PERGAMON

Phytochemistry 52 (1999) 1731–1734

PHYTOCHEMISTRY

Two alkaloids from Chinese bittersweet *Celastrus angulatus*

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Received 10 April 1998; received in revised form 9 March 1999

Abstract

Two alkaloids, named Chinese bittersweet alkaloid I and II, were isolated from the seeds of Chinese bittersweet (*Celastrus angulatus M.*). Their structures were identified as (−)-2α-(2′-pyrrolyl)-4α-methoxycarbonyl-1,3-oxazacyclohexane and 2α-(2′-pyrroly1)-4α-methoxycarbonyl-5β-[(2″-methyl)-hepty1]-1,3-oxazacyclohexane, respectively, by spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Bittersweet alkaloids; *Celastrus angulatus*; Celastraceae; Structural elucidation; NMR spectroscopy

1. Introduction

Chinese bittersweet (*Celastrus angulatus M.*) is widely distributed in China and has been used as a traditional plant insecticide (Tu et al., 1993; Wakbayashi et al., 1988; Wu & Gao, 1985; Zhong, 1950) and a medicinal herb (Shizuri, Wada, Sugiura, Yamada & Hirata, 1973) for many years in the folk medicine of China. In previous studies, most of its chemical constituents from the root bark have been β-dihydroagarofuran sesquiterpene polyol esters (Liu, Han, Jia, Ju & Wang, 1991; Manske, 1977; Tu, Huang, Ma, Wu & Song, 1992). It was reported that most of β-dihydroagarofuran sesquiterpene polyol esters from Chinese bittersweet have inhibitory effects on EBV activation (Takaishi, Ohshima, Nakano & Tomimatsu, 1992) and antitumor activity (Liu, Jia, Wu, Zhou & Zhou, 1989). In an attempt to find novel bioactive compounds in Chinese bittersweet, we have studied the constituents of its seeds. Apart from several sesquiterpene polyol esters, we obtained two new type alkaloids, named Chinese bittersweet alkaloid I (**1**) and Chinese bittersweet alkaloid II (**2**) (Fig. 1). Both **1** and **2** contain a 1,3-oxazacyclohexane heterocyclic moiety, which displayed a novel skeleton not found in the natural products literature. Here, we wish to report the isolation and structure elucidation of these two alkaloids.

2. Results and discussion

Chinese bittersweet (*C. angulatus*) seeds (1 kg dry wt) were extracted with petroleum ether two times at room temperature. The extract was concentrated and then chromatographed on a silica gel column. The middle polar constituents were further separated on ready-made silica gel plates, affording alkaloid I (30 mg) and alkaloid II (6 mg).

The molecular formula of alkaloid I was determined to be $C_{10}H_{14}O_3N_2$ from the EIMS spectrum. Its ^{13}C NMR spectrum showed 10 lines, in agreement with the molecular formula. A DEPT experiment revealed that there was one carbonyl carbon at 172.6 ppm, one unsaturated quaternary carbon at 132.8 ppm, two saturated and three unsaturated methines at 54.1, 85.8, 108.5, 114.2 and 118.9 ppm, two saturated methylenes at 71.6 and 29.7 ppm and one methoxyl group at 55.9 ppm. Its IR spectrum also suggested the presence of an ester carbonyl (1750 cm^{-1}) group. The UV spec-

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PII: S0031-9422(99)00216-2

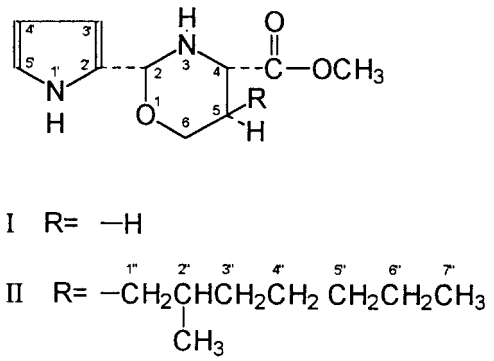

Fig. 1. Structures of Chinese bittersweet alkaloids I and II.

trum in $CHCl_3$ displayed maximum absorbance at 280.2 and 239.6 nm, suggesting a pyrrole moiety.

