137.0, qC
13	68.1, CH	73.5, CH	66.8, CH	73.8, CH	68.3, CH	67.3, CH
14	38.9, CH_2	37.0, CH_2	34.5, CH_2	36.5, CH_2	34.9, CH_2	33.7, CH_2
15	42.8, qC	43.7, qC	40.3, qC	43.5, qC	41.1, qC	41.0, qC
16	26.8, CH_3	26.4, CH_3	29.1, CH_3	27.0, CH_3	28.4, CH_3	29.0, CH_2
17	19.8, CH_3	22.5, CH_3	22.9, CH_3	22.9, CH_3	22.2, CH_3	22.9, CH_3
18	15.1, CH_3	112.7, CH_2	128.0, CH_2	112.9, CH_2	122.9, CH_2	128.0, CH_2
19	9.8, CH_3	8.8, CH_3	12.5, CH_3	11.5, CH_3	12.5, CH_3	12.9, CH_3
20	77.3, CH_2	76.4, CH_2	77.2, CH_2	65.7, CH_2	66.3, CH_2	67.1, CH_2
OBz	167.2, qC	163.8, qC	164.0, qC	166.8, qC	166.0, qC	166.0, qC
	133.7, CH	133.2, CH	133.3, CH	134.1, CH	134.1, CH	134.4, CH
	130.2, CH	129.9, CH	129.9, CH	130.1, CH	130.1, CH	130.3, CH
	129.6, CH	129.6, CH	129.6, CH	129.0, CH	129.2, CH	129.9, CH
	128.7, CH	128.5, CH	128.5, CH	128.7, CH	128.7, CH	129.0, CH
OAc-4	170.8, qC	172.1, qC	170.5, qC			
	22.7, CH_3	22.7, CH_3	21.9, CH_3			
OAc-5						170.4, qC[c]
						21.2, CH_3[b]
OAc-7			171.1, qC[c]			169.9, qC[c]
			21.7, CH_3[b]			21.8, CH_3[b]
OAc-10			169.7, qC[c]			170.3, qC[c]
			20.8, CH_3[b]			22.5, CH_3[b]
OAc-13			169.5, qC[c]			169.7, qC[c]
			20.6, CH_3[b]			20.8, CH_3[b]
OAc-20				170.7, qC	171.4, qC	171.1, qC
				21.0, CH_3	20.9, CH_3	21.0, CH_3

[a] Assignments are based on heteronuclear HSQC and HMBC experiments. [b] Assignments may be interchanged. [c] Assignments may be interchanged.

Table 3. Long-Range Proton−Carbon Correlations for Compounds **2a** and **3a** in $CDCl_3$[a]

	2a	3a
proton	correlated carbons	correlated carbons
H-3	2,4,7,8,9,19	2,4,5,7,8,9,20
H-5	3,4	3,4,6,7
H-6a, H-6b	4,5,7,8	4,7
H-7	3,5,19	3,6,9,19
H-10	8,12,15	8,12,15
H-11	1,9,10,12,13,15,16,17,18	1,9,10,12,13,15,16,17,18
H-13	1,9,12	1,11,12,14,18
H-14a, H-14b	1,2,12,13,15	1,2,12,13,15
CH_3-16	1,11,15,17	1,11,15,17
CH_3-17	1.11,15,16	1,11,15,16
H-18a, H-18b	11,12,13	11,12,13
CH_3-19	3,7,8,9	3,7,8,9
H-20a, H-20b	3,4,5	4,5,20-OAc (CO)
4-OCO**Me**	4-O-**CO**-Me	
20-O-CO**Me**		20-O-**CO**-Me

[a] HMBC correlations are optimized for 8 Hz.

experiments were carried out on **2b**, which provides much more clear and intense NOEs than **2a**. The reason for this is the presence of numerous OH groups in **2a** that produced broadening of the signals and strong exchange peaks in the NOE spectra. The NMR data demonstrated that **2a** had the same skeleton as 10-DAB (**1**). In particular, the network of heterocorrelations of H-3, H-6a, H-6b, H-11, H-14a, and H-14b with the surrounding carbons (Table 3) are in complete agreement with retaining the A, B, C, and D fused ring arrangement. The occurrence of the four-membered-ring D in **2a** was evident from the value of the CH_2-20 geminal coupling constant (8.8 Hz), which is smaller than that normally observed for methylene groups and is typical of oxetane rings.

