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Lipase catalyzed regioselective hydrolysis of Crotepoxide isolated from *Piper cubeb Cass DC*

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ABSTRACT

Regioselective hydrolysis of biologically active natural product crotepoxide (**1**), isolated from *Piper cubeb Cass DC*, has been studied employing various commercially available lipases. No conversions were observed with *Aspergillus niger* lipase (APL), *Pseudomonas fluorescens* lipase (PFL) and *Lipase-M-Amano* (LM). However, regioselective hydrolysis was achieved with *Mucor miehei* lipase (MML), *Pseudomonas cepacia* lipase (PSL), *Candida cylindraceae* lipase (CCL) and lipase from *Porcine pancreas* (PPL). The active enzymes were found to have distinct regioselectivity towards **1** with respect to hydrolysis of benzoyl and acetyl groups. While CCL was non-selective and hydrolysed both acetyl as well as benzoyl groups giving dideacetyl debenzoyl crotepoxide (**4**) as the only product, the reaction with PPL lead to formation of two products viz. 3-deacetyl crotepoxide (**2**) and 3-deacetyl-7-debenzoyl crotepoxide (**3**). MML catalysed reaction, though kinetically slow, afforded **3** as the only product while as PSL was successful in librating 7- and 3-hydroxyls, leaving 2-acetyl group intact, and showing 100% conversion of **1** to **3**. This is the remarkable result as it can be employed to selectively liberate hydroxyl groups of **1** so as to make them available for selective derivatization even under mild conditions of neutral pH and room temperature without disturbing the epoxide rings.

1. Introduction

Natural sources offer a wealth of chemically diverse compounds that exhibit potential biological activities and desirable pharmacological profile. As biologically validated starting points binding to protein receptor surfaces, natural products can be considered privileged structures. There are only a few instances wherein sets of structurally related natural compounds were isolated and found appropriate for the analysis of structure–activity relationships (SARs). Synthesizing analogues that possess appropriate structural variation can elude this obstacle and as a result, natural product guided library synthesis increases the likelihood of finding lead compounds [1]. Several industrial and academic groups are accessing natural sources and have developed methods to generate large and diverse natural product libraries optimized for high throughput screening and for a fast discovery process. Natural product chemistry and organic synthesis are powerful tools for optimizing natural leads and for generating new diversity from natural scaffolds. The amalgamation of both may be expected to become an important strategy in future drug design. Thus after isolation, the natural products are chemically transformed to be orthogonally protected or to undergo chemoselective reactions so that a diverse substitution bearing pharmacophoric groups are introduced into lead-like natural product core yielding libraries of what are named as diversity modified natural scaffolds (DYMONS) [2]. Extensive libraries of complex natural products like Epithilones—the novel anticancer drug have been produced [3,4].

Since biologically active molecules typically contain multiple substituents that are chemically reactive and, therefore, to modify the structure, proper introduction and removal of protecting groups is one of the most important and widely carried out transformations in preparative organic chemistry. Functionalities of similar reactivity as well as presence of structures sensitive to acids, bases, oxidation and reduction renders the task of protection–deprotection greatly difficult by using classical chemical tools. To add up, chemical transformations generate copious amount of waste i.e. they have high E-factors [5] and employ environmentally unattractive reagents and solvents. Thus currently much attention is being paid to the application of biocatalytic methods as cleaner alternative to the additional organic syntheses. The goal is the development of green, sustainable methodologies for the manufacture of value added chemicals like pharmaceuticals.

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Biocatalysts offer mild reaction conditions, environmentally attractive systems, high activities and the most important chemo-, regio- and stereo-selectivities enabling traditionally difficult chemical syntheses to be circumvented.