The signals at 108.5, 114.2, 118.9 and 132.8 ppm in the ^{13}C NMR spectrum of alkaloid I showed that it contained four aromatic carbons. The multiple peaks at δ 6.80–6.90 ppm in the ^{1}H NMR spectrum of alkaloid I were assigned to the three hydrogens of a 2′-substituted pyrrole ring, which corresponded with its ^{13}C NMR spectral data. The single peak at 3.88 ppm in the ^{1}H NMR spectrum and the signal at 55.9 ppm in the ^{13}C NMR spectrum showed there was a methoxyl group. The signal at 172.6 ppm was characteristic of an ester carbonyl carbon, which was attributed to a carbomethoxy group. This result was also supported by the characteristic absorption of its carbonyl group in the IR spectrum.

If a carbonyl group and a pyrrole ring account for four degrees of unsaturation, from its molecular formula we know that alkaloid I must have a saturated ring. The ^{13}C NMR resonances at 29.7 (CH_2), 54.1 (CH), 71.6 (CH_2) and 85.8 (CH) ppm showed the presence of an oxygenated methylene, a subamino-group and an unsubstituted methylene in alkaloid I. The downfield signal at 85.8 ppm suggested the presence of a methine located between an oxygen and a nitrogen atom. So, the structure was deduced to be a 2,4-disubstituted 1,3-oxazacyclohexane. Signals at 4.72 (d, 1H), 4.23 (dd, 1H), 3.85 (dd, 1H) and 3.10 (m, 1H) ppm in the ^{1}H NMR spectrum were in agreement with our interpretation. The 1,3-oxazacyclohexane structure has never been reported in the literature.

In the HMBC spectrum of alkaloid I, there are obvious correlations between δ_C 108.5 ppm and δ_H 4.72 ppm, between δ_C 114.2 ppm and δ_H 4.72 ppm which indicated that the 2′-pyrrolyl was connected to the C-2 position and the methoxycarbonyl was joined to the C-4 position.

The configurations of two substituted groups on the 1,3-oxazacyclohexane ring were determined by study of the coupling constants in the ^{1}H NMR and NOESY spectra. Double peaks at 4.72 ppm (J = 3.6 Hz, H-2) were split by the *ortho*-hydrogen on the

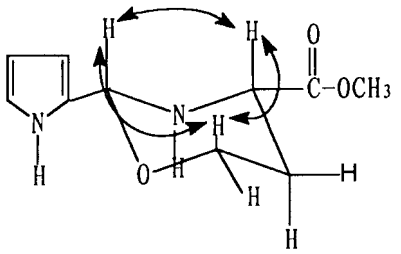

Fig. 2. NOESY correlations in **1**.

nitrogen. The double–double peaks at 4.23 and 3.85 ppm were assigned to H-6a and H-6e. The signal at 3.10 ppm displayed multiple peaks with a larger half-peak width (> 10 Hz), which showed that the carbomethoxy group was located at C-4 in equatorial configuration. In the NOESY spectrum of alkaloid I, there were significant correlations between H-2, H-4 and H-6a. Therefore, both the pyrrolyl and the methoxycarbonyl were connected with 1,3-oxazacyclohexane ring in an equatorial configuration. Finally, the structure of alkaloid I was identified as (−)-2α-(2′-pyrrolyl)-4α-methoxycarbonyl-1,3-oxazacyclohexane (Fig. 2). The complete interpretation of its spectra is shown in Section 3.

The characteristic fragments of alkaloid I in its MS spectrum, m/z 195 (15), 194 (100), 178 (12.5), 177 (96), 150 (7), 149 (33) and 130 (6) (Fig. 3), further confirmed the deduced structure. The mechanism of fragmentation of alkaloid I is described in Fig. 3.

The molecular formula of alkaloid II was determined to be $C_{18}H_{30}O_3N_2$ from its high resolution mass spectrum. Its IR spectrum suggested the presence of an ester carbonyl group (1750 cm^{-1}). The UV spectrum in $CHCl_3$, displaying maximum absorbance at 279.4 and 240.0 nm, indicated that it also had a pyrrole moiety. Comparing the data in the ^{1}H NMR spectrum with those of alkaloid I, we found that alkaloid II displayed a similar structure to alkaloid I.