The biotransformation of **1** to **2a** involves several structural and stereochemical modifications, namely, (a) shift of the C_{11}═C_{12} double bond from endo- to exocyclic (C_{12}═C_{18}); (b) migration of the benzoyl group from C-2 to C-1, with oxidation of the C-2 secondary alcohol to a ketone; and (c) inversion of configuration at C-10 with formation of a cyclic hemiacetal between the 13-hydroxyl and the 9-keto group. The occurrence of a hemiacetal at C-9 was suggested by its ^{13}C chemical shift (δ 98.2) typical of a carbon linked to two oxygen atoms. The long-range correlation of C-9 with H-13 indicated that the cyclic hemiacetal involved the 13-OH group. Furthermore, the occurrence of a new carbonyl group in the molecule with respect to **1** was indicated by the presence of the signal at δ 209.3 in the ^{13}C spectrum of **2a**. The interactions C-2/H-3 and C-2/H-14 observed in the HMBC spectrum and the lack of any vicinal coupling constant for H-3 demonstrated that C-2 was part of the above-mentioned carbonyl function. Evidence for the remaining structural changes cited above was obtained from NOE experiments on **2b**. Compound **2b** lacked the hemiacetal structure and instead was a diketo derivative, as indicated by the ^{13}C spectrum, which showed, with respect to **2a**, the presence of an additional carbonyl group at δ 202.3 attributable to C-9. The occurrence of the exocyclic double bond in **2b** involving C-18 was inferred by NOE interactions between the methylene olefin protons and the methine protons H-13 and H-11. The configuration at C-10 and C-11 was determined from the observation of NOEs involving the nuclei H-10, H-11, and CH_3-19. Indeed, the strong mutual NOE observed between H-10 and H-11 and the small vicinal coupling constant ($J_{10,11}$ = 1.8 Hz) showed that they were *cis*-oriented. Moreover, H-10 and CH_3-19 displayed a strong NOE, indicating that they were spatially close. Thus, assuming that the B, C ring junction is the same in **1** and **2b**, these observations indicate that

the groups CH_3-19, H-10, and H-11 in **2b** lie on the same β-face of the molecule. Finally, the benzoyl group in **2b** was linked to C-1. Evidence of this structural modification with respect to **1** was based on the absence of the OH-1 signal in the proton spectrum together with the observation of clear NOEs between the CH_3-16 and -17 groups and the protons H-2′ and H-6′ of the phenyl ring (Table 4, Supporting Information).

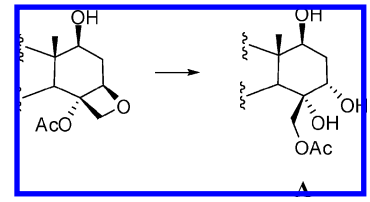
Compound **3** was isolated as a powder, and the mass spectrum exhibited a molecular ion at *m/z* 560, 18 units higher than **2a**, indicating the addition of a water molecule. Compound **3** exists in solution as a temperature-dependent mixture of the hemiacetal and keto forms **3a** and **3b** in slow equilibrium. The two forms are equally populated in chloroform solution at the temperature of 30 °C. Increasing the temperature, the population of the hydroxyketone form **3b** increased, reaching 60% at 40 °C. This behavior allowed us to analyze and completely assign the spectra of both forms, whose NMR data are collected in Tables 1 and 2. It is noteworthy to observe that, contrary to **3**, compound **2** did not undergo any appreciable tautomeric equilibrium and exists in solution only in the hemiacetal form **2a**. Possibly the conformation of the ring C, which is different for **2a** with respect to **3a** (see below), may be important for the formation of the hydrogen bond between CO-9 and OH-7 and thus may play a role in stabilizing the hydroxyketone form **3b**. The proton−carbon correlations were determined only for the isomer **3a** and are reported in Table 3, while the NOE measurements were carried out on the tetraacetyl derivative **3c** and are collected in Table 5 (Supporting Information).

