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Regioselective Synthesis of Polymerizable Vinyl Guaifenesin Esters Catalyzed by an Alkaline Protease of *Bacillus subtilis*

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Abstract: Three polymerizable vinyl guaifenesin esters with different acyl donor carbon chain lengths (C4,C6,C10) were regioselectivly synthesized by an alkaline protease from *Bacillus subtilis* in pyridine at 50°C for 1, 3, 5 days respectively.

Keywords: Vinyl guaifenesin ester, regioselectivity, alkaline protease, transesterification.

Guaifenesin (I) [3-(*o*-methoxyphenoxy)-1,2-propanediol] has been extensively used as an expectorant and presented in a variety of pharmaceutical formulations[1]. In recent years, there has been considerable interest in developing biodegradable polymer drugs as they can effectively control the rate of the drug release and increase the therapeutic benefit, while minimizing side effects. So preparing monomers of polymeric drug**s** is of more significance, and the enzymatic synthesis can be considered as being highly regioselective and achieved in mild conditions[2,3]. We have reported that alkaline protease from *Bacillus subtilis* effectively catalyzed the synthesis of vinyl sugar esters and the preparation of polymers containing sugar branches through free radical polymerization[4-6]. In the present study, we prepared three polymerizable vinyl guaifenesin esters which can be polymerized to obtain polymer drugs containing guaifenesin in good regioselectivity and yields.

Experimental

Materials

Alkaline protease from *Bacillus subtilis* was purchased from *Wuxi* Enzyme Co. Ltd (Wuxi, P. R. China). The pyridine was dried over molecular sieves for 24 h *prior to* use. Divinylsuccinate, divinyladipate and divinylsebacate were produced and purified as described by the patent literature[7]. Guaifenesin, vinyl acetate and all other chemicals were of the highest purity commercially available.

Analytical methods

The progress of reaction was monitored by TLC with an eluent consisting of ethyl acetate/petroleum ether (3/7, v/v). Infrared spectra were measured with a Nicolet

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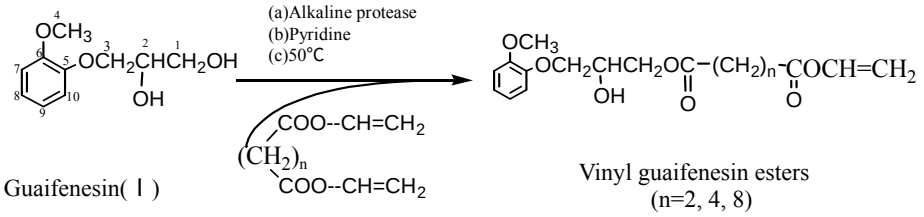
Nexus FTIR 670 spectrophotometer. The position of acylation in enzymatically prepared guaifenesin esters was established by ^{13}C-NMR (Bruker AVANCE DMX 500). $CDCl_3$ was used as a solvent and chemical shifts being expressed in ppm with reference to Me_4Si, coupling constants (J) in Hz. Mass spectrometry was carried out on HP5973 GC/MS.

Transesterification reaction

Guaifenesin 1.0 g (0.005 mol) was dissolved in 20 mL pyridine, 0.02 mol divinyl dicarboxylates (C4, C6, C10) were added in the mixture. The reaction was initiated by adding alkaline protease from *Bacillus subtilis* (50 mg/mL) and the suspension was shaken at 50℃ for 1, 3, 5 days respectively. The reactions were terminated by filtering off the enzyme and then the pyridine was evaporated. Formation of the vinyl guaifenesin ester was confirmed by TLC. The products were isolated by silica gel column chromatography with an eluent consisting of ethyl acetate/petroleum ether (3/7, v/v). Enzymatic synthesis of vinyl guaifenesin esters **(1, 2, 3)** was shown in **Scheme 1**.

Scheme 1 Enzymatic synthesis of vinyl guaifenesin esters **(1, 2, 3)**



1-Vinylsuccinyl -guaifenesin (1)

Tan oil; Yield: 55%, 0.90 g. ^{1}H-NMR ($CDCl_3$), δppm: 7.21 (dd, 1H, J=6.3, 14Hz, –CH=), 6.90 (m, 4H, H–7, 8, 9, 10), 4.86 (d, 1H, J=14Hz,=CH_2), 4.55 (d, 1H, J=6.3Hz, =CH_2), 4.30 (m, 2H, –CH_2O), 4.20 (m, 1H, –CH–), 4.03 (m, 2H, –CH_2OOC), 3.81 (s, 3H, –OCH_3), 3.00 (s, 1H, –OH), 2.67 (m, 4H, –CH_2–CH_2–). ^{13}C-NMR assignments are shown in **Table 1**. IR (neat, cm^{-1}): 3493 (ν_{OH}), 1747 ($\nu_{C=O}$), 1646 ($\nu_{C=C}$), 1593, 1506 (ν_{arom}), 1155 (ν_{CO}). GC-MS: 324, 307, 281, 201, 163, 124, 109, 99, 77, 55.

