Chinese Chemical Letters Vol. 14, No. 2, pp 163 – 166, 2003
http://www.imm.ac.cn/journal/ccl.html

Enzyme-catalyzed Transesterification of Unusual Substrate: Synthesis of Acyclovir and L-ascorbic Acid (Vitamin C) Vinyl Esters

Xing Tao XUE, De Shui LÜ, Zhi Chun CHEN, Qi WU, Ying CAI, Xian Fu LIN*

Department of Chemistry, Zhejiang University, Hangzhou 310027

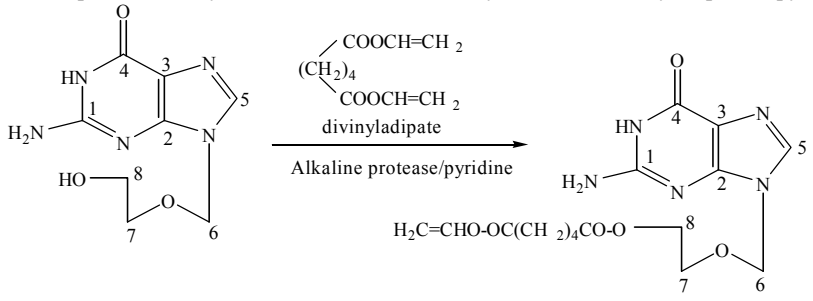
Abstract: The synthesis of acyclovir and L-ascorbic acid with divinyladipate was performed with alkaline protease from *Bacillus subtilis* and lipase from Lipozyme (immobilized from *Mucor miehei*) in different anhydrous organic solvents. Two corresponding derivatives were obtained.

Keywords: Alkaline protease, lipase, transesterification, acyclovir, vitamin C, vinyl ester.

The structural modification of the old drugs is an active research field due to its potential application. Acyclovir (9-[2-hydroxyethoxy methyl]-9H-guanine, ACV) is an antiviral drug, which selectively inhibits replication of herpes viruses with low cell toxicity. But the low aqueous solubility and poor absorption are its deficiency. The esters of acyclovir as its prodrug can solve these problems[1,2]. L-ascorbic acid (vitamin C, 2-oxo-L-gulonic acid-γ-lactone) is one of the most important antioxidants. Esterified vitamin C, like 6-O-palmitoyl-L-ascorbic acid, is used in food *e.g.* creams and baby milk. However, chemical modifications of vitamin C are limited by its distinct instability. An enzymatic synthesis under mild reaction conditions can be accepted, several vitamin C fatty acid esters have been obtained[3,4].

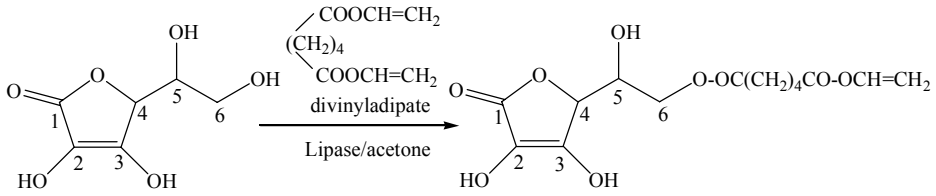
We focused on the enzymatic synthesis for modification of these drugs. Here we choose two common hydrolytic enzymes as the catalysts for the transesterification. The vinyl esters of these drugs have been successfully obtained.

Scheme 1 protease catalyzed transesterification of acyclovir with divinyladipate in pyridine



*E-mail: llc123@css.zju.edu.cn

Scheme 2 lipase catalyzed transesterification of vitamin C with divinyladipate in acetone



6-O-Vinyladipoyl L-ascorbate

Experimental

Materials

Alkaline protease from *Bacillus subtilis* was purchased from Wuxi Enzyme Co. Ltd. Lipozyme (immobilized from *Mucor miehei*) lipase was gained from Japan. Divinyladipate was produced and purified as described by the patent literature[5]. The pyridine and acetone were dried over 3Å molecular sieves for 24 h *prior* to use. Acyclovir, L-ascorbic acid and all other chemicals were of the highest purity commercially available and used without further purification.

Alkaline protease synthesis of acyclovir vinyl ester

5 mmol (1.125 g) acyclovir was dissolved in 10 mL pyridine containing 20 mmol (3.96 g) divinyladipate. The reaction was initiated upon addition of 0.4 g alkaline protease from *Bacillus subtilis* and the suspension was placed in a shaking incubator with a stirring speed of 250 rev/min for 3 days at 60°C. Filtering off the enzyme terminated the reaction. The pyridine was evaporated off. The product was separated by silica gel chromatography with an eluent consisting of chloroform/methanol (8:1 by vol) to give white powder in yield 30% (0.61 g).

Lipase synthesis of 6-O-vinyladipoyl L-ascorbate

5 mmol (0.88 g) vitamin C, 20 mmol (3.96 g) divinyladipate, 0.25 g lipozyme lipase, 1 g molecular sieves (3 Å, activated by heating overnight at 250°C under reduced pressure) was dissolved in 25 mL acetone. The reaction mixture was incubated in a capped vial, placed in an oil bath, thermostated at 45°C and stirred for 48 h. The lipase and molecular sieves were filtered off to terminate the reaction. The acetone was evaporated off. The product was separated by silica gel chromatography with an eluent consisting of chloroform/methanol/water (75:15:2 by vol) to give brownish oil in yield 33% (0.62g).

