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Design and Synthesis of Novel Hapten and Antigen for Dichlorvos: A Famous Organophosphorus Pesticide

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Dichlorvos, a famous organophosphorus pesticide, was widely used in agriculture to defend insects. It was difficult to obtain the antigen of dichlorvos because of its little molecular without active group. In this paper, a good strategy was carried out for designing hapten and complete antigens of dichlorvos. Hapten was synthesized facilely and simply in good yield. The novel antigen of dichlorvos was prepared successfully by active ester method.

Keywords: Organophosphorus pesticide, Dichlorvos, Hapten, Molecular modeling.

INTRODUCTION

Organophosphorus pesticides have been successfully used in agriculture and forestry in China now days. Particularly, it made a great contribution to improvements in agricultural output and the control of disease vectors[1]. Dichlorvos (dimethyl-dichloro-vinyl-phosphate, DDVP) is a famous organophosphorus pesticide and has been widely used in a variety of agricultural pest control because of its wide insecticidal spectrum and high efficiency. Although dichlorvos degrades relatively rapidly, it is urgent to monitor dichlorvos residues in crops and vegetables in the view of its high acute toxicity with LD50 80 mg/kg[2].

In organophosphorus pesticides, the common structure was a phosphate group. A few haptens with a phosphate group were designed to prepare the broad class antibodies for multi-residue detection[3,4]. But the most research focused on the relatively bigger molecular which had a phosphate and an aromatic ring, for examples, fenitrothion[5], fenthion[6,7], chlorpyrifos[8], parathion[9] and azinphos[10]. To our best of knowledge, only one paper reported the preparation of dichlorvos-protein complete antigen[11].

It was difficult to modify and evoke immune response for a simple molecular. Dichlorvos was obtained from trichlorfon in the presence of sodium hydroxide by elimination and rearrangement reaction, while trichlorfon has a hydroxyl group in the structure (Fig. 1). Based on the consideration, the similarity of the two molecules was studied by molecular modeling. Therefore, the hapten was prepared from succinic anhydride and trichlorfon and the artificial antigen was obtained by active ester method.

EXPERIMENTAL

Dichlorvos, trichlorfon, succinic anhydride, N,N-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), keyhole limpet hemocyanin (KLH) were purchased from Sigma (USA). All solvents were of analytic grade. UV spectra (200-800 nm) were recorded on a Cary 50-Bio UV spectrometer (Victoria, Australia). Fourier transform infrared (FT-IR) spectra (4000-400 cm^{-1}) were recorded using KBr pellets in a Vector 22 FT-IR spectrophotometer (Bruker, Germany). ^{1}H and ^{31}P NMR spectra were recorded on an AV-300 spectrometer (Bruker, Germany). Thin-layer chromatography (TLC) was performed on silica gel sheets and detected by a ZF-C ultraviolet-visible detector (Shanghai, China).

General procedure

Preparation of hapten: In 50 mL flask, trichlorfon (257 mg, 1 mmol) was added in the solution of succinic anhydride (100 mg, 1 mmol) in 20 mL pyridine. The mixtures were stirred in room temperature for 22 h. And then the solution was removed by reduced pressure. The residues were washed by 2M HCl, then extracted by ethyl acetate twice. The combined organic phases were dried over anhydrous $MgSO_4$. Following the concentration, the product was obtained with recrystallization in the THF-hexane.

Preparation of active ester of hapten: In 50 mL flask, the hapten was added in the solution of N-hydroxysuccinimide

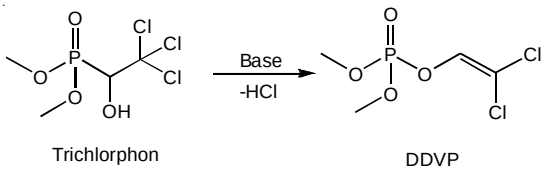

Fig. 1. Preparation of dichlorvos from trichlorphon

(127 mg, 1 mmol) in 5 mL freshly distilled THF. After the mixtures were stirred 0.5 h in ice-bath, the solution of DCC (227 mg, 1.1 mmol) in THF was added under nitrogen atmosphere. The mixtures were stirred 2 h in ice-bath and then warmed to the room temperature. The reaction process was monitored by TLC. After stirring 12 h, the reaction mixture was filtered to remove the precipitate of dicyclohexylurea. After the concentration, the residues were purified by column chromatography (hexane/ethyl acetate 1:6) to give the product as yellow oil.

Preparation of the artificial antigen: The solution of the active ester (2 mg) in 200 µL DMF was added to the KLH solution (10 mg keyhole limpet hemocyanin in 4 mL of 0.13 mol/L $NaHCO_3$ buffer, pH 8.1) drop by drop. The reaction was stirred overnight in 4 °C and then dialyzed against 0.01 mol/L PBS (pH 7.4) for 3 day at 4 °C with three buffer changes per day to give immunogens and then stored at -20 °C until used. Similarly, the coating antigens were prepared by the same method with OVA instead of keyhole limpet hemocyanin.

