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Synthesis and Insecticidal Activities of Novel Spin-Labeled Derivatives of Camptothecin

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ABSTRACT: *Continuing our search for natural product based compounds for the control of Brontispa longissima larvae, eight spin-labeled camptothecin derivatives* **7a–h** *and the intermediate* **2** *were first tested for their insecticidal activities against fifth-instar larvae of Brontispa longissima. Among all the tested compounds, especially compounds* **7a** *and* **2** *showed promising insecticidal activities with the corrected mortality rates of 69.55% and 74.07% against fifth-instar larvae of B. longissima, respectively. The different insecticidal activity ranges of these compounds indicated that the variation of the structures of* L*-amino acids in these compounds markedly affected the activity profiles of this compound class, and some important SAR information has been revealed from it.* © 2011 Wiley Periodicals, Inc. Heteroatom Chem 22:687–691, 2011; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20734

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INTRODUCTION

Brontispa longissima (Gestro) is one of the most serious insect pests of palm plants [1,2]. Control of the *B. longissima* larvae is primarily dependent on repeated applications of conventional insecticides such as organochlorine, organophosphorus, carbamate, and formamidines insecticides. Although effective, their extensive use for decades has produced risks in the development of insect resistance and residues to humans and to the environment [3,4]. These problems have highlighted the need for the development of new strategies for their selective control.

Plants may be an alternative source of insecticidal agents because they constitute a rich source of bioactive chemicals. Much effort has been focused on plant secondary metabolites as potential sources of commercial insect control agents or as lead compounds. Especially, the discovery of new insecticidal leads from plant sources, followed by using them as the useful prototypes for further modification and structure optimization, has recently been one of the important ways for the research and development of new insecticides [5–8].

Camptothecin (**1**), a naturally occurring indole alkaloid, besides its use as the molecular precursor for the development of potent antineoplastic drugs and antiviral agents [9–11], exhibited the

promising insecticidal activity [12]. Meanwhile, it has been reported that the introduction of a stable nitroxyl radical to the bioactive molecule would usually potentiate the biochemical or pharmacological properties of the original molecule [13]. More recently, the introduction of the L-amino acids containing a nitroxyl radical moiety into camptothecin by esterifying the 20-hydroxyl group as potential insecticides has been reported by our group and some compounds showed more promising insecticidal activity than camptothecin [14]. During investigation of structure–insecticidal activity relationships of **1**, interestingly, 5-(2'-hydroxythoxy)-20(*S*)-camptothecin (DRF-1042) **2** was found to display more potent insecticidal activity than **1** and toosendanin, a commercial insecticide derived from *Melia azedarach*. These encouraging results, therefore, prompted us in the present work to use DRF-1042 as a lead model for further synthesis of novel spin-labeled camptothecin derivatives as insecticidal agents.

RESULTS AND DISCUSSION

Chemistry

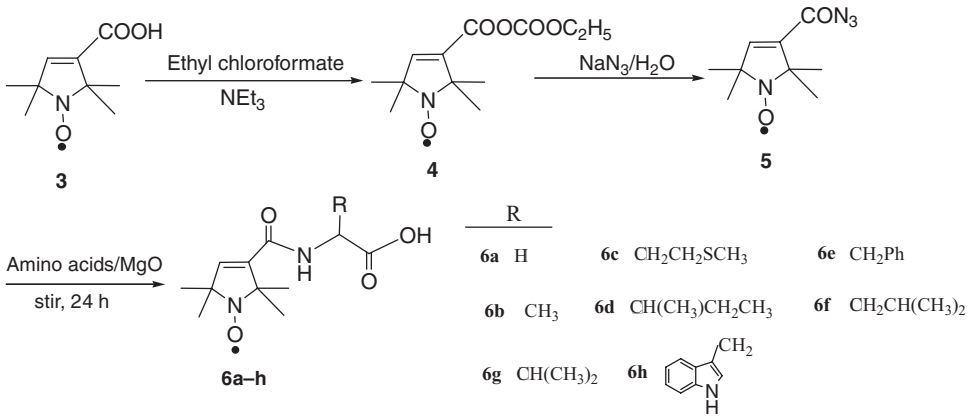
As shown in Scheme 1, the reactive anhydride **4** was prepared in almost quantitative yield by addition of ethyl chloroformate in the presence of catalytic amount of triethylamine to 2,2,5,5-tetramethylpyrroline-3-carboxylic acid-1-oxyl **3.** Compound **4** was further converted into the corresponding acid azide **5** [15,16] when dissolved in an aqueous acetone solution of sodium azide within a few minutes at 0°C, without further purification, the reaction of the free-radical acid azide **5** with the corresponding amino acids in the presence of magnesium oxide at room temperature afforded *N*-(1-oxyl-2,2,5,5-tetramethyl pyrroline-3-carbonyl)-amino acids **6a–h**. Spectral data for **6a–h** are identical to those reported by Hankovszky and coworkers [17].