Being particularly interested in the regioselective enzymatic modifications of natural compounds, we have studied a biocatalysed regioselective hydrolysis and a subsequent regioselective derivatization of crotepoxide (**1**)-*a* cyclohexane bisepoxide belonging to a biologically important class of compounds called cyclitols (Polyhydroxy cycloalkanes). Naturally occurring cyclohexane epoxides have attracted considerable attention from synthetic and natural product chemists due to their unusual structures, stereochemistry, biogenesis and biological activities including tumor-inhibitory, anti-leukemic and antibiotic activities [6]. Crotepoxide was first isolated by Kupchan et al. [7] from fruits of *Croton macrostachys* and thereafter has been isolated from a number of medicinal plants [8–10]. It displays significant tumor-inhibitory activity against Lewis lung carcinoma and Walker intramolecular carcinosarcoma [6]. For performing regioselective modification of its structure, we studied a number of commercially available lipases for hydrolysis and then prepared various acyl derivatives of hydrolyzed products to generate a series of related compounds.

2. Experimental

2.1. General

Melting points are uncorrected and were determined in one end open capillaries on Buchii 570 mp apparatus. $[\alpha]_D^T$ was determined on Autopol IV automatic polarimeter (Rudolph Research Analytical, USA). ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz respectively on a Bruker 200 MHz spectrometer using TMS as internal standard. Mass spectra were determined on JOEL-MS D300. Column chromatography was carried with Merck silica gel (60–120 mesh), analytical as well as preparatory TLC was performed on Merck silica gel 60-F_{254} precoated plates and compounds were visualized by fluorescence under UV light (254 nm). Ceric ammonium sulphate spray followed by heating was used for detecting the spots on TLC plates.

2.2. Enzymes and chemicals

All the solvents were of analytical grade. Lipases from *Pseudomonas fluorescens* (PFL, immobilized on Sol–Gel-AK) and *Mucor miehei* (MML, immobilized on Sol–Gel-AK) were Biochemika products. *Pseudomonas cepacia* lipase (PSL, immobilized on Hyflo Super Gel), Lipase-M-Amano (LM) and *Aspergillus niger* lipase (APL, free enzyme) were purchased from Amano (Japan) while as *Candida cylindraceae* lipase (CCL, free enzyme) and lipase from *Porcine pancreas* (PPL, free enzyme) were purchased from Sigma.

2.3. Isolation of crotepoxide (*1*)

Dried powdered fruits (500 g) of *Piper cubeb Cass DC* (Piperaceae) were soxhlet extracted with petroleum ether (60–80 °C) for 30 h. The extract was partially concentrated in vacuo and on standing gave a crystalline material which on filtration and recrystallization from ethylacetate-*n*-hexane gave crystals of crotepoxide (4 g). The compound was authenticated by comparing its spectral data with that available in literature [7].

2.4. Biocatalysed hydrolysis of crotepoxide ***(1)***

Small scale biocatalysed reactions were carried out in 5 ml screw capped bottles for screening enzymes active for regioselective hydrolysis of **1**. 5 mg of substrate was dissolved in 200 μL of toluene. 500 μL of phosphate buffer (pH 7) was added. The reaction was started by adding 5 mg of lipase and was maintained at 25 °C in a shaker at 300 rpm. Control reaction was conducted without adding enzyme. The progress of reaction was monitored by TLC using precoated silica gel F_{254} TLC plates and were visualized by UV-light (254 nm) and by spraying with ceric ammonium sulphate followed by heating.

After screening experiment, large scale reaction was started with all those enzymes found active for hydrolyzing **1**. For this purpose 200 mg of substrate (0.55 mmol) was dissolved in 6 ml of toluene and 15 mL of phosphate buffer (pH 7). The reaction was started by adding 200 mg of enzyme as discussed earlier. The reaction was quenched either after TLC showed complete conversion of reactants into product(s) or latest by 72 h. However, the time of completion of reaction was observed to vary with the nature of enzymes. The reaction was quenched by adding 10 ml of ethylacetate and then centrifuged. The reaction mixture was extracted with more of ethylacetate (2 × 50 ml) and organic layer dried over anhydrous sodium sulphate. Solvent was evaporated under vacuum and the mixture finally purified by chromatography on silica gel column (2 cm × 40 cm) using an increasing gradient of petether:dichloromethane (9:1 to 1:20) followed by 2% methanol in dichloromethane. The % age yield was calculated on the basis of amount of **1** used in the reaction.

