Pergamon

Tetrahedron Letters, Vol. 38, No. 39, pp. 6871-6874, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01582-7

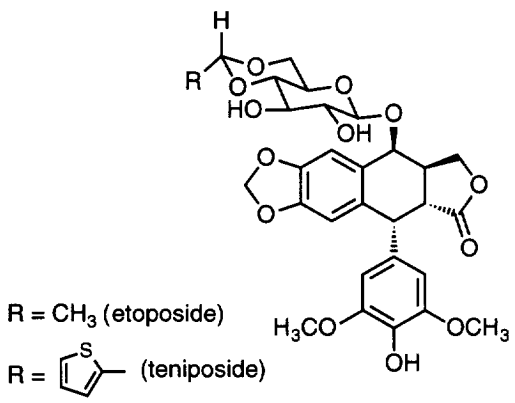
Stereo and Chemoselective Enzymatic Reduction of Azido Functionality: Synthesis of 4-ß-Aminopodophyllotoxin Congeners by Baker's Yeast

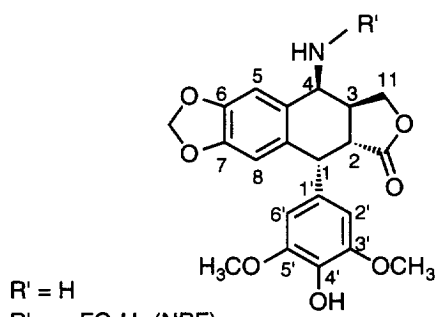
Ahmed Kamal *, B. Laxminarayana and N. Lakshmi Gayatri

Division of Organic Chemistry
Indian Institute of Chemical Technology, Hyderabad, 500 007, India

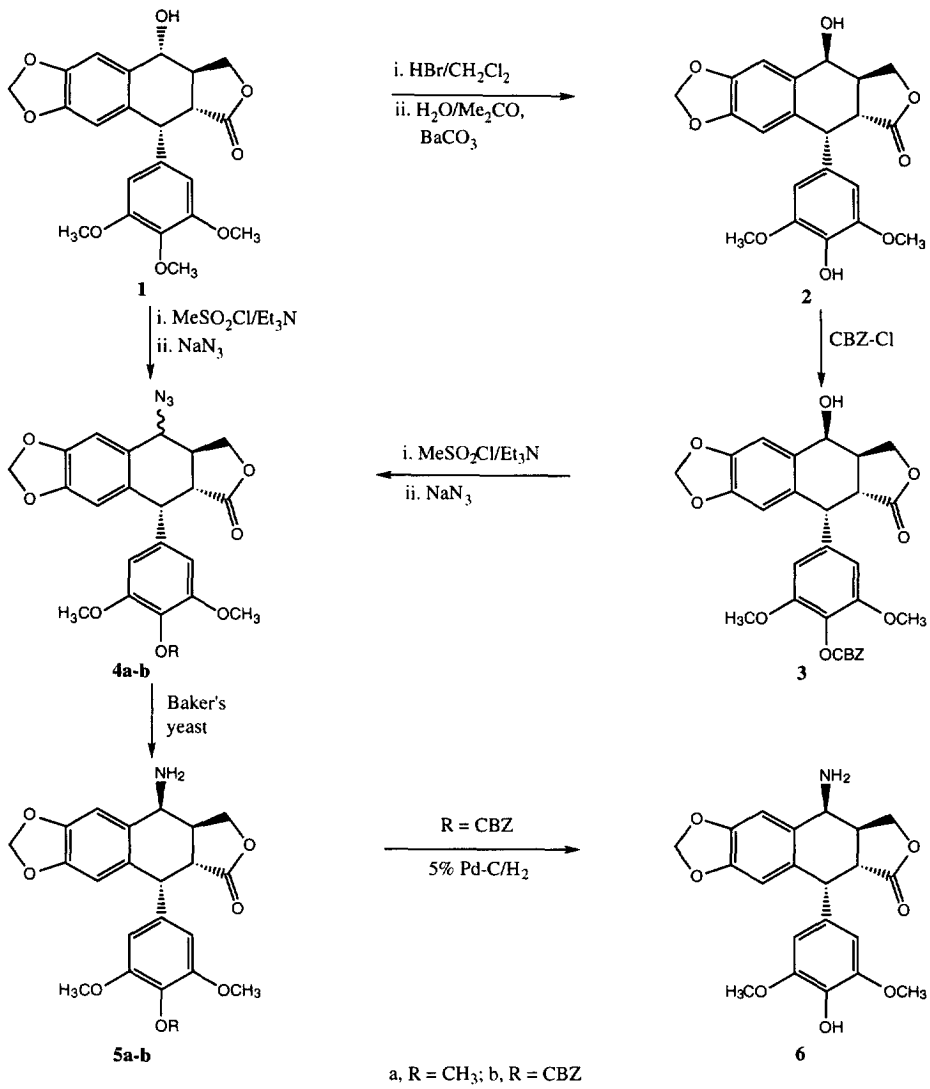
Abstract : 4ß-Aminopodophyllotoxin congeners have been synthesized by the stereoselective biocatalytic reduction of the 4-azidopodophyllotoxins employing baker's yeast in excellent yields under mild conditions. © 1997 Elsevier Science Ltd.