Results and Discussion

Synthesis of acyclovir vinyl ester

Enzymatic synthesis of vinyl acyclovir ester is shown in **Scheme 1**. Transesterification of acyclovir with divinyladipate catalyzed by alkaline protease from *Bacillus subtilis* in pyridine shows more efficiency in the preliminary screening test of enzymes (alkaline protease and lipozyme lipase) and solvents (acetone, pyridine, acetonitrile, DMF). The obtained acyclovir vinyl ester was characterized by IR and NMR spectroscopy [6], and compared with the literature data [7]. IR spectra indicated that the acyclovir vinyl ester was obtained. ^{13}C-NMR revealed that acyclovir ester was substituted at C-8 position of acyclovir. The signals for C-8 of acyclovir vinyl ester shifted downfield from 59.46 ppm to 63.23 ppm and C-7 positions shifted upfield from 69.94 ppm to 67.14 ppm compared with acyclovir. Furthermore, the ^{1}H-NMR spectroscopy also confirmed this conclusion. The proton signal was downshifted from 3.48 ppm to 4.09 ppm at C-8. This acyclovir derivative would be useful as a monomer of functional material such as anti-sense RNA compound and/or polymeric cofactor[8]. We also have obtained the vinyl sugar esters from glucose with different acyl donor carbon chain length (C4, C6, C10) in pyridine by an alkaline protease from *Bacillus subtilis*[9], and the polymer containing sugar ester[10]. Synthesis of co-polymer with acyclovir and sugar branches and the polymerization of acyclovir are in progress. It can be expected that with the same strategy, the polymerization reaction of vinyl acyclovir ester would be available.

Synthesis of 6-O-vinyladipoyl L-ascorbate

Transesterification of vitamin C with divinyladipate was shown in **Scheme 2**. The obtained vitamin C vinyl ester was characterized by IR and ^{13}C-NMR spectra[11], and compared with the standard spectroscopy data of vitamin C. IR and ^{13}C-NMR spectra revealed that acylation took place with excellent regioselectivity towards the primary hydroxyl function. The signals for C-6 of vitamin C ester in ^{13}C-NMR shifted downfield from 62.4 ppm to 64.65 ppm and C-5 signal shifted upfield from 68.9 ppm to 67.59 ppm compared with vitamin C. The polymerization of 6-O-vinyladipoyl L-ascorbate would be obtained in the near future.

References and Notes

1. G. Arnold, Y. Milka, Y. Ivan, *Int. Appl.*, WO 9851692 A1 19 NOV **1998**, 70pp.
2. L. M. Beauchamp, G. F. Orr, *et al.*, *Antiviral Chem. Chemother.*, **1992**, *3* (3), 157.
3. Y. C. Yan, U. T. Bornscheuer, R. D. Schmid, *Biotechnol. Lett.*, **1999**, 1051.
4. C. Humeau, B. Rovel, M. Girardin, *Biotechnol. Lett.*, **2000**, 165.
5. E. D. M John, W. S. Henry, J. P. Robert, **1960**, Brit 827 718.
6. Spectral data of acyclovir vinyl ester:
   ^{1}H-NMR (DMSO-d6, δ, ppm): 7.25 (dd, 1H, J=5.9Hz and J=13.2Hz, -CH=,), 4.91 (d, 1H, J=14.2Hz, CH_2=), 4.66 (d, 1H, J=5.4Hz, CH_2=), 2.44 (t, 2H, J=6.9Hz, -CH_2-(COO-CH=CH_2)), 2.27 (t, 2H, J=6.8Hz, -CH_2- (COO-acyclovir)), 1.50-1.52 (m, 4H, other 2 CH_2 of the adipoyl part), 10.75 (s, 1H, N-H), 7.81 (s, 1H, N=C-H), 6.61 (s, 2H, -NH_2), 5.35

(s, 2H, -N-CH_2-O), 4.09 (t, 2H, J=4.7Hz, CO-O-CH_2), 3.66 (t, 2H, J=4.8Hz, -CH_2-O-). ^{13}C-NMR (DMSO-d6, δ, ppm): 23.96, 24.21, 33.20, 33.49 (-CH_2), 173.26, 170.91 (C=O), adipic acid moiety; 98.74, 141.78 (-CH=CH_2), vinyl moiety; 154.99 (C-1), 152.06 (C-2), 116.91 (C-3), 157.89 (C-4), 138.17 (C-5), 72.37 (C-6), 67.14 (C-7), 63.23 (C-8). IR (KBr): 3439 cm^{-1}(N-H), 1733 cm^{-1} (C=O), 1690 cm^{-1} (C=N), 1647 cm^{-1} (C=C).

7. H. W. Gao; A.K. Mitra, *Magn. Reson. Chem.*, **1999,** *37* (9), 687.
8. M. Kitagawa, H. Fan, T. Raku, *et al.*, *Biotechnol. Lett.*, **2000,** *22*, 883.
9. Q. Wu, D. S. Lu, X. F. Lin, *Biotechnol. Lett.*, **2001,** *23*, **1981**.
10. Q. Wu, J. Y. Feng, X. F. Lin, *et al.*, *Chin. Chem. Lett.,* **2002**, *13* (5), 416.
11. Spectral data of 6-*O*-vinyladipoyl L-ascorbate:
^{13}C-NMR ($CDCl_3$, δ, ppm): 24.13, 24.25, 33.65, 33.80 (-CH_2), 173.94, 171.03 (C=O), adipic acid moiety; 98.14, 141.31 (-CH=CH_2), vinyl moiety; 172.67 (C-1), 153.43 (C-2), 118.87 (C-3), 76.44 (C-4), 67.59 (C-5), 64.65 (C-6). IR: 3406 cm^{-1} (O-H), 1723 cm^{-1} (C=O), 1645 cm^{-1} (C=C).

Received 20 May, 2002