2.5. Physical and spectroscopic data

2.5.1. Crotepoxide (*1*)

Recrystallized from ethylacetate-*n*-hexane as colourless needles; yield 0.8% of dry weight of plant material; mp 152 °C (lit 150–151 °C) [7]; $[\alpha]_D^{25} + 70^\circ$ (c 1.2, $CHCl_3$) (lit + 74° (c 1.7, $CHCl_3$)

Table 1
^{1}H NMR signals of crotepoxide (**1**) and its derivatives (**2, 3, 5–8**) in $CDCl_3$ (δ in ppm; *J* in Hz). For compound **4** solvent is MeOH-d_4.[a].

Proton	1	2	3	4	5	6	7	8
H-2	5.77 d (9.0)	5.46 d (8.7)	4.79 d (8.1)	3.91 d (9.2)	5.62 d (9.3)	5.93 d (10.0)	5.92 d (9.5)	5.46 d (9.4)
H-3	4.99 dd (1.6, 9.0)	3.97 dd (1.7, 8.7)	3.95 dd (1.5, 8.1)	3.86 dd (1.5, 9.3)	4.92 dd (1.5, 9.3)	5.54 dd (2.5, 10.1)	5.18 dd (1.6, 9.5)	4.93 dd (1.5, 9.3)
H-4	3.10 dd (1.6, 3.9)	3.17 dd (1.7, 3.9)	30.1 dd (1.5, 4.0)	2.98 dd (1.5, 3.9)	3.10 dd (1.5, 3.8)	3.46 dd (2.5, 3.5)	3.19 dd (1.6, 3.9)	3.13 dd (1.5, 3.9)
H-5	3.45 dd (2.7, 3.9)	3.43 dd (2.7, 3.9)	3.40 dd (2.7, 4.0)	3.40 dd (2.6, 3.9)	3.45 dd (2.3, 3.8)	3.60 dd (2.7, 3.5)	3.44 dd (2.7, 3.9)	3.44 dd (2.3, 3.9)
H-6	3.67 d (2.7)	3.62 d (2.7)	3.54 d (2.7)	3.55 d (2.6)	3.57 d (2.3)	3.74 d (2.7)	3.72 d (2.7)	3.55 d (2.3)
H-7A	4.24 d (12.1)	4.24 d (12.0)	3.60 d (12.3)	3.57 d (12.3)	3.85 d (12.1)	4.49 d (12.0)	4.26 d (12.0)	3.84 d (12.3)
H-7B	4.57 d (12.1)	4.60 d (12.0)	4.17 d (12.4)	4.03 d (12.3)	4.43 d (12.1)	4.61 d (12.0)	4.60 d (12.0)	4.40 (12.3)
m-ArH	7.47 t (7.1)	7.46 t (7.3)	–	–	–	7.38 m	7.42 m	–
p-ArH	7.60 t (7.1)	7.59 t (7.2)	–	–	–	7.55 m	7.54 m	–
o-ArH	8.03 d (7.0)	8.02 d (7.1)	–	–	–	7.99 m	8.04 m	–
OAcyl	2.03 s 2.12 s	2.19 s –	2.10 s	–	2.07 s 2.08 s 2.11 s	–	2.06 s	2.11 s 0.9–1.7

[a] In case of compounds from **9** to **12**, the ^{1}H NMR signals were similar to that of **8** with the difference in the integral for methelene protons of the acyl chain.

Table 2
^{13}C NMR signals of crotepxoide (**1**) and its derivatives (**2,3, 5–8**) in $CDCl_3$ (δ in ppm). For compound **4** solvent is MeOH-d_4.[a].

