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Enzymatic Selective Dehydration and Skeleton Rearrangement of Paclitaxel Precursors

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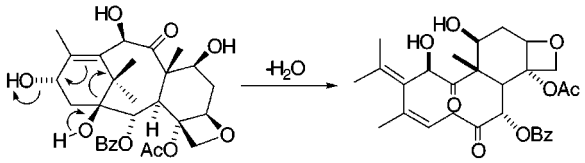
ABSTRACT



Enzymatic dehydration reactions to remove selectively the chemically least reactive 13-hydroxyl group of 10-deacetylbaccatin III and its derivatives 2a–c are described. The elimination of the 13-OH takes place with skeleton rearrangement, allowing us to synthesize some interesting diterpenoids in good yields.

The semisynthesis of paclitaxel (**1**), a diterpene anticancer agent isolated from the bark of the Pacific yew, *Taxus brevifolia*, requires first selective protection of 10-deacetylbaccatine III (10-DAB, **2a**) and then coupling with the 13-side chain (**3**) or equivalents.[1,2] We recently reported an enzymatic method for the selective protection of **2a** and its 7-triethylsilyl derivative (7-TES-10-DAB, **2b**) using *Psuedomomas cepacia* lipase (PCL) as the catalyst and moderately activated acid anhydrides such as dichloroacetic and trichloroacetic anhydride as the acyl donors.[3] The procedure isparticularly useful for the selective acylation of the 7- and 10-hydroxyl groups in unprotected **2a**[4,5] and of the 13-hydroxyl group in 7-protected **2b**. No procedure, however, is currently available for the selective transformation of the chemically least reactive 13-hydroxyl group in unprotected **2a**. We now wish to report an enzymatic transformation to remove selectively the 13-hydroxyl group of **2a**−**c** with skeleton rearrangement. This reaction allows us to synthesize several taxane molecues in good yields. Previously, some chemical reactions including similar skeleton rearrangements were described.[6] However, to the best of our knowledge, no reports have so far appeared to describe the enzyme-mediated dehydration and skeleton rearrangement.

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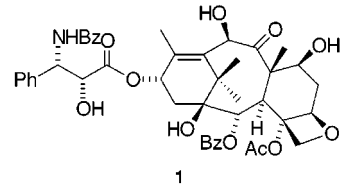
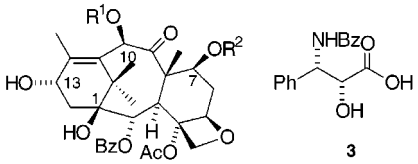


1



3

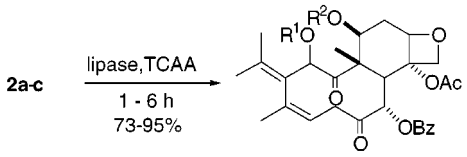
2a $R^1 = R^2 = H$
2b $R^1 = H$, $R^2 = TES$
2c $R^1 = R^2 = TES$

Initially, we screened several different lipases to search for an enzyme catalyzing the selective acylation or esterification of the 13-hydroxyl group in **2a**. Unexpectedly, we observed the formation of an unknown compound as the major product in the reaction with *Rhizopus delemar* lipase (RDL) in the presence of trichloroacetic anhydride. Similar results also were observed in the reactions of **2b**,**c** with RDL. Accordingly, we explored further these reactions with more detail to determine the structures of products and elucidate reaction mechanisms.

In typical experiments, the enzymatic reactions were carried out at room temperature with heterogeneous solutions containing the taxane substrate, trichloroacetic anhydride (TCAA, 6−18 equiv), and RDL (10 units/mmol substrate) in THF.[7] The reactions were monitored by TLC and stopped by the filtration of the insoluble enzymes. The isolated products were characterized on the basis of analyses by mass and NMR spectroscopies including H−H and C−H COSY experiments.