RESULTS AND DISCUSSION

For the small molecule, the design and synthesis of hapten is the most important for preparation of the antibody. As we all know, it is better that the structures of the hapten and the target are more similar. Dichlorvos is a simple molecular without active group and it is difficult to obtain its hapten by direct modification. While its precursor, trichlorfon, has a hydroxyl group in the chemical structure. The synthetic route was shown in Fig. 2. The similarity of the two molecular was expressed by molecular modeling shown in Fig. 3(a). From the alignment images of dichlorvos and trichlorfon, it was clearly shown that the two molecules were well overlapped in the dimethoxyphosphoryl group and there was a little difference in the other group. Therefore, it was feasible that trichlorfon was chosen to synthesis of hapten. At the same times, the hapten was also aligned in Fig. 3(b). It was found the common structures were in good coincidence only except for the armers. So the structure of trichlorfon was well exposed and it was favorable to obtain the antibody with high specificity.

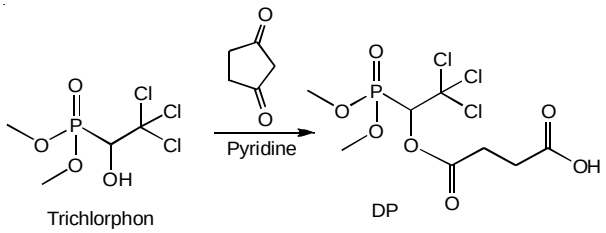

Fig. 2. Synthetic route of haptens

Hapten structure was confirmed by spectral data: MS (ESI) calcd. for $C_8H_{12}C_{l3}O_7P$ (Mr^+ 357.51); found: 357.27. ^1H NMR ($CDCl_3$) δ: 7.851 (s, -COOH, 1H), 5.950 (s, $-CHCCl_3$, 1H), 4.517-4.488 (s, $-CH_2COOH$, 1H), 3.940-3.890 (t, $(CH_3O)_2$-, 6H), 2.851-2.631 (dd, $-COOCH_2CH_2$-, 2H), 2.097 (s, $-CH_2COOH$, 1H). ^{31}P NMR ($CDCl_3$) δ: 18.815 (s, POCH-OH, 1P). Infrared spectrum of hapten is shown in Fig. 2. The observed 3161 cm^{-1} indicated O-H at carboxyl group and a C-H bond vibration around 2960 and 2876 cm^{-1}. The bonds around 1861 and 1729 cm^{-1} resulted from C=O ester and carboxyl group, respectively. The observed features around 1203 and 805 cm^{-1} indicated P=O stretch and C-Cl vibration, respectively. Characterization of the product by IR analysis was consistent of the characteristic peak of functional group of hapten.

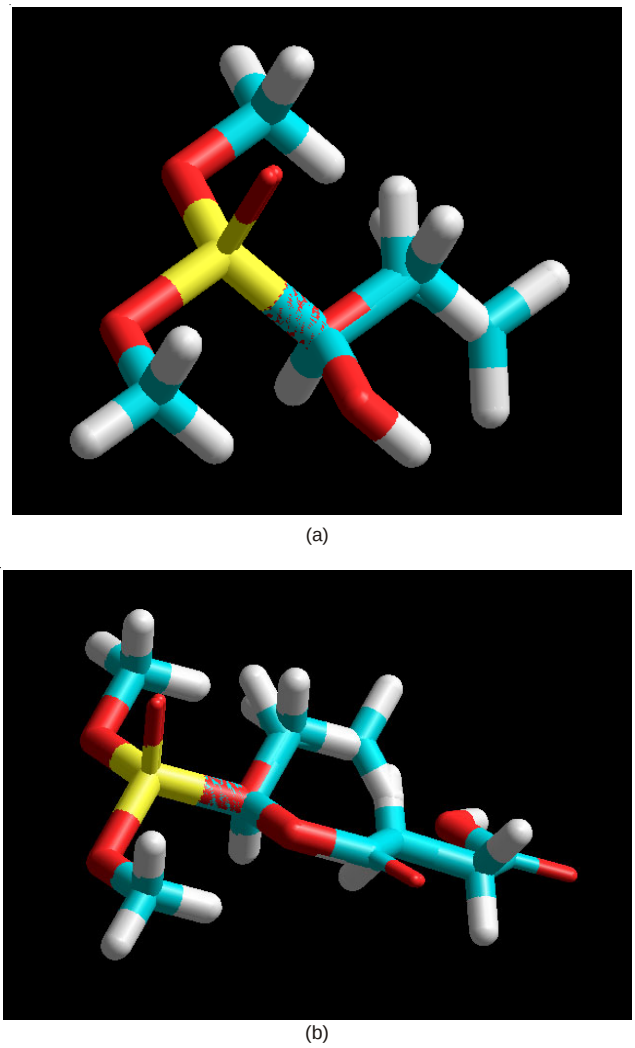

Fig. 3. Alignment images of molecular: (a) dichlorvos and trichlorphon, (b) dichlorvos, trichlorphon and hapten

Active ester of hapten was confirmed by spectral data: MS (ESI) calcd. for $C_{12}H_{15}C_{l3}NO_9P$ (Mr^+ 454.58); found: 478.25 (Mr^+ Na). Infrared spectrum of active ester of hapten was shown in Fig. 2. The O-H bond stretching vibration at 3161 cm^{-1} was disappeared because of the formation of the active ester. The observed 1781 cm^{-1} indicated C=O bond in the N-hydroxysuccinimide.

The artificial antigen were detected by UV in 200-400 nm and found it had maximum absorbed peak at 251 nm obviously different from the keyhole limpet hemocyanin (KLH) at 279 nm.

Conclusion

In a word, dichlorvos and trichlorfon were in good structural similarity by molecular modeling. Base on the consideration, a novel hapten for dichlorvos was successfully designed and synthesized.

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