Carbon no.	1	2	3	4	5	6	7	8
1	59.4	59.8	58.3	58.1	59.7	60.0	60.1	59.6
2	70.4	73.3	73.8	72.7	70.3	73.8	71.9	70.3
3	69.5	69.1	69.0	69.3	69.2	70.0	70.1	69.5
4	52.6	53.7	53.9	53.4	52.7	53.6	53.7	52.7
5	48.0	48.1	48.3	48.3	48.1	48.0	48.9	48.1
6	53.8	54.7	54.9	54.7	53.3	54.3	54.6	53.3
7	62.5	62.6	61.3	61.5	60.7	62.7	63.1	60.8
ArC'1	129.2	129.1	–	–	–	129.3	129.8	–
ArC'2,6	129.8	129.8	–	–	–	129.8	130.5	–
ArC'3,5	128.6	128.6	–	–	–	128.6	129.3	–
ArC'4	133.5	133.6	–	–	–	133.6	134.2	–
2 COMe	170.0, 20.6	171.5, 20.8	172.3, 21.1	–	170.1, 20.7	168.5	170.7	170.2
3 COR	169.7, 20.4	–	–	–	169.8, 20.5	168.3	166.5	169.5, 18.3–20.5
7 COR	165.8	165.9	–	–	169.3, 20.2	165.7	166.2	169.1, 18.4–20.3

[a] In case of compounds from **9** to **12**, the ^{13}C NMR signals were similar to that of **8** except for the number of signals for the carbons in the acyl chain.

[7]); IR KBr pellets ν_{max} = 1752, 1738, 1602, 1270, 1219, 1110 cm^{-1}; MS (ESI) *m/z* 362; ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.2. 3-Deacetyl crotepoxide (**2**)*

Obtained as one of the products of hydrolytic reaction catalyzed by PPL (25 mg, 14.2% based on **1** used); mp 134 °C (lit 134–136 °C) [11]; $[\alpha]_D^{25}$ + 71.3° (c 1.1, $CHCl_3$) (lit + 75.8° (c 0.3, $CHCl_3$) [11]); IR KBr pellets ν_{max} = 3300, 1722 cm^{-1}; MS (ESI) *m/z* 320; ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.3. 3-Deacetyl-7-debenzoyl crotepoxide (**3**)*

Obtained as one of the products of reaction catalyzed by PPL (92 mg, 76.6% yield) and the only product from PSL and MML catalyzed reactions with amount 98 mg (82.5%) and 34 mg (28.6%) respectively; mp 122–123 °C; $[\alpha]_D^{25}$+51.7° (c 1.01, $CHCl_3$); IR KBr pellets ν_{max} = 3260, 1720 cm^{-1}; MS (ESI) *m/z* 216; ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.4. Dideacetyl debenzoyl crotepoxide (**4**)*

Obtained as hydrolysed product of reaction catalyzed by CCL (83 mg, 87% yield); mp 98 °C (lit 101–102 °C) [7]; $[\alpha]_D^{25}$ + 29° (c 1.00, CH_3OH); IR KBr pellets ν_{max} = 3226, 1404 cm^{-1}; MS (ESI) *m/z* 174; ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.5. Acetylation of **4***

Compound **4** (20 mg, 0.11 mmol) in acetic anhydride (1 ml) and DMAP (5 mg) was warmed for 4 h and kept at room temperature overnight. Usual work up and purification on silica gel gave a white solid (23 mg, 70% yield) of **5.** mp 70 °C [12]; $[\alpha]_D^{25}$ + 47.3° (c 1.07, $CHCl_3$); ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.6. Benzoylation of **4***

Compound **4** (20 mg, 0.11 mmol) was benzoylated with benzoyl chloride (200 μL) in pyridine (1 ml) at 0 °C for half an hour and then kept at room temperature overnight. Usual work up and purification on silica gel gave a pale yellowish solid (28 mg, 51.8%) of **6.** mp 183 °C; $[\alpha]_D^{25}$ + 31.3° (c 1.01, $CHCl_3$); ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.7. Benzoylation of **3***

Compound **3** (30 mg, 0.14 mmol) on benzoylation in same manner gave a white solid (28 mg, 47.1%) of Boesenoxide (**7**)**.** mp 170 °C (lit. 170–171 °C) [11]; $[\alpha]_D^{25}$ + 29.9° (c 0.3, $CHCl_3$) (lit. + 34.9°) [11]; ^{1}H and ^{13}C NMR (Tables 1 and 2).