1-Vinyladipate-guaifenesin (2)

Tan oil; Yield: 45%; 0.80 g. ^{1}H-NMR ($CDCl_3$), δppm: 7.26 (dd, 1H, J=6.3, 14Hz, –CH=), 6.94 (m, 4H, H–7, 8, 9, 10), 4.87 (d, 1H, J=14Hz, =CH_2), 4.56 (d, 1H J=6.3Hz, =CH_2), 4.27 (m, 2H, –CH_2O), 4.23 (m, 1H, –CH–), 4.05 (m, 2H, –CH_2OOC), 3.84 (s, 3H, –OCH_3), 3.00 (s, 1H, –OH), 2.40 (m, 4H, –CH_2COO), 1.70 (m, 4H, –CH_2–CH_2–). ^{13}C-NMR assignments are shown in **Table 1**. IR (neat, cm^{-1}): 3493 (ν_{OH}), 1747 ($\nu_{C=O}$), 1646 ($\nu_{C=C}$), 1593, 1506 (ν_{arom}), 1155 (ν_{CO}). GC-MS: 352, 335, 309, 229, 185, 163, 124, 111, 83, 55.

1-Vinylsebacate-guaifenesin (3)

Tan oil; Yield: 54 %; 1.10 g. ^{1}H-NMR ($CDCl_3$), δppm: 7.27 (dd, 1H, J=6.3, 14Hz, –CH=), 6.92 (m, 4H, H–7, 8, 9, 10), 4.85 (d, 1H, J=14Hz, =CH_2), 4.55 (d, 1H,

J=6.3Hz, $=CH_2$), 4.26 (m, 2H, $–CH_2O$), 4.25 (m, 1H, –CH–) , 4.07 (m, 1H, $–CH_2OOC$), 3.83 (s, 3H, $–OCH_3$), 3.20 (s, 1H, –OH), 2.35 (m, 4H, $–CH_2COO$), 1.63, 1.27(m, 12H, $–CH_2–$). ^{13}C-NMR assignments are shown in **Table 1**. IR (neat, cm^{-1}): 3493(ν_{OH}), 1747($\nu_{C=O}$), 1646 ($\nu_{C=C}$), 1593, 1506(ν_{arom}), 1155(ν_{CO}). GC-MS: 408, 365, 285, 241, 185, 163, 124, 109, 97, 69, 55.

Results and Discussion

In the transesterification reactions, we selected guaifenesin as the drug substrate and three divinyl dicarboxylates with different carbon chain length (C4, C6, C10) as the acyl donors **(Scheme 1)**. Transesterification of guiafenesin with divinyl dicarboxylates occurred in the presence of the protease, and only one product was found by TLC.

The products were characterized by ^{13}C-NMR analysis, as shown in **Table 1 (1, 2, 3)**. The general strategy was the same as described by Yoshimoto *et al.*[8]. As established by them, acylation of a hydroxyl group of substrate results in a downfield shift of the peak corresponding to the O-acylated carbon and an upfield shift of the peak corresponding to the neighboring carbon. Characterization of the products **(1, 2, 3)** by ^{13}C-NMR revealed that vinyl guaifenesin esters were substituted at C-1 position of guiafenesin. Thus signals for C-1 of guaifenesin shifted downfield from 63.5 ppm to 65.3 ppm and $\delta_{C\text{-}2}$ of guaifenesin shifted upfield from 70.2 ppm to 68.5 ppm. Furthermore, the ^{1}H-NMR of the corresponding guaifenesin derivatives also confirmed the regioselective acylation at the primary OH (downfield shift of the signal). This result implies that alkaline protease from *Bacillus subtilis* shows an effective regioselectivity in the transesterification of guaifenesin with divinyl dicarboxylates (C4, C6, C10).

Table 1 Chemical shifts of ^{13}C-NMR ($CDCl_3$) of guaifenesin and its vinyl esters

Carbon number	Guaifenesin	**1**	**2**	**3**
1	63.3	65.5	65.3	65.2
2	70.2	68.1	68.5	68.7
3	70.6	70.8	71.4	71.7
4	55.6	55.7	56.0	56.1
5	148.5	147.9	148.0	148.2
6	149.4	149.5	150.0	150.2
7	112.4	112.0	112.2	112.3
8	121.1	122.1	122.5	122.7
9	120.9	121.0	121.1	121.1
10	113.8	114.8	115.1	115.4
─CH_2		28.7, 28.8	33.7, 33.8	24.8, 25.1
			24.1, 24.3	29.1, 29.2
				34.1-34.5
C═O		169.0	170.5	171.0
		172.0	173.4	174.0
─CH=		98.0	97.8	97.7

The effects of enzyme resource, solvents and temperature were investigated. It has been showed that the reaction system that we selected was optimal for an enzyme-catalyzed synthesis of vinyl guaifenesin esters. The guaifenesin derivatives would be useful as monomers of functional polymer drugs. Vinyl guaifenesin esters

can be polymerized through free radical polymerization for biodegradable polymer drugs. The polymerization of vinyl guaifenesin esters and preparation of copolymers with sugar branches are in progress.

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