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Effects of introducing theanine or glutamic acid core to tralopyril on systemicity and insecticidal activity

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ABSTRACT

Tralopyril was the active agent of a pro-insecticide chlorfenapyr. To simultaneously solve the problems of the phytotoxicity and non-systemic insecticidal activity of tralopyril, four new tralopyril conjugates containing theanine or glutamic acid moieties were designed and synthesized. Their phytotoxicity to tea shoot, phloem systemicity, and insecticidal activity were evaluated. Phytotoxic symptoms were not observed after the tea shoots were exposed to the four conjugates at concentrations of 2 mM. The phloem mobility test on *Ricinus communis* L. seedlings confirmed that all four conjugates were mobile in the sieve tubes. Results of insecticidal activity against the third-instar larvae of *Plutella xylostella* showed that only conjugate **20** exhibited activity with an LC_{50} value of 0.5882 ± 0.0504 mM. After root application to tea seedlings, conjugate **20** showed obviously systemic insecticidal activity against *Dendrothrips minowai* Priesner, while chlorfenapyr showed no attribute of that. A new conjugate as potential phloem mobile pro-insecticide candidate was provided and so a novel strategy of pro-insecticide for improved phloem systemicity was proposed.

1. Introduction

Tralopyril (Fig. 1) is a biocide optimized from dioxapyrrolomycin that shows excellent insecticidal activity [1,2]. Tralopyril uncouples oxidative phosphorylation in the mitochondria to cause disruption of ATP production and loss of energy, which can lead to cell dysfunction and subsequent death of the organism [3]. However, this compound exhibits phytotoxic effects. Chlorfenapyr is the first commercial pro-insecticide developed by introducing ethoxymethyl group into the pyrrole N of tralopyril to improve its intolerable phytotoxicity [1–4]. Chlorfenapyr is activated by the cleavage of an *N*-ethoxymethyl side chain to form tralopyril in the mid-gut of insects and mites through mixed-function oxidases. Owing to the success of chlorfenapyr, studies on the structural modification of tralopyril have been carried out [5–8]. However, no works have focused on the structural optimization of tralopyril to simultaneously solve its tough phytotoxicity and phloem mobility transformation problems.

Phloem-mobile insecticides are preferred for insect control efficacy. However, few phloem-mobile synthetic insecticides are available except spirotetramat [9]. Developing a novel phloem mobile insecticide is time-consuming and expensive, but attempts have been made to achieve phloem-mobile insecticides by introducing a carboxyl group, amino acid, or sugar to the parent compounds of existing non-phloem-mobile products [10–12]. In previous attempts, Hsu and his team took the lead to achieve phloem-mobile pro-nematicide by introducing glucuronic acid moiety to oxamyl [11]. However, the strict reactive conditions limited the further development of hydroxymethyloxamyl glucurzonide, which showed more phloem-mobile attributes and greater nematicidal activity than oxamyl alone. Yang et al. reported the phloem mobility and insecticidal activity of a conjugate containing both fipronil and glucose moieties (GTF) [12]. The main employed synthesis method for GTF was click chemistry, which made the conjugation between glucose and insecticides very efficient. However, no studies reported on the conjugation between amino acids and insecticide cores via click chemistry.

The effectiveness of introducing an amino acid to the parent compound to convert a non-mobile crop protection product into a phloem-mobile type was proven [10,13]. In this approach, the uptake and translocation of conjugates may involve two components of passive transport and specific active carrier-mediated systems. For example, 2,4D-Lys uptake involves an active carrier-mediated system aside from diffusion [13]. Theanine is a unique non-protein amino acid found almost exclusively in tea and accounts for about 50% of the total free amino acids and 1%–2% of the dry weight of tea leaves [14]. Theanine is synthesized in tea under a unique theanine synthase

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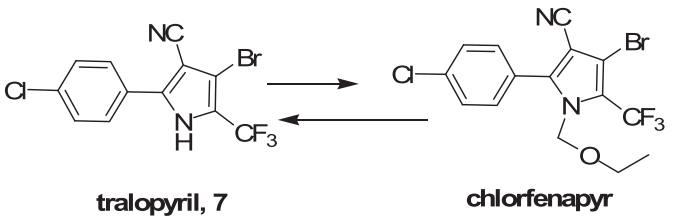

Fig. 1. Chemical structure of tralopyril and chlorfenapyr.

(L-glutamate: ethylamine ligase), which is capable of ligating glutamic acid and ethylamine to form theanine [14,15]. Theanine is hydrolyzed into glutamic acid and ethylamine by an enzyme called theanine hydrolase after being transported to growing tea shoots [14,15], where many tea infection insect species settle, such as *Dendrothrips minowai* Priesner [16] and *Empoasca (Matsumurasca) onukii* Matsuda [17].

These observations led us to consider whether tralopyril can be used as another non-phloem-mobile candidate, which is imparted phloem mobility by introducing theanine or glutamic acid moiety and consequently exhibits phloem-mobile pro-insecticide properties. In the present study, four conjugates with both tralopyril and amino acid moieties (theanine or glutamic acid) were synthesized, and their phytotoxicity to tea and phloem mobility were assessed. Insecticidal activity assessments of the four conjugates against the third-instar larvae of *Plutella xylostella* and systemic insecticidal activity of the most activated conjugate against *D. minowai* Priesner were also studied.

2. Materials and methods

2.1. Synthesis

2.1.1. General information for synthesis

Solvents were of analytical grade and were dried according to the methods of the Purification of Laboratory Chemicals, Fifth Edition. Reagents were purchased from a commercial company. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA- 400 instrument. Chemical shifts were expressed in δ (ppm) values, with TMS as an internal standard. The mass spectra of new conjugates were obtained by high-performance liquid chromatography (HPLC)–mass spectrometry (MS). Analytical thin-layer chromatography (TLC) was performed on silica gel GF254. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) was used for column chromatography.