In the ^{1}H NMR spectra of alkaloid II, signals at 0.85 ppm (t, 3H, CH_3) and 1.25 ppm (s, n, CH_2) indicated the presence of a saturated hydrocarbon chain. The presence of a series of peaks which had a difference of 14 in mass (m/z 43, 57, 71, 99, 113) and the absence of a fragment ion peak at m/z 85 in its mass spectrum suggested that a saturated 2-methyl-heptane hydrocarbon chain was present. Also in the mass spectrum of alkaloid II, there was a fragment at m/z 211 (2.60) but no fragment at m/z 210 or m/z 209, which suggested that alkaloid II underwent β-cleavage of the carbomethoxy group and hydrogen rearrangement of the 2-methyl-heptane group. So, we deduced that the 2-methyl-heptane group was connected to C-5, which was in agreement with the data in the ^{1}H NMR spectrum. The mechanism of fragmentation of alkaloid II is described in Fig. 4.

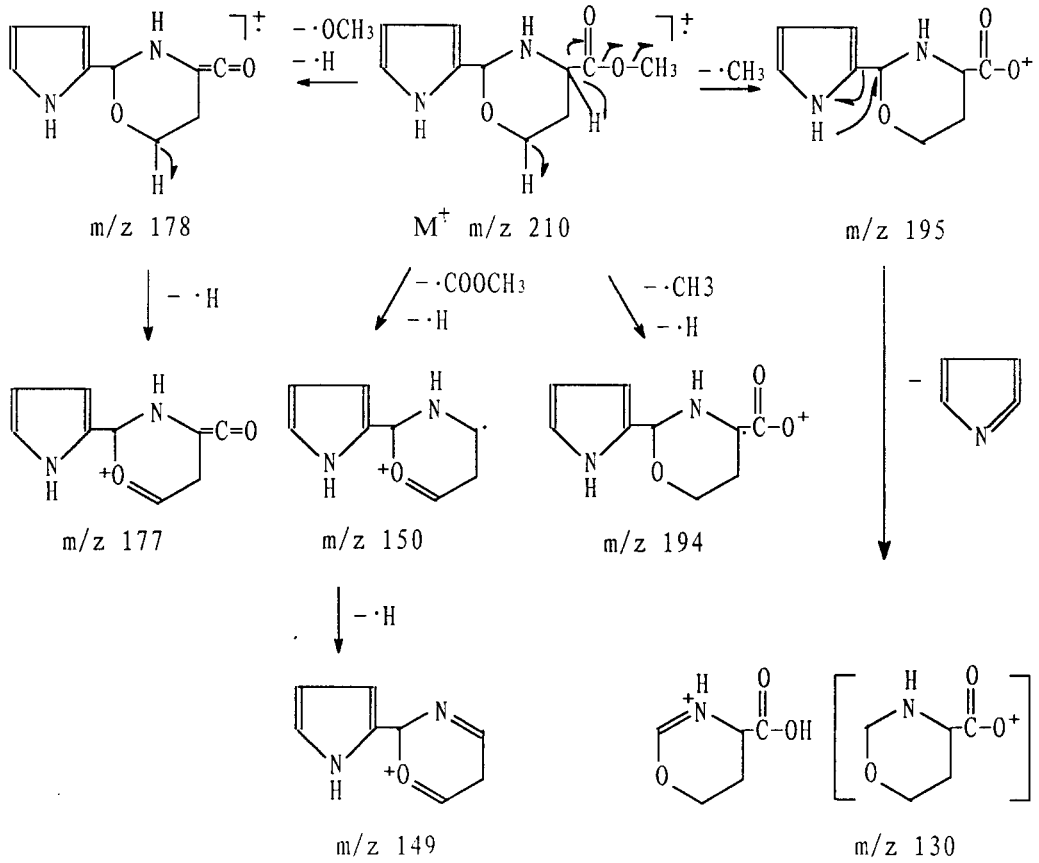

Fig. 3. Proposed MS fragmentation of Chinese bittersweet alkaloid I.