*2.5.8. Acylation of **3***

Compound **3** (20 mg, 0.09 mmol) was acylated with palmitic acid (47 mg, 0.18 mmol) in dry dichloromethane using DCC (45 mg, 0.22 mmol) and DMAP (5 mg) at room temperature for 24 h. The crude mixture was purified by silica column chromatography to give 8 mg (13.3%) of dipalmitate (**8**)**.** Similarly dimyristate (**9**), dideconoate (**10**), dihexanoate (**11**) and dibutanoate (**12**) were also prepared and characterized using ^{1}H and ^{13}C NMR (Tables 1 and 2).

3. Results and discussion

Chemically crotepoxide has a cis 1,6:4,5-diepoxy functionalities in addition to trans 2,3 diacetoxy-1-benzoyloxy methyl functionality and all its functional groups are easily hydrolysable even under mild conditions. For construction of a library of related compounds starting from crotepoxide, these functional groups have to be manipulated selectively. Although numerous chemical techniques are available for masking and librating hydroxyl groups, development of enzymatic methods as a better option for such transformations prompted us to subject crotepoxide to selective hydrolysis using lipases. To identify biocatalyst capable of regioselectively hydrolyzing specific site in **1**, we tested about eight commercially available enzymes in a biphasic system composed of toluene as organic phase and phosphate buffer (pH 7) as the aqueous phase. The selectivity of different enzymes under study towards functionalities of **1** was found to be different. No conversions were observed in case of reactions catalyzed by lipases APL, PFL and LM even after 72 h of incubation at 30 °C. Further, rate of hydrolysis catalyzed by MML was quite slow in comparison to that catalyzed by lipases from PSL, CCL and PPL. It was observed that while with PSL and CCL the reactions were completed in 38 h, only 30–35% of conversion was observed in case of reactions catalysed by MML even after 72 h. However, completion of reaction with PPL was observed only after 60 h. The active enzymes were found to have distinct regioselectivity towards **1** with respect to hydrolysis of benzoyl and acetyl groups (Fig. 1). While CCL was non-selective and hydrolysed both acetyl as well as benzoyl groups giving **4** as the only product, the reaction with PPL as biocatalyst gave **2** as the minor product (14.2%) and **3** as the major product (76.6%). The follow up of kinetics of this reaction showed that **3** always remained as a major product. MML catalysed reaction, though kinetically slow, afforded **3** as the only product. Particularly remarkable is the regioselectivity displayed by biocatalyst PSL in librating 7- and 3-hydroxyls, leaving 2-acetyl group intact, and showing 100% conversion of **1** to **3**.

The hydrolyzed products (**2–4**) obtained from these biocatalyzed reactions were characterized on the basis of their physical