2.1.2. Synthesis of compound ***4***

Synthesis of 2-azidoethanamine followed a procedure previously reported by Blatti et al. [18], where 2-bromoethylamine hydrobromide (2.0 g, 9.76 mmol) was added to a solution of sodium azide (1.9 g, 29.3 mmol) in H_2O (10 mL). The stirred solution was heated to 75 °C for 24 h before it was cooled to 0 °C. Et_2O (10 mL) was added and followed by solid KOH (3 g). The organic phase was separated, and the aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic layers were dried with Na_2SO_4, and the solvent was concentrated at 35 °C to afford 2-azidoethanamine (**2**) as a colorless liquid.

Ethyl dimethylaminopropyl carbodiimide (EDC) (0.758 g, 3.95 mmol) was added to a solution of Boc-Glu-O^{t}Bu (1.00 g, 3.30 mmol) in CH_2Cl_2 (5 mL), followed by 2-azidoethanamine (0.312 g, 3.63 mmol). The mixture was stirred at rt for 2 h, followed by extraction with H_2O (3 × 5 mL) and drying with MgSO4. After the drying agent was filtered, the filtrate was concentrated to furnish crude Boc-Glu(azide)-O^{t}Bu(**4**), which was used without further purification.

2.1.3. Synthesis of compound ***6***

Synthesis of 2-Azidoethanol followed the procedure previously reported by Lu and Bittman [19]. In a 50 mL flask, 2-bromoethanol (7.51 g, 60.5 mmol), NaN_3(5.13 g, 122 mmol), and n-Bu_4NBr (500 mg, 1.5 mmol) were mixed and stirred for 15 h at 110 °C. After the mixture had cooled, the product was taken up with Et_2O (20 mL), and the precipitate (consisting of NaBr, unreacted NaN3, and phase-transfer catalyst) was removed by filtration. The salt was washed with Et_2O (20 mL). Evaporation of the solvents resulted in a yellow residue that was purified by distillation at 35 °C to yield 2-Azidoethanol (**6**) as a colorless liquid.

2.1.4. Synthesis of compound ***9***

NaH (60% in oil, 0.144 g, 3.6 mmol) was added to the solution of tralopyril (1.05 g, 3 mmol) in dry THF (15 mL). The mixture was then stirred at room temperature for 2 h. Propargyl bromide (3.3 mmol) in THF (15 mL) was added drop wise at room temperature. The mixture was then stirred at 65 °C for 48 h. The reaction mixture was quenched by adding ice — water, and the resultant mixture was extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with aqueous sodium hydrogen carbonate and brine, dried with sodium sulfate, filtered, and evaporated in vacuo. chromatography to obtain the desired product **9** as a white solid with 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (m, 4H,Ar-H), 4.66 (d, J = 1.96 Hz, 2H), 2.51 (t, J = 1.96 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.55, 137.43, 131.09 * 2, 129.79 * 2, 124.90, 121.32, 118.62, 113.30, 103.26, 99.04, 76.54, 75.20, 37.31. LC-MS (APCI, Pos) m/z: 388.00 $[M + 1]^+$.

2.1.5. Synthesis of compound ***12***

NaH (60% in oil, 0.288 g, 7.2 mmol) was added to the solution of tralopyril (1.05 g, 3 mmol) in a mixture solvent of dry THF (20 mL) and bromochloromethane (1.95 g, 15 mmol, 5 equiv). The mixture was then stirred at room temperature for 2 h. propargyl alcohol (3 mmol) in THF (10 mL) was added drop wise and stirred at 65 °C for 48 h. The reaction mixture was quenched by adding ice — water, and the resultant mixture was extracted with ethyl acetate (20 mL × 3). The combined organic layers were washed with aqueous sodium hydrogen carbonate and brine, dried with sodium sulfate, filtered, and evaporated in vacuo. The residues were purified by column chromatography to obtain the desired product **12** as a white solid with 61% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (m, 4H,Ar-H), 5.38 (s, 2H), 4.05 (d, J = 2.4 Hz, 2H), 3.37 (t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.60, 135.88, 132.04 * 2, 129.32 * 2, 125.26, 121.31, 118.66, 113.61, 103.44, 97.96, 78.10, 77.96, 74.77, 55.65. LC-MS (APCI, Pos) m/z: 441.00 $[M + Na]^+$.

2.1.6. General procedures for compounds ***10***, ***11***, ***13*** and ***14***

Compounds **10**, **11**, **13**, and **14** were synthesized using this procedure. Tralopyril derivative 9 or 12 (1 mmol) and crude azide 4 or 6 (1.2 mmol) were dissolved in 6 mL ^{t}BuOH. The reaction was initiated by the addition of $CuSO_4 \cdot 5H_2O$ (0.2 mmol) and sodium ascorbate (0.4 mmol) in 6 mL distilled water. The mixture was stirred at 60 °C until TLC indicated the disappearance of the starting materials. The mixture was poured into distilled water (20 mL), and the product was extracted three times with ethyl acetate (15 mL × 3). The organic layer was dried with Na_2SO_4 and filtered. The solvent was removed under

Table 1
Experimental parameters and UHPLC–MS/MS conditions of conjugate **20** and chlorfenapyr in ESI$^+$ mode and tralopyril in ESI$^-$ mode.

Compound	Parent	product	S-Lens(v)	MRM collision energy(v)
Conjugate **20**	634.909	130.026	123	29
		246.881		60
		327.767		43
Chlorfenapyr	407.005	271.368	80	15
		377.526		16
Tralopyril	348.916	131.152	100	47
		270.099		31