Camptothecin **1** was isolated from a Chinese medicinal plant *Camptotheca acuminata*, and the conversion of the isolated available camptothecin to DRF-1042 (**2**) was accomplished in suitable yields by using the combination of sulfuric acid and $FeCl_3$ in the presence of $HOCH_2CH_2OH$ [18]. The desired compounds **7a–h** was achieved by treating DRF-1042 with the corresponding *N*-(1-oxyl-3-carbonyl-2,2,5,5-tetramethylpyrroline) amino acids **6a–h** in the presence of *N*,*N*′-dicyclohexyl carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Synthesized target compounds **7a–h** were characterized by melting point, electron spin resonance (ESR), IR, and HRMS spectral analyses (Scheme 2).

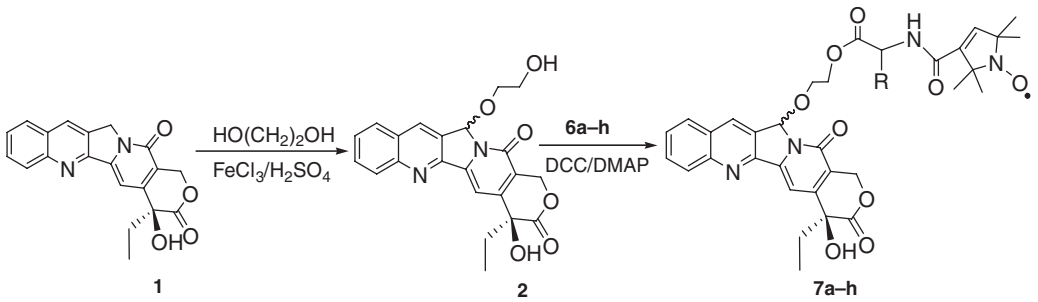
Bioassay

On the basis of the methodology described in Scheme 2, with eight spin-labeled camptothecin derivatives **7a–h** and the intermediate **2** in hand, we next examined their insecticidal effects against the fifth-instar larvae of *B. longissima* in vivo at the concentration of 0.1 mg/mL. The results are summarized in Table 1.

As shown in Table 1, the corresponding corrected mortality rates caused by these compounds after 9 days were far higher than those after 3 and 6 days. For example, the corrected mortality rate of **7a** against *B. longissima* after 3 days was only 35.93%, after 9 days the corresponding mortality rate was increased to 69.55%, which was nearly two times of the mortality rate after 3 days. That is, these compounds, different from those conventional neurotoxic insecticides, such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal



SCHEME 1 Synthesis of *N*-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carb onyl)-amino acids **6a–h**.



SCHEME 2 Synthesis of target compounds **7a–h**.

activity, which coincided very well with our previous studies [14], Thus it further showed that the delayed insecticidal activity would be a common property of camptothecins' derivatives.

From the screening, all these compounds exhibited less potent than the intermediate **2**, whereas the corrected mortality rates of these compounds were either similar or better than those of the prototypical compound camptothecin **1**. Among all the test compounds, compounds **7a** and **2** possessed the highest overall insecticidal potency, with the corrected mortality rates of 69.55% and 74.07% against fifth-instar larvae of *B. longissima*, respectively. From insecticidal activity values, it emerged that the relative relationship between bioactivity and substituents at α-carbon of amino acid, for example, compound **7a** containing the L-glycine group exhibited more promising and pronounced insecticidal activity than **1**; whereas compounds **7d** and **7h** bearing the L-leucine and L-proline groups would be reduced as compared to **1.** The results also clearly underlined the insecticidal difference that could be ascribed to a combination of factors, such as the nature of the substitutes (which may depend on the size of substitutes, electronic characteristics of substitutes, and other factors) or by a different interaction at the site. Hence, a systemic, predictable correlation could be made between the nature of amino acids and insecticidal activities. As can be seen, as a whole, the introduction of a stable nitroxyl radical into the molecule of **2** with L-amino acids led to potentiate their insecticidal activity, which also indicated that the design and synthesis of these compounds might be beneficial for camptothecin as insecticides and further studies would be taken to reveal the mode of insecticides of these interesting compounds and to survey quantitative structure–activity relationship as to find the biorational pesticide.