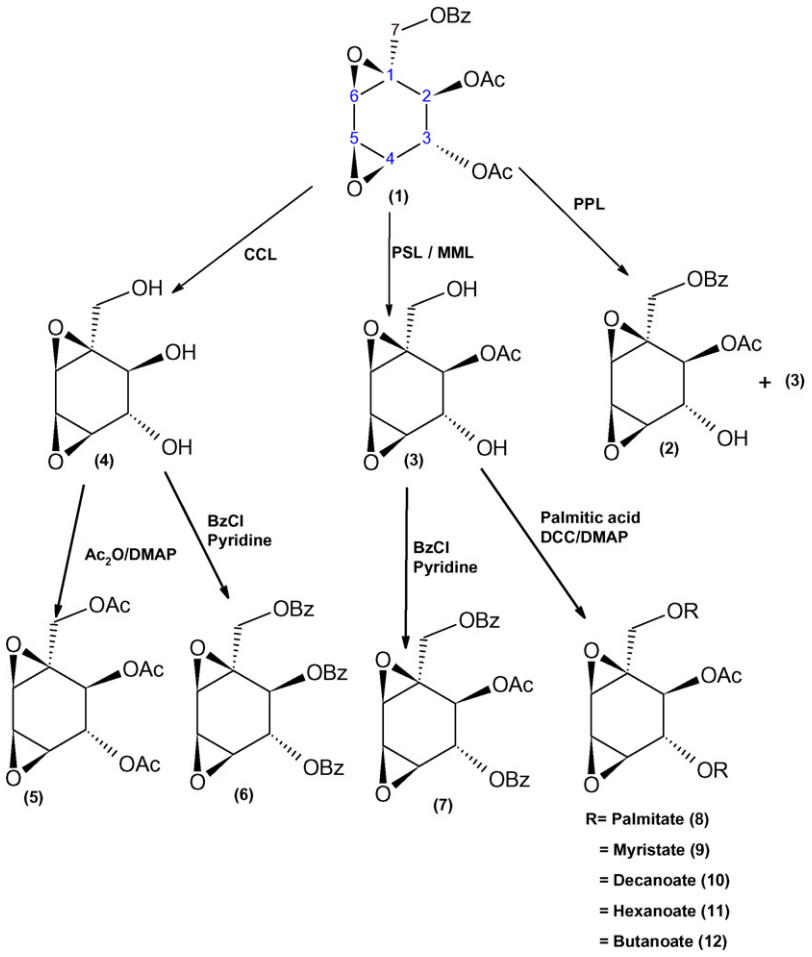

Fig. 1. Regioselective hydrolysis of crotepoxide catalyzed by lipases and the subsequent derivatization of hydrolyzed products.

and spectral data. The mass spectrum of **2** showed molecular ion peak at *m/z* 320 suggesting the removal of one of the acetyl groups. It was supported by the appearance of a signal at 3300 cm^{-1} in its IR spectrum. Downfield shift of signals for C-2 and C-4 in ^{13}C NMR spectrum and upfield shift of C-3 signal suggest the removal of C-3 acetyl group. It was further authenticated by ^{1}H NMR spectrum in which H-3 signal shifted upfield by δ 1.00 ppm relative to **1** (Tables 1 and 2). Thus **2** was characterized as 3-deacetyl crotepoxide.

^{1}H and ^{13}C NMR spectra of **3** were similar to that of **2** except for absence of signals for aromatic protons and carbons. In mass spectra [M^{+}] at *m/z* 216 confirmed **3** to be 3-deacetyl-7-debenzoyl crotepoxide. Molecular ion peak at *m/z* 174 in mass spectrum of **4** together with chemical shifts of ^{1}H NMR signals for H-2, H-3 and H-7 confirmed **4** to be dideacetyl debenzoyl crotepoxide.

