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Regiospecific Addition of Uracil to Acrylates Catalyzed by Alkaline Protease from *Bacillus subtilis*

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Abstract: Michael addition reactions of uracil to acrylates were catalyzed by an alkaline protease from *Bacillus subtilis* in dimethyl sulfoxide at 55 ℃ for 72 h. The adducts were determined by TLC, IR and ^{1}H NMR.

Keywords: Alkaline protease, Michael addition, uracil, pyrimidine.

In recent years, synthesis of bioactive compounds has gained considerable attention of many researchers in the field of organic synthesis. Many derivatives of purine and pyrimidine are used as antiviral agents[1¬3], *e.g.*, penciclovir, acyclovir, famciclovir, ganciclovir, HPMPC and HPMPA. These compounds can be prepared by alkylation and addition reactions[4¬5]. Synthesis of nucleosides analogues or derivatives as antiviral and anticancer agents has attracted great attention in the past decade[6¬9], for example, Michael additions of nucleosides to acrylonitrile, ethyl acrylate and ethyl crotonate were achieved[10]. But all of above reports are chemosynthesis.

To our best knowledge, enzymatic reactions in non-aqueous media have been reported more and more because of the high chemo-, regio- and enantioselective activity of enzymes[11¬13]. In this paper, we investigated the Michael addition reactions of uracil to acrylates catalyzed by an alkaline protease from *Bacillus subtilis*, and all adducts were N-1 alkylated.

Experimental

Materials

Alkaline protease from *Bacillus subtilis* was purchased from *Wuxi* Enzyme Co. Ltd (Wuxi, P. R. China). Uracil, acrylates and all other chemicals were AR.

Analytical methods

Infrared spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. NMR spectra were recorded on $CDCl_3$ solutions using Bruker AVANCE DMX 500 with

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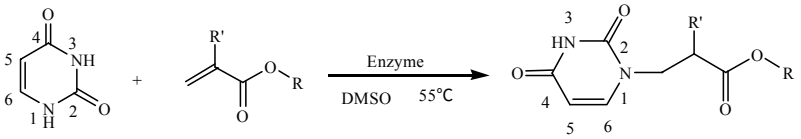
$(CH_3)_4Si$ as internal reference.

Scheme 1 Addition reactions of uracil to acrylates



a R=CH_3, R'=H
b R=CH_2CH_3, R'=H
c R=$CH_2CH_2CH_2CH_3$, R'=H
d R=CH_3, R'=CH_3

Enzymatic addition reactions

0.224 g uracil (0.002 mol) and 0.01 mol acrylates (methyl acrylate, ethyl acrylate , *n*-butyl acrylate and methyl methacrylate) were dissolved in 10 mL dimethyl sulfoxide (DMSO), 0.15 g (15 mg/mL) alkaline protease from *Bacillus subtilis* was added to the solution. The whole mixture was shaken at 55 ℃ for 72 h. Filtered the enzyme and evaporated DMSO in reduce pressure (0.5 mmHg 60℃). The residue was dissolved in chloroform and washed with water. Evaporated chloroform and the rude product was recrystallized from acetone. The product was determined by TLC, IR and ^{1}H NMR.

Results and Discussion

Results in **Table 1** show that the alkaline protease from *Bacillus subtills* can catalyze Michael addition reaction of uracil and acrylates. Adducts were characterized by IR and ^{1}H NMR, and results were shown in **Table 2**. Results of ^{1}H NMR showed that the adducts were N-1 alkylated[14], the peak corresponding to N-1 proton at the shift of 10.60 disappeared and the peak corresponding to the alkyl group were observed at the same time while the peak corresponding to N-3 proton at the shift of 8.56 still existed. The protease from *Bacillus subtills* has high regioselectivity of addition reaction between uracil and acrylates.

All of three adducts (**a**, **b**, **c**) were obtained in appropriate yields as hazel crystals. Yield of adduct decreased when the acrylate had a longer alcohol chain.

Table 1 Enzymatic addition of uracil to acrylates

Entry	Acceptor	Enzyme	Adduct	Yield (%)	m.p.(℃)
1	MA	Alkalin protease from *Bacillus subtills*	**a** Hazel crystal	53.3	127-128
2	EA	Alkalin protease from *Bacillus subtills*	**b** Hazel crystal	49.5	79-81
3	BA	Alkalin protease from *Bacillus subtills*	**c** Hazel crystal	43.3	64-66
4	MMA	Alkalin protease from *Bacillus subtills*		0	
5	MMA	proteinase from *Aspergillus oryzae*	**d** Hazel crystal	<5%	59-61

MA: Methyl acrylate; EA: Ethyl acrylate; BA: *n*-Butyl acrylate; MMA: Methyl methacrylate

Table 2 IR and ^{1}H NMR data of products **a-d**

		a	b	c	d
IR (cm^{-1},KBr)	-COOR	1733.14	1728.36	1736.06	1736.09
	uracil CO-NH-	1696.05 1662.03	1698.80 1683.49	1677.19	1716.64 1669.70
NMR ($CDCl_3$,δppm)	uracil **H**-3	9.03	8.56	8.56	9.13
	uracil **H**-6, J=7.9	7.40	7.40	7.39	7.29
	uracil **H**-5, J=7.9	5.68	5.66	5.66	5.66
	N-C**H**$_2$	4.00	3.99	3.99	3.94
	CO-C**H**$_2$	2.80	2.78	2.78	
	CO-C**H**				3.06
	O-C**H**$_3$	3.71			3.70
	O-C**H**$_2$		4.16	4.16	
	CH_2-C**H**$_3$		1.27	0.92	
	C**H**$_2$-CH_2-CH_3			1.60	
	CH_2-C**H**$_2$-CH_3			1.36	
	CH-C**H**$_3$				1.24

Structure of acceptor can also effect the addition reaction, when we used methyl methacrylate as acceptor, no adduct was found after 72 h at the same conditions as the addition reaction of uracil to acrylates.

Proteinase from *Aspergillus oryzae* was used to catalyze the reaction of uracil and methyl methacrylate too. After shaken the whole mixture (uracil/methyl methacrylate 1/5, mol/mol, 5 mg/mL enzyme in DMSO) at 55 ℃ for 72 h, adduct was obtained in a very low yield and it was also N-1 alkylated.

The results show hydrolase can catalyze Michael addition reaction of uracil and acrylates, and methyl methacrylate in non-aqueous media. Hydrolase catalyzed Michael additions of imidazole, purine and other nitrogen nucleophiles to α, β-ethylenic compounds are in progress.

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