The enzymatic transformation of **2a** for 1−1.5 h provided **4a** as the major product in 73% yield (Scheme 1). Mass

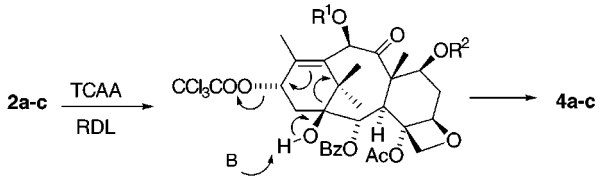
Scheme 1



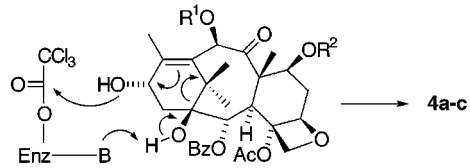
4a $R^1 = R^2 = H$
4b $R^1 = H$, $R^2 = TES$
4c $R^1 = R^2 = TES$

spectroscopic (MS) data indicated that it has exact mass corresponding to the loss of one molecule of H_2O from the substrate.[8] The two signals (201.99 and 213.02 ppm) below 200 ppm in the ^{13}C NMR spectra clearly indicated the presence of two C-1 and C-9 carbonyls. Two double bonds were characterized by four ^{13}C NMR signals [C(11), 142.00; C(12), 138.80; C(13), 120.56; C(15), 130.32 ppm] and one ^1H NMR signal [C(13)−H, 5.44 ppm, J = 4 and 10 Hz, coupled to the C-14 methylene]. The C-10 proton was characterized by the singlet peak at 5.80 ppm in the ^1H NMR spectrum. All these data combined with other NMR data supported the structure of **4a** shown. The product structure thus indicates that among three secondary OH groups at the 7-, 10-, and 13-positions in **2a** the chemically least reactive 13-OH was selectively removed and the A and B rings were expanded into a 10-membered ring in the enzymatic reaction. It was confirmed that the dehydration and skeleton rearrangement did not take place in the absence of RDL.

The enzymatic reactions of **2b** and **2c** under similar conditions afforded, respectively, the products **4b** and **4c** in 85% and 95%. MS data indicated that both of these products had the masses expected when one molecule of H_2O was removed from the substrates. The desilylation of them with HCl in MeOH gave the same products which were identical to **4a**. Accordingly, all the results from the reactions of **2a**−**c** demonstrate that RDL catalyzed the 13-selective dehydration and skeleton rearrangement.



Mechanism **A**



Mechanism **B**

Two mechanisms could be proposed for the dehydration and skeleton rearrangement reaction. In mechanism A, the reaction takes place in a stepwise fashion. The 13-hydroxyl group is first enzymatically acylated and then eliminated with deprotonation of the 1-hydroxyl group and cleavage of the C(1)−C(15) bond. The deprotonation could be done enzymatically or chemically. In mechanism B, the reaction takes place in a concerted fashion, where the elimination of the 13-hydroxyl group and the deprotonation of the 1-hydroxyl

(6) Similar skeleton rearrangements were observed in the chemical reactions of 7-triethylsilyl-13-oxobaccatine III and 2-debenzoly-4,13-dideacetylbaccatine VI. See: (a) Py, S.; Khuong-Huu, F. *Bull. Soc. Chim. Fr*. **1993**, *130*, 189. (b) Pinciroli, V.; Ceccarelli, W.; Fusar-Bassini, D.; Menichincheri, M.; Mongelli, N.; Vanotti, E, *Tetrahedron Lett*. **1996**, *37*, 9365.

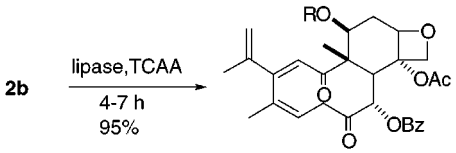
(7) **Representative Experimental Procedure.** To a stirred solution of 63 mg (0.12 mmol) of **2a** and 4.5 mg of RDL (45.6 units/mg, Fluka) in 1.5 mL of dried THF was added 133 mL (6 equiv) of TCAA. The reaction mixture was allowed to be stirred for 1.5 h. The reaction was stopped by filtering off the enzymes and then saturated aqueous $NaHCO_3$ was added dropwise until no gas was produced. The solution was diluted with 5 mL of ethyl acetate and extracted twice with the same organic solvent. The combined organic layers were dried with anhyrous Na_2SO_4, evaporated, and subjected to column chromatography to give 46.2 mg (0.088 mmol, 73%) of **4a** as a white powder.