Crotepoxide being a tumor-inhibitory compound [6] with interesting stereochemistry and a structure easy to derivatize in a number of ways generating a library of related compounds, its SAR can be studied and a better drug candidate can emerge with potential biological activity. Reports are available in literature where modification of structure of crotepoxide has been achieved by chemical methods. Kupchan et al. [7] have reported that hydrolysis with methanolic potassium hydroxide resulted in removal of both of its acetyl groups while as under acidic conditions all ester functionalities as well as epoxide rings were hydrolyzed without any selectivity. In another report [13], milder basic condition, however, can leave benzoyl group intact but it cannot discriminate between the acetyl groups, thereby hydrolyzing both. Our experiment on biocatalysed regioselective hydrolysis of its ester groups has revealed that using various lipases, all its hydroxyl groups can be selectively liberated and made available for manipulation even at mild conditions of neutral pH and room temperature without disturbing the epoxide rings. Thus biocatalyzed reactions can easily and ecofriendly replace the arsenal of chemical tools for selective modification of crotepoxide. The hydrolyzed products (**2–4**) obtained from these biocatalyzed reactions were derivatized by chemically acylating the liberated hydroxyl groups. Compound **3** obtained from biocatalyzed reactions and compound **6** and **8–12** obtained by chemical acylation of **4** and **3** respectively are new to literature and are reported for the first time. Compound **7** obtained by benzoylating **3** is a natural product (Boesenoxide) isolated for the first time by Tuntiwachwuttikul et al. [14].

4. Conclusion

Crotepoxide (**1**)-*a* cyclohexane bisepoxide belonging to a biologically important class of compounds called cyclitols (Polyhydroxy cycloalkanes) has been successfully subjected to regioselective hydrolysis using commercially available lipases. No conversions were observed with lipases APL, PFL and LM. Regioselective hydrolysis was achieved with MML, PSL, CCL and PPL. The active enzymes were found to have distinct regioselectivity towards **1** with respect to hydrolysis of benzoyl and acetyl groups. While CCL was nonselective and hydrolysed both acetyl as well as benzoyl groups giving **4** as the only product, the reaction with PPL lead to formation of two products viz. **2** and **3**. MML catalysed reaction, though kinetically slow, afforded **3** as the only product while as PSL was successful in librating 7- and 3-hydroxyls, leaving 2-acetyl group

intact, and showing 100% conversion of **1** to **3**. Our results show that the lipase catalysed regioselective hydrolysis of **1** can be employed to selectively liberate its hydroxyl groups so as to make them available for manipulation even under mild conditions of neutral pH and room temperature without disturbing the epoxide rings.

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References

[1] R. Breinbauer, M. Manger, M. Scheck, H. Waldmann, Curr. Med. Chem. 9 (2002) 2129–2145.
[2] U. Abel, C. Koch, M. Speitling, F.G. Hansske, Curr. Opin. Chem. Biol. 6 (2002) 453–458.
[3] G. Hofle, M. Sefkow, Heterocyles 48 (1998) 2485–2488.
[4] K.C. Nicolaou, N. Winssinger, D. Vourloumis, T. Oshima, S. Kim, J. Pfefferkorn, J.Y. Xu, T. Li, J. Am. Chem. Soc. 120 (1998) 10814–10826.
[5] R.A. Sheldon, Pure Appl. Chem. 72 (2000) 1233.
[6] S.M. Kupchan, R.J. Hemingway, R.M. Smith, J. Org. Chem. 34 (1969) 3898.
[7] S.M. Kupchan, R.J. Hemingway, P. Coggon, A.T. McPhail, G.A. Sim, J. Am. Chem. Soc. 90 (1968) 2982–2983.
[8] S. Takahashi, Phytochemistry 8 (1969) 321.
[9] O. Pancharoen, P. Tuntiwachwuttikul, W.C. Taylor, Phytochemistry 43 (1996) 305–308.
[10] S.C. Taneja, S. Koul, P. Pushpangadan, K.L. Dhar, W.M. Daniewski, W.M. Schilf, Phytochemistry 30 (1991) 871–874.
[11] T.K.M. Shing, E.K.W. Tam, J. Org. Chem. 63 (1998) 1547–1554.
[12] S. Ogawa, T. Takagaki, Bull. Chem. Soc. Jpn. 60 (1987) 800–802.
[13] S.M. Kupchan, W.L. Sunshine, J. Org. Chem. 43 (1978) 171–173.
[14] P. Tuntiwachwuttikul, O. Pancharoen, W.A. Bubb, T.W. Hambley, W.C. Taylor, V. Reutrakul, Aust. J. Chem. 40 (1987 2049).