The signal at 3.10 ppm in the ^{1}H NMR spectrum of alkaloid II also displayed multiple peaks with a larger half-peak width (>10 Hz), which indicated that the methoxycarbonyl was located at an equatorial position on C-4. The NOESY spectrum of alkaloid II revealed significant correlations between H-2, H-4 and H-6a and between the 2-methyl-heptane group and OCH_3 group. Therefore, the 2-methyl-heptane group was deduced to be attached to C-5 in an equatorial configuration and H-2, H-4 and H-5 were in a axial configurations (Fig. 5). Finally, the structure of alkaloid II was identified as 2α-(2′-pyrroly1)-4α-methoxycarbonyl-5β-[(2″-methyl)-hepty1]-1,3-oxazacyclohexane. The absolute configuration of C-2″ remains to be clarified. The completed interpretation of its spectra is shown in Section 3.

3. Experimental

3.1. General

^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT and 2D correlation spectra: Bruker DPX400; MS: HP5859A, 70 eV; IR: PE-580B; UV: Shimadzu UV-2100 spectrophotometer; optical rotation: PE-341.

3.2. Plant material

The seeds of bittersweet (*C. angulatus*) were collected in the Song Xian County, Henan Province, People's Republic of China in October 1996, with a voucher specimen deposited at the Laboratory of Natural Products, Institute of Chemistry, Henan Academy of Sciences.

3.3. Extraction and isolation

The air-dried and pulverized seeds of Chinese bittersweet (*C. angulatus*), dry wt 1 kg, were extracted with petroleum ether (2 × 5 l) at room temperature. The extract was concentrated and the mixture chromatographed on a silica gel column, eluted with cyclohexane–EtOAc (6:4) to give a crude product (120 g). The fraction from CC was partitioned between C_6H_6 and H_2O and the oil layer was concentrated in vacuo to give a yellow sticky residue. The constituents were separated on ready-made silica gel plates using cyclohexane–EtOAC (8:2) affording alkaloid I (30 mg) and alkaloid II (6 mg).

Chinese bittersweet alkaloid I, colorless oil. $[\alpha]_D^{20}$ −0.12° ($CHCl_3$, *c*, 0.45). $C_{10}H_{14}O_3N_2$. MW 210. UV λ^{CHCl_3} nm: 280.2 and 239.6. IR (film) ν cm^{-1}, 3440,