Inspection of the NMR data for the rings A and B of **3a** revealed that they were almost identical to the data of **2a**. However, marked changes were observed for rings C and D. Diagnostic was the change of the geminal coupling constant of the C-20 methylene protons from 8.8 Hz in **2a** to 12.3 Hz in **3a**, suggesting the opening of the oxetane ring. Compound **3a**, in principle, could be derived from **2a** by addition of H_2O and concomitant opening of the four-membered ring with the formation of two new OH groups. However, this reaction is much more complex because it is accompanied by two additional structural modifications, namely, migration of the acetyl group from C-4 to C-20 and inversion of configuration of C-5. Evidence that the acetyl group is linked at C-20 was obtained from the $^1H-^{13}C$ connectivities of the H-20a and H-20b methylene protons, showing a clear correlation with the acetyl group, a correlation lacking in **1** and **2a**. The configuration at C-4, C-5, and C-7 of ring C was inferred from the value of the proton vicinal couplings around the fragment $C_5-C_6-C_7$ and through an extensive NOE study carried out on the tetraacetyl derivative **3c** (Table 5, Supporting Information). This product (**3a**), as observed also for **2b**, exists also as the 2,9-diketo derivative.

The vicinal coupling constants of protons on carbons $C_5-C_6-C_7$ of **3c** are typical of a six-membered ring in the chair conformation. Thus, the values of $J(6\alpha,7) = 12.9$ Hz, $J(5,6\alpha) = 2.5$ Hz, and $J(5,6\beta) = 4.2$ Hz show that H-6α and H-7 are *trans* diaxially oriented, and H-5β is equatorial. As a consequence, the oxygen atom at C-5 lies on the α-face of the molecule, opposite that observed for **2a** and **2b**. Finally, the NOEs observed among the protons CH_3-19, CH_2-20, and H-6β show that they are located on the same β-face of the molecule, while the strong contact between H-3 and H-7 confirms that they both lie on the α-face. These observations strongly suggest that the configurations of C-4 and C-7 in **3c** are the same as those found in **1** and **2a**.

In conclusion, we have structurally elucidated compounds **2a** and **3**. Their formation requires a series of reactions, whose exact sequence is difficult to establish. A shift of the endocyclic double bond from ring A to ring B (from $\Delta^{11(12)}$ to $\Delta^{11(10)}$) had been observed in the biotransformation of taxinine by *Absidia coerulea*, while a $\Delta^{11(12)}$ to $\Delta^{12(18)}$ migration had been observed in a derivative of yunnanxane, a 4(20),11(12)-taxadiene, upon fermentation with the same microorganism.[5] In our case, it could be assumed that

Scheme 1. Oxetane Ring-Opening by the Bioagent *C. lunata*



the rearrangement is triggered by the formation of a C-10 allylic cation, followed by loss of one proton from the C-18 methyl group with the generation of a 9,10-double bond favored by release of steric strain. Hydration in an anti-Markovnikov and anti-configurational fashion would lead to the 10α-hydroxy, 11β-H structural element of **2a**.

The shift of the double bond allowed a more flexible structure and, therefore, the formation of an intramolecular hemiacetalic bond between the C-9 carbonyl and the OH group in position 13. Although more than 500 natural taxanes have been reported to date, **2a** and **3** are unique in having this hemiacetal ring; only a taxane isolated from *Taxus cuspidata*[6] presents an ethereal intramolecular bond between C-13/C-9.

The other important feature of compounds **2a** and **3** is the shift of the benzoyl group from the oxygen at C-2 to the OH group at C-1; a similar intramolecular benzoyl migration has been reported to take place chemically in basic medium (DBU, NaH).[7] Successively, the free OH group at C-2 was bio-oxidized to a carbonyl.