(8) HRMS: calcd for $C_{29}H_{35}O_9$ ($M + H^+$) 527.2281, found 525.2245.

group occur at the same time and thus no acyl intermediate is involved. Some evidence seems to disprove mechanism A and support mechanism B. The 13-acylated intermediate prepared from **1b** using our previous procedure[3] was stable in the presence of RDL, trichloroacteic acid, or triethylamine in THF for a few days, indicating that it is inert toward enzyme, general acid, and base-catalyzed deprotonation. More evidence, however, is needed to verify mechanism B.

Interestingly, the enzymatic reaction of **2b**, when allowed for the longer time (4−7 h), gave the new product **5a** in 95% yield (Scheme 2), which has a mass corresponding to

Scheme 2

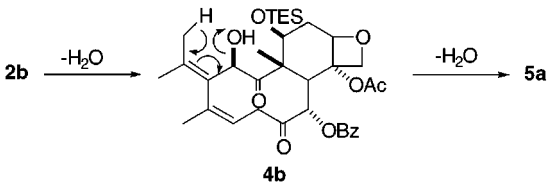


5a R = TES
5b R = H

the loss of two molecules of H_2O from the substrate. In time-dependent NMR experiments, the fast formation and slow breakdown of the intermediate **4b** was observed. The desilylation of **5a** with HCl in MeOH gave the molecule **5b** with the exact mass expected when two water molecules were lost from **2a**.[9] Two ^{13}C NMR signals (205.99 and 203.00 ppm) below 200 ppm strongly indicate the presence of two C-1 and C-9 carbonyls in **5b**. The olefinic C-10 and C-13 protons are characterized by two signals at 6.67 (s) and 5.51 (m, coupled to the C-14 methylene) ppm and the presence of the olefinic C-16 methylene is clearly indicated by the signals at 5.25 (br s) and 5.33 (br s) in the ^1H NMR spectrum. These data combined with other NMR data support the structure of **5b** shown.

These results suggest that in the complete enzymatic reaction of **2b** dehydration and skeleton rearrangement took place twice as shown in Scheme 3. The first dehydration/

Scheme 3

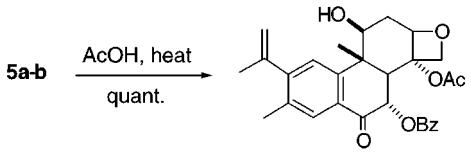


4b

rearrangement provided **4b** and then, in the second dehydration with **4b**, the 10-hydroxyl group was removed with deprotonation of C(16)−H and migration of the C(11)−C(15) double bond. The detailed mechanism for the second dehydration is unclear although two mechanisms would be proposed similarly as suggested for the first dehydration.

Finally, the enzymatically produced **5a** and its desilylated derivative **5b**, when treated separately with AcOH at elevated temperature, readily underwent intramolecular aldol condensation to yield the same tetracyclic diterpene **6** with an aromatic nucleus (Scheme 4).[10] The aromatic A ring in **6** is

Scheme 4



6

characterized by two singlet peaks (7.88 and 8.35 ppm) and the olefinic methylene protons of the isopropenyl group attached to the A ring by the signals at 4.88 and 5.24 ppm in the ^1H NMR spectrum. The presence of the C-1 carbonyl is indicated by the downfield signal at 192.44 ppm in the ^{13}C NMR spectrum. The structure of **6** was further confirmed by X-ray crystallography.

In summary, we have demonstrated unprecedented enzymatic dehydration and skeleton rearrangement of the taxane core leading to the synthesis of new tricyclic and tetracyclic diterpenes (**4a**, **5b**, and **6**). It is noteworthy that the tricyclic diterpenes (**4a** and **5b**) have multiple fuctionality in the 10-membered ring which are amenable to a wide range of chemical transfomations, which will allow us to synthesize more taxane derivatives. The studies toward this end are under progress in this laboratory.

Acknowledgment. This work was supported by research grants from KOSEF-CBM and POSTECH/BSRI.

Supporting Information Available: Details on the synthesis and characterization of **4a**, **5b**, and **6** and crystal structure and tables of crystal structure data for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) HRMS: calcd for $C_{29}H_{33}O_8$ 509.2175 ($M + H^+$), found 509.2179.
(10) Mass (CI): calcd for $C_{29}H_{31}O_7$ 491.2047 ($M + H^+$), found 491.20.