Podophyllotoxin and desoxypodophyllotoxin are two well known naturally occuring aryltetralin lignans and the former is the major constituent of a number of plant species of the *Podophyllum* family[1,2]. Both these compounds are cytoxic, and their derivatives are in use as antitumor agents, e.g., etoposide[3,4] and teniposide[4b]. These semisynthetic podophyllum lignans block the catalytic activity of DNA topoisomearse II and concurrent enzyme-mediated production of lethal DNA strand breaks leading to DNA damage and cytotoxicity[5]. This lead has generated a renewed interest in the chemical and biochemical studies of podophyllotoxin derived antitumour agents[6]. The 4-amino congeners, have also been reported to possess significant cytotoxicity and potent inhibitory activity on human DNA topoisomerase II [7].





As a result of our interest in the podophyllotoxin congeners[8] coupled with our recent explorative investigation of biocatalytic reactions[9], we envisioned an efficient synthesis of 4-aminopodophyllotoxin congeners by the biotransformation of an azido functionality. We have recently observed a novel biocatalytic



reduction of arylazides to the corresponding anilino compounds[10]. Based on this finding 4-azidopodophyllotoxin and 4-azido-4'-demethylpodophyllotoxin have been reduced by baker's yeast to the 4-aminopodophyllotoxins in a stereoselective manner.

The precusors, 4-azidopodophyllotixins **4a** and **4b** were prepared from podophyllotoxin (**1**) and 4'-O-carbobenzyloxyepipodophyllotoxin (**3**) by the literature method[7a]. **3** inturn was prepared from 1 by bromination and selective demethylation employing a modified Kuhn's method[11] followed by the protection of phenolic group with carbobenzyloxy chloride[12]. In a typical experiment, to a solution of **4a**[13] (100 mg, 0.23 mmol) in aqueous ethanol (50%, 1ml) was added to a preincubated suspension of baker's yeast (4 g) in

phosphate buffer solution of pH 7.2 (20 ml) and stirred vigorously for 4h. The reaction was monitored by TLC ($CHCl_3$-MeOH, 9.8:0.2). On completion of the reaction, EtOAc (60 ml) was added to the reaction mixture. The organic phase was separated, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue obtained was subjected to column chromatography (silica gel, $CHCl_3$-MeOH, 9.9:0.1) to afford the reduced product, 4ß-aminopodophyllotoxin **5a** in 88% yield. Similarly, **4b** has produced **5b**, i.e., ß-isomer exclusively in 84% yield..

It is interesting to note that this biocatalytic reaction is highly stereoselective process as it affords 4B-aminopodophyllotoxins alone in quantitative yield[14]. In contrast to already reported chemical reductive process, wherein α and ß isomers have been obtained in 3:7 ratio respectively. The ß-selectivity in the present method could be because of the isomerization of the amine functionality via its Schiffs base formation in the presence of oxido-reductases in the yeast. It is shown in the literature[7a] that the biological activity is generaly retained or predominant in the case of 4ß-substituted podophyllotoxin congeners when compared to their α-isomers, thus illustrating the importance for the synthesis of ß-isomers.

Furthermore, this biotransformation is chemoselective as well, and this is depicted in case of **4b**, wherein the O-carbobenzyloxy (CBZ) group remains totally intact unlike most of the chemical azido reductive procedures, e.g. Pd-C/H_2. The main advantage of this type of selectivity in the present method is of potential application for the preparation of newer analogues of podophyllotoxin class of compounds. That is in some cases, wherein by employing this protocol the 4-amino functionality could alone be reacted without the interference of the phenolic hydroxy group, and the O-CBZ could be deprotected at a later stage.

In conclusion, the present biocatalytic method employing baker's yeast is a significant development with remarkable stereo and chemoselectivity under mild conditions with excellent yields for the reduction of azido functionality in the biologically important podophyllum lignans. This finding will be of assistance to the investigators involved in the synthesis of various new analogues of biologically significant podophyllotoxin congeners.

Acknowledgements: BL and NLG are greatful to UGC, New Delhi for the award of Research Fellowships.

References and Notes:

1. Lee, K. H. Antineoplastic Agents from Chinese Traditional Medicine and their Analogs. In *Human Medicinal Agents from Plants* : American Chemical Society, Symposium Series; Kinghorn, A. D.; Balandrin, M., Eds.; American Chemical Society : Washington, DC, 1993; pp 170-190.
2. MacRae, W. D.; Hudson, J. B.; Towers, B. H. N. *Planta Medica* **1993**, *55*, 531.
3. Jardine, I. In *Anticancer Agents based on Natural Prouduct Models*; Cassady, J. M.; Douros, J.Eds.; Academic Press : New york, 1980; pp 319-351 and references sited therein.
4. a) Lee, K.; Beers, S. A.; Mori, M.; Wang, Z.; Kuo, Y.; Li.;L.; Liu, S.; Chang, Y.; Han, F.;Cheng, Y. *J. Med. Chem.* **1990**, *33*, 1364.
   b) Wang, Z.; Kuo, Y.; Schnur, D.; Bowen, J. P.; Liu, S.; Han, F.; Chang, J.;Cheng, Y.; Lee, K. *J. Med. Chem.* **1990**, *33*, 2660.
   c) Stahelin, H.; Von Wartburg, A. *Prog. Drug. Res.* **1989**, *33*, 169.

5. Macdonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K. C., Ross, W. E., On the Mechnaism of Interaction of DNA Topoisomerase II with Chemotherapeutic Agents. In *DNA Topoisomerases in Cancer*, Potmesil, M.; Kohn, K. W.; Eds.; Oxford University Press, New York, 1991; pp 199-214.
6. a) Ward, R. S. *Synthesis* **1992**, 719 and references therein,
b) Sauliner, M. G.; LeBoulluec, K. L.; McGee, D. P. C.; Long, B. H.; Crosswell., A. R.; Vyas, D. M. *Bioorg. Med. Chem. Lett* .**1993**, *3*, 1619.
c) Terrada, T.; Fujimotom, K.; Nomura, M.; Yamazaki, R.; Shibata, J.; Sugimoto, Y.; Yamada, Y. *J. Med Chem.* **1993**, *36*, 1689.
d) Bush, J. E.; Jones, D. W. *J.Chem. Soc., Chem. Commun.* **1993**, 1200.
e) Tomioka, K.; Kubota, Y.; Kogo, K. *Tetrahedron Lett.* **1993**, *49*, 1891.
f) Watt , P. M.; Hickson, I. D. *Biochem. J.* **1994**, *303*, 681 and references therein.
g) Hitosuyangi, Y.; Kobayashi,M.; Takeya, K.; Itokawa, H. *J. Chem. Soc., Perkin Trans. 1* **1995**,1387.
h) Hadimani, S. B..; Tanpure, R. P.; Bhaat, S. V. *Tetrahedron Lett.* **1996**, *37*, 4791.
i) Tawa, R.; Takami, M.; Imakura, Y.; Lee, K. H.; Sakuri, H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 489.
7. a) Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L.S.; Dai, H.J.; Chen, C.-H. *J. Nat.Prod.* **1989**, *52*, 606.
b) Zhang, Y,-L.; Shen, Y-C.; Wang, Z.-Q; Chen, H.-X; Guo,X.; Cheng, Y.-C.; Lee,K.-H. *J. Nat. Prod.***1992**, *55*, 1100.
c) Zhang, Y. L.; Guo, Z.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 446.
8. a) Kamal, A.; Atchison, K.; Daneshtalab, M.; Micetich, R.G. *Anti-cancer Drug Design* **1995**, *10*, 545.
b) Kamal, A.; Gayatri, N. L. *Tetrahedron Lett.* **1996**, *37*, 3359.
c) Kamal, A.; Damayanthi, Y. *Bioorg. Med. Chem. Lett.***1997**, *7*, 657.
9. a) Kamal, A.; Rao, M. V.; Meshram, H. M. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 2056.
b) Kamal, A.; Reddy, B. S. P. *Bioorg. Med. Chem Lett.* **1991**, *1*,159.
c) Kamal, A.; Rao, M. V. *Tetrahedron Assymet.* **1994**, *5*, 1881.
10. Kamal, A.; Damayanthi, Y.; Reddy, B. S. N.; Lakshminarayana, B.; Reddy, B. S. P. *Chem. Commun.* **1997**, 1015.
11. Kuhn. M.; Keller-Juslen, C.; von Wartburg, A. *Helv. Chem. Acta* **1969**, *52*, 948.
12. 4'-CBZ protected epiodophyllotoxin was prepared by the reaction of epipodophyllotoxin with carbobenzoxychloride in presence of triethylamine as described earlier [7a].
13. 4α-and 4ß-azido isomers were in the ratio of 1:3 as determined by NMR studies. Compound **4** has been used directly without the separation of the isomeric azido forms for the baker's yeast mediated process.
14. The assignment of the configuration at C-4 was based on the difference of $J_{3,4}$ coupling constants and compared to the sample prepared by reported method for **6**. Selected spectral data for **5a** : 1H-NMR (200MHz, $CDCl_3$) : 6.74 (s, 1H, H-5), 6.41 (s,1H,H-8), 6.22 (s, 2H, H-2', 6'), 5.9 (Abq, J=1Hz, OCH_2O), 4.45 (d, J=5.2Hz, 1H, H-1), 4.2 (d, J=9.5Hz, 2H, H-11 and H-11'), 4.1 (d, J=4.1Hz, 1H, H-4)3.7 (d,9H, 3', 4', 5'-OMe), 3.2 (dd, J=5.2, 14Hz, 1H, H-2), 2.75 (m, 1H, H-3).

(*Received in UK* 22 *April* 1997; *revised* 31 *July* 1997; *accepted* 1 *August* 1997)