In conclusion, eight novel spin-labeled camptothecin derivatives **7a–h** and the intermediate **2** have been synthesized and evaluated for their insecticidal Activity against the fifth-instar larva *B. longissima* in vivo at the concentration of 0.1 mg/mL. The bioactivity assay for these analogues showed that some of them are either similar or better than camptothecin itself. Especially, **7a** and **2** exhibited the most potent insecticidal activity compared with camptothecin. The results suggested that the design and synthesis of these compounds may be beneficial for the insecticidal activity of camptothecin and related analogues, and this study will be of assistance to investigators involved in the design and preparation of biologically useful camptothecin congeners of this class as insecticidal agents in the future.

TABLE 1 Insecticidal Activity of Compounds **7a–h** against Fifth-Instar Larvae of *B. Longissima* in vivo at the Concentration of 0.1 mg/mL

	Corrected Mortality Rate (%)		
Entry	*3 Days*	*6 Days*	*9 Days*
7a	35.93 ± 7.56	37.57 ± 7.25	69.55 ± 12.18
7b	13.70 ± 5.48	31.85 ± 8.98	48.15 ± 6.42
7c	17.41 ± 6.51	17.78 ± 5.88	37.04 ± 6.42
7d	6.67 ± 5.77	7.41 ± 6.42	25.93 ± 6.42
7e	19.01 ± 7.46	31.40 ± 6.29	44.44 ± 6.17
7f	14.07 ± 12.24	14.07 ± 5.13	29.63 ± 6.42
7g	14.07 ± 7.06	21.11 ± 9.49	22.22 ± 11.11
7h	13.33 ± 11.55	24.81 ± 4.49	44.44 ± 11.11
2	20.74 ± 1.28	42.96 ± 2.57	74.07 ± 6.42
1	25.93 ± 6.42	27.16 ± 5.66	51.85 ± 14.24

EXPERIMENTAL

General

Melting points were taken on a Kofler melting point apparatus and were uncorrected. Mass spectra were recorded on a ZAB-HS and Bruker Daltonics APEXII49e instrument, and the infrared spectra were recorded on a NIC-5DX spectrophotometer. The ESR spectra were obtained with a Bruker A300 X-band EPR spectrometer. IR spectra

were measured on a Nicolet 5DX-FT-IR spectrometer on neat samples placed between KBr plates. The synthetic compounds were purified by flash chromatography on Merck silica gel (70–230 mesh). Thin-layer chromatography (TLC) involved the use of silica gel plates with a fluorescent indicator (Merck Silica Gel 60 F_{254} 0.25 mm thick). *N*-(1-Oxyl-2, 2, 5, 5-tetramethyl pyrroline-3-carbonyl)-amino acids **6a–h** were synthesized by employing previous procedures [15,16]. The intermediate **3** was prepared from **1** by a modified previous procedure [15].

General Procedure of Synthesis of **7a–h**

A mixture of the corresponding *N*-(1-oxyl-2,2,5,5-tetramethyl pyrroline-3-carbonyl)-amino acids **6a–h** (0.5 mmol), compound **2** (0.5 mmol), and DMAP (20 mg) was stirred in dry CH_2Cl_2 (10 mL) for 5 min at room temperature under nitrogen. *N,N*-Dicyclohexylcarbodiimide (DCC, 106 mg, 0.5 mmol) was added, and the mixture was stirred for 4 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was separated by column chromatography on silica gel with CH_2Cl_2–acetone to afford compounds**7a–h**.

Compound **7a**: yield: 90%; mp 115–117°C; IR (KBr) υ (cm^{-1}): 3346 (NH), 1715 (NHCO), 3062, 1626, 1490 (ArH), 1662 (C=O), 1242, 1155,1090 (C—O), 1364(NO·); MS *m/z*: 632 $[M + H]^+$; HRMS: *m/z* calcd for $C_{33}H_{35}N_4O_9$: 632.2481 $[M + H]^+$, Found: 632.2477 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Compound **7b**: yield: 86%; mp 120–122°C; IR (KBr) υ (cm^{-1}): 3429 (NH), 1743 (NHCO), 3056, 1624, 1552 (ArH), 1662 (C=O), 1087, 1156, 1231 (C—O), 1362 (NO•); MS *m/z* : 646 $[M + H]^+$; HRMS: *m/z* calcd for $C_{34}H_{37}N_4O_9$: 646.2625 $[M + H]^+$, Found: 646.2633$[M + H]^+$; ESR: $g_0 = 2.0058$, $A_N =$ 14.62Gs (triplet peak in 1×10^{-4} M, DMF).