The four-membered oxetane D ring is one of the structural features deemed to be essential for bioactivity.[8] Compound **3** was obtained for the first time in a biotransformation process. This process seems to proceed with mechanisms similar to those reported for compounds of this class in the presence of acid reagents. Thus **3** can be derived from **2a** by an acetate-assisted intramolecular opening of the oxetane ring, a reaction well precedented in oxetane-type taxoids and known to occur with acyl rearrangement. It is reported that 9-dihydro-13-acetylbaccatin III under acidic conditions (trichloroacetic acid in CH_2Cl_2)[7] can lead to a mixture of taxanes where the oxetane ring is opened and acetyl migration occurs, as shown in partial structure A (Scheme 1) with epimerization at C-5. In the same way, a reaction of Taxol with electrophilic reagents (Meerwein's reagent) gave the same structure A.[9] Although **2a**, **3**, and **4** showed only marginal cytotoxicity with respect to the starting material **1** (Experimental Section), their structural novelty highlights how fermentation can complement chemical transformations to expand the diversity of the natural products pool.

Experimental Section

General Experimental Procedures. Mass spectra were obtained with Bruker Esquire 3000 and, for HRMS, with Bruker APEX-QZT ICR spectrometers. The proton and carbon NMR spectra were carried out on a Bruker DMX-500 instrument at 303 K in $CDCl_3$. The assignment of the proton signals is based on the chemical shift correlation experiments (COSY), while the carbon nuclei were assigned from the heteronuclear correlation experiments via one-bond (HSQC)

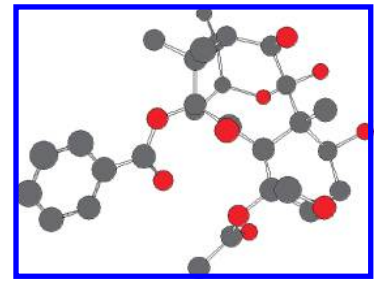


Figure 1. Model of compound **2a** (MM2) showing the 3D spatial arrangement of the molecule.

and long-range (HMBC) coupling constants. The nuclear Overhauser effects were determined by monodimensional NOE difference spectra or by NOESY experiments using a mixing time of 700−900 ms. Flash CC was performed on Merck silica gel; TLC and PLC were performed with Merck HF_{254} silica gel. The purity of products was checked by TLC, NMR, and MS and deemed sufficient for the purpose of structural determination. 10-DAB (**1**) was kindly provided by Indena S.p.a.-Milano.

Microorganisms and Fermentation Procedures. The microorganisms used in this work were two strains of fungus *C. lunata,* deposited in the collection of our Institute (CNR-ICRM) as 176-ICRM and 198-ICRM. Both fungi were maintained at 4 °C as a stock culture on agar slants containing PDA medium (potato dextrose agar), where they grow forming white (176-ICRM) or black (198-ICRM) colonies; the white strain is a morphogenetic variation of the original black strain formed during subsequent transfers in pure culture and has lost the capability of spore formation The cultures were grown according to standard two-stage fermentation protocol. Experiments were performed in conical Erlenmeyer flasks (300 mL) containing 100 mL of the sterile medium CSB (per L): corn steep 10 g, glucose 30 g, $NaNO_3$ 2 g, K_2HPO_4 2 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.02 g. Stage I cultures were inoculated with a water−spore suspension obtained from freshly grown agar slants. Flasks were incubated at 24 °C on a rotary shaker at 180 rpm. After 72 h, a 1−2% inoculum from stage I cultures was transferred to 60 flasks with baffles containing 100 mL of the same medium (CSB) and incubated at 28 °C at 180 rpm. After 24−48 h of growth, 10-DAB (**1**) (1.2 g), dissolved in DMSO (12 mL), was evenly distributed among the 60 flasks containing stage II cultures (final concentration 0.2 g/L). The speed of agitation during the phase of biotransformation was 250 rpm. Culture controls consisted of fermentation blanks in which microorganisms were grown under identical conditions but without substrate. Substrate controls consisted of sterile medium containing the same amount of substrate and incubated under the same conditions. The fermentations were sampled periodically by TLC analysis, with pure samples as reference. Metabolites **2a** and **4** were reproducibly produced by fermentations of both strains 176-ICRM and 198-ICRM; metabolite **3** was produced by cultures of the black strain (198-ICRM).