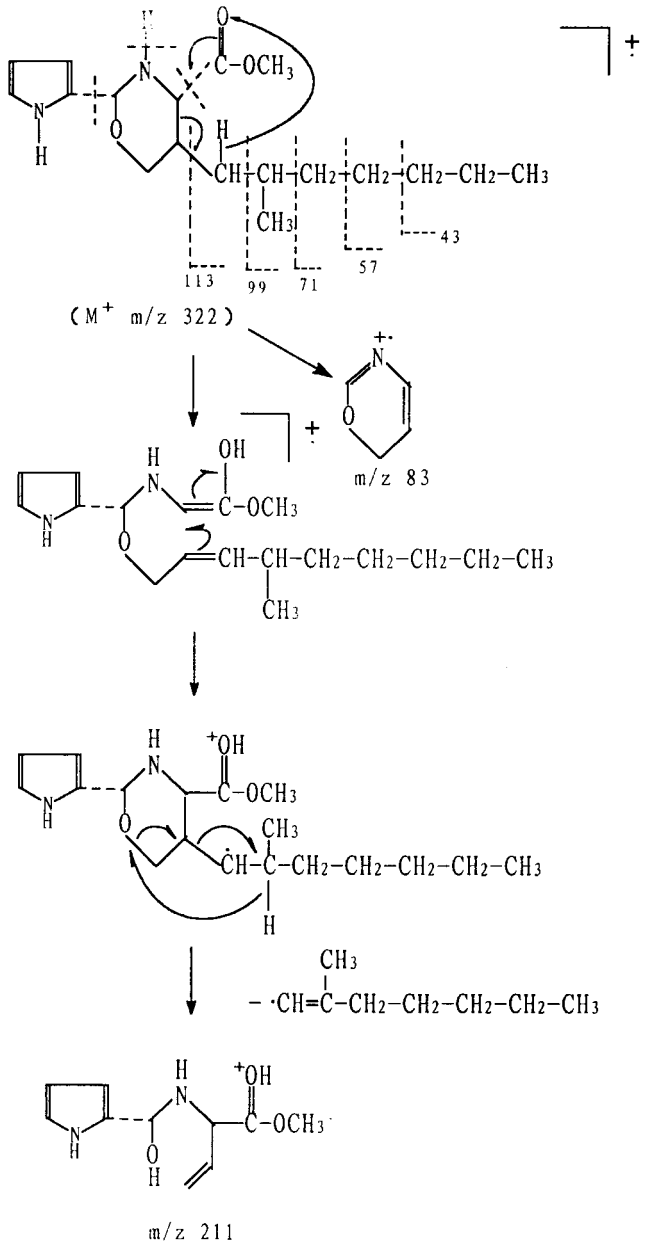

Fig. 4. Proposed mass spectral fragmentation of **2**.

2860, 1750, 1630, 1610, 1460, 1440, 1375, 1240, 1160, 1040. EIMS *m/z* (%): 210, $[M]^+$, 195 (15), 194 (100), 178 (12.5), 177 (96), 150 (7), 149 (33), 130 (6). ^1H NMR (400 MHz, $CDCl_3$, ppm): 3.10 (m, 1H, H-4), 3.85 (dd, J = 8.8, 3.2 Hz, 1H, H-6e), 3.88 (s, 3H, OCH_3), 4.23 (dd, J = 8.8, 6.8 Hz, 1H, H-6a), 4.72 (d, 1H, J = 3.6 Hz, H-2), 6.82 (dd, J = 6.8, 1.6 Hz, 1H, H-4′), 6.88 (d, J = 6.8 Hz, 1H, H-3′), 6.89 (d, J = 1.6 Hz, 1H, H-5′), 5.40 ~ 5.70 (br, 2H, N-H). ^{13}C NMR

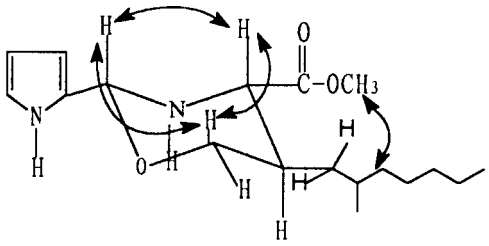

Fig. 5. NOESY correlations in **2**.

(100 MHz, $CDCl_3$, ppm): 29.7 (C-5), 54.1 (C-4), 55.9 (OCH_3), 71.6 (C-6), 85.8 (C-2), 108.5 (C-4′), 114.2 (C-3′), 118.9 (C-5′), 132.8 (C-2′) and 172.6 (C=O).

Chinese bittersweet alkaloid II, yellowish liquid. $C_{18}H_{30}O_3N_2$, MW 322. HR-MS: *m/z* 307.2014 $[M-15]^+$ (Δ, −0.8 mmu for $C_{17}H_{27}O_3N_2$). UV λ^{CHCl_3} nm: 279.4 and 240.0. IR (film) ν cm^{-1}, 3480, 3040, 2940, 2860, 1750, 1630, 1610, 1460, 1440, 1375, 1240, 1160, 1050, 720. EIMS *m/z* (%): 323 (0.96) $[M+1]^+$, 307 $[M-15]^+$, 211 (2.60), 185 (0.30), 153 (0.43), 113 (13.6), 99 (100), 83 (2.71), 71 (11.71), 57 (23.01), 43 (22.14). ^1H NMR (400 MHz, $CDCl_3$, ppm): 0.87 (t, 3H, 7″-CH_3), 1.25 (s, 8H, –(CH_2)-4), 1.30 (d, 3H, branch-CH_3), 3.10 (m, 1H, H-4), 3.85 (dd, J = 8.8, 3.2 Hz, 1H, H-6e), 3.88 (s, 3H, OCH_3), 4.23 (dd, J = 8.8, 6.8 Hz, 1H, H-6a), 4.72 (d, J = 3.6 Hz, 1H, H-2), 6.82 (dd, J = 6.8, 1.6 Hz, 1H, H-4′), 6.88 (d, J = 6.8 Hz, 1H, H-3′), 6.89 (d, J = 1.6 Hz, 1H, H-5′), 5.40 ~ 5.70 (br, 2H, N-H).

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