Compound **7c**: yield: 82%; mp 155–157°C; IR (KBr) υ (cm^{-1}): 3423 (NH), 1711 (NHCO), 3060, 1625, 1563 (ArH), 1663 (C=O), 1088, 1153, 1236 (C—O), 1362 (NO•); MS *m/z*: 706 $[M + H]^+$; HRMS: *m/z* calcd for $C_{36}H_{41}N_4O_9S$: 706.2656 $[M + H]^+$. Found: 706.2267 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Compound **7d**: yield: 75%; mp 160–162°C; IR (KBr) υ (cm^{-1}): 3423 (NH), 1746 (NHCO), 3091, 1621, 1562 (ArH), 1664 (C=O), 1114, 1155, 1228 (C—O), 1359 (NO·); MS *m/z* : 688 $[M + H]^+$; HRMS: *m/z* calcd for $C_{37}H_{43}N_4O_9$: 688.3098 $[M + H]^+$; Found: 688.3103 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Compound**7e**: yield: 87%; 142–144°C; IR (KBr) υ (cm^{-1}): 3425 (NH),1744 (NHCO), 3093, 1623, 1564 (ArH), 1664 (C=O), 1113, 1153, 1234 (C—O), 1361 (NO•); MS *m/z* : 722 $[M + H]^+$; HRMS: *m/z* calcd for $C_{40}H_{41}N_4O_9$: 722.2944 $[M + H]^+$, Found: 722.2946 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Compound **7f**: yield: 68%; mp 117–119°C; IR (KBr) υ (cm^{-1}): 3418 (NH), 1747 (NHCO), 3065, 1621, 1562 (ArH), 1664 (C=O), 1114, 1157, 1227 (C—O), 1359 (NO•); MS *m/z*: 688 $[M + H]^+$; HRMS: *m/z* calcd for $C_{37}H_{43}N_4O_9$: 688.3110 $[M + H]^+$, Found: 688.3103 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Compound **7g**: yield: 76%; mp 120–122°C; IR (KBr) υ (cm^{-1}): 3416 (NH), 1750 (NHCO), 3092, 1620, 1538 (ArH), 1665 (C=O), 1106, 1157, 1231 (C—O), 1359 (NO•); MS *m/z*: 674 $[M + H]^+$; HRMS: *m/z* calcd for $C_{36}H_{41}N_4O_9$: 674.2956 $[M + H]$+, Found: 674.2946 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Compound **7h**: yield: 54%; mp 127–129°C; IR (KBr) υ (cm^{-1}): 3413 (NH), 1745 (NHCO), 3061, 1619, 1522 (ArH), 1663 (C=O), 1086, 1158, 1229 (C—O), 1356 (NO•); MS *m/z*: 761 $[M + 1]^+$; HRMS: *m/z* calcd for $C_{42}H_{42}N_5O_9$: 761.3040 $[M + H]^+$, Found: 761.3055 $[M + H]^+$; ESR: $g_0 = 2.0055$, $A_N = 14.62$Gs (triplet peak in 1×10^{-4} M, DMF).

Biological Assay

The insecticidal activity of compounds **7a–h** against the fifth-instar larvae of *B. longissima* was assessed by the leaf-dipping method as described previously [19]. For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of **7a–h**, camptothecin (used as a positive control), were prepared at the concentration of 0.1 mg/mL. Fresh coconut leaves were dipped into the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with acetone alone were used as a control group. Several treated leaves were kept in each dish, where every 10 larvae were raised. If the treated leaves were consumed, corresponding ones were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until the adult emergence. The experiment was carried out at 25 ± 2°C and relative humidity (RH) 65%–80% on a 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the fifth-instar larvae of *B. longissima* was calculated by the formula

$$\text{Corrected mortality rate (\%)} = (T - C) \times 100/(1 - C)$$

where T is the mortality rate in the treated group expressed as a percentage and C is the mortality rate

in the untreated group expressed as a percentage, and the results are presented in Table 1.

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