Extraction Procedures. Two-week-old cultures were harvested and filtered; the filtrate containing the metabolites was extracted two times with equal volumes of EtOAc/MeOH (100:1) and evaporated to dryness under reduced pressure, to give a brown oil (from strain 176-ICRM) or a white-beige solid (from strain 198-ICRM).

Isolation of 2a and 4 from Cultures of White Strain 176-ICRM. The crude residue (1.7 g) was purified by column chromatography on silica gel with a stepwise elution using CH_2Cl_2/i-PrOH from 30:1 to 1:1. The fractions obtained, were further separated on preparative plates (PLC) in hexane/diethyl ether (1:15) as eluent to afford metabolite **4** (114 mg, 12.3%).[4] Compound **2a** was further purified on preparative plates (PLC) in reversed-phase (RP-18) using H_2O/MeCN (1:1) as eluent to give 40 mg of metabolite **2a** (4%) and unreacted 10-DAB (**1**) (260 mg, 22%).

Compound 2a: white solid, mp 200−204 °C; $[\alpha]^{20}_D$ −35 (*c* 0.3, MeOH); UV (EtOH) λ_{max} 202, 232, and 274sh (ε 15.130, 14.050, and 1350); ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS, *m/z* 565 [M + Na]$^+$, 543 [MH]$^+$, and 525 [MH − 18]$^+$; HREIMS 542.2152 (calcd for $C_{29}H_{34}O_{10}$, 542.2148).

Isolation of 3 from Cultures of Black Strain 198-ICRM. Metabolites **2a** and **4** were also obtained from cultures of the black strain of *C. lunata* (198-ICRM) although in different quantity; after purification of the crude residue (1.6 g), at first by column chromatography eluting with CH_2Cl_2/i-PrOH from 30:1 to 1:1, then on preparative plates (PLC), 32 mg of **2a** (3.6%) and 39 mg of **4** (4.3%) were obtained. The greater difference observed in the black cultures was the presence of the new metabolite **3**. Compound **3** (28 mg, 3%) was obtained by PLC (RP-18), using H_2O/MeCN (55:45) as eluent; unreacted 10-DAB (310 mg 26%) was recovered.

Compound 3: white solid, mp 158 °C; $[\alpha]^{20}_D$ +12 (*c* 0.15, MeOH); UV (EtOH) λ_{max} 203, 233 and 274sh (ε 16 400, 15 920, and 2350); ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS, *m/z* 583 [M + Na]$^+$; EIMS, 560 (24%), 542 (33), 524 (50), 482 (100), and 464 (45); HREIMS 560.2046 (calcd for $C_{29}H_{36}O_{11}$, 560.2075).

Acetylation of 2a and 3. Compound **2a** (30 mg) dissolved in CH_2Cl_2 (5 mL) was acetylated in the presence of acetic anhydride (0.5 mL), pyridine (0.2 mL), and 4-dimethylaminopyridine (DMAP) (10 mg); the reaction was shaken at rt and monitored by TLC (CH_2Cl_2/i-PrOH, 20:1). After 2 h, compound **2a** was completely converted to compound **2b**. After evaporation of solvent, the residue obtained (24 mg, 68%) was analyzed; ^{1}H and ^{13}C NMR data are in Tables 1 and 2; ESISM, *m/z* 691 [M + Na]$^+$. Compound **3** (30 mg) was acetylated as above to obtain **3c** (18 mg, 49%), after purification by PLC (hexane/EtOAc, 2:1); ^{1}H and ^{13}C NMR data are in Tables 1 and 2; ESIMS, *m/z* 751 [M + Na]$^+$ and 292.

Cytotoxicity Bioassay. Compounds **2a**, **3**, **4**, and 10-Dab (**1**) were tested for their cytotoxicity against the non-small cell lung tumor cell line H 460 [IC_{50} (μM): 51.5, 95.2, 60, and 3.1, respectively].

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Supporting Information Available: NOEs for compounds **2b** and **3c** (Tables 4 and 5) and the original ^{1}H, ^{13}C, and HMBC NMR spectra for compounds **2a**, **3a/3b**, and **3c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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