COMMUNICATION

Structures of Us-7 and Us-8: a new type of oxindole alkaloids isolated from *Uncaria attenuata* Korth

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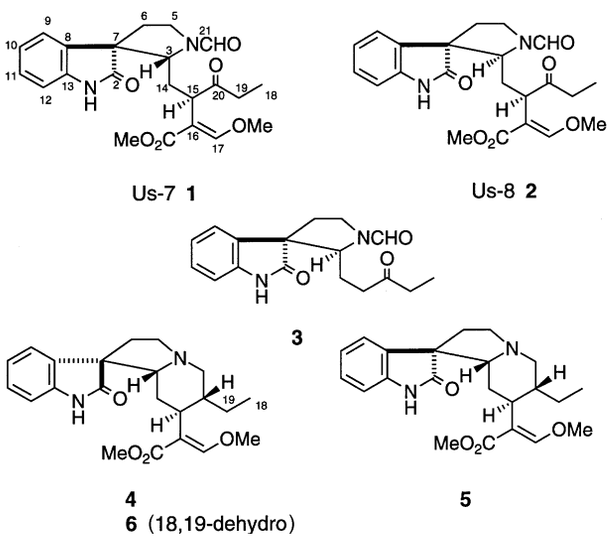
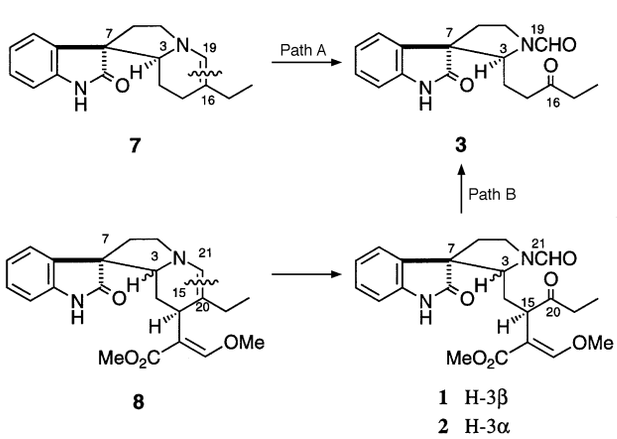
Novel D-seco corynanthé-type oxindole alkaloids Us-7 1 and Us-8 2 have been isolated from *Uncaria attenuata* Korth. Their structures have been deduced by detailed analysis of their spectroscopical and chiroptical properties.

Uncaria attenuata Korth (*U. salaccensis* Bakh. f. nom prouis) is known to contain corynanthé- and heteroyohimbine-type indole and oxindole alkaloids.[1,2] In 1990, we reported the isolation and synthesis of salacin **3**, a new type of oxindole alkaloid.[3] devoid of a β-methoxyacrylic ester moiety, has already been reported.[4] The existence of **3** led us to expect similar D-seco alkaloids derived from corynanthé-type alkaloids, which are major metabolites in this plant. We report here the first isolation and structure elucidation of the anticipated alkaloids from the stem bark and hooks of the same plant. We now believe **3** is formed from these newly characterised molecules (**1** and **2**) *via* loss of a β-methoxyacrylate moiety (Scheme 1, path B).



When considering how **3** might be formed in the plant, we can postulate the existence of a precursor molecule **7** which undergoes an oxidative cleavage at the enamine double bond (Scheme 1, path A). In fact this class of tetracyclic alkaloid,



Scheme 1

Results and discussion

A methanol extract of this plant was submitted to a DIAION HP-20 column. The fractions were eluted with H_2O–MeOH and were further purified with silica gel columns (open column, flash column and HPLC). Two new alkaloids **1** and **2** were isolated along with three known oxindole alkaloids, rhynchophylline **4**, isorhynchophylline **5** and corynoxine **6**.

The molecular formula of Us-7 **1** was proved to be $C_{22}H_{26}N_2O_6$ by the molecular ion peak at *m/z* 414.1788 in the high resolution mass spectrum. The UV spectrum of **1** indicated an oxindole chromophore and a β-alkoxyacrylic ester moiety in the molecule. Three peaks in the ^{1}H NMR spectrum at δ 3.62 (3 H, s, CO_2Me), 3.78 (3 H, s, OMe) and 8.20 (1 H, s, H-17) were characteristic of a β-methoxyacrylate methyl ester moiety. The ^{13}C NMR spectrum indicated an aliphatic ketone (δ 208.4). The FG-HMQC spectrum demonstrated the location of the ketone carbonyl group at C-20 by the observed coupling between H-19 (δ 1.86, 2.62) and C-20 (δ 208.4) and between H-18 (δ 0.84) and C-20 (δ 208.4). A two-bond C, H-coupling between the oxindole carbonyl carbon at δ 167.3 (C-2) and the N(a)-H at δ 8.20 (1 H, s), and one-bond coupling between the N(b)-formyl carbon at δ 161.3 (C-21) and the formyl proton at δ 8.37 (1 H, s) indicated the presence of amide (oxindole) and *N*-formate moieties. The FG-HMBC spectrum of **1** showed that C-21 (δ 161.3) makes a three-bond coupling with H-3 (δ 4.02). Similarly, C-7 (δ 56.3) correlated to the protons at δ 2.62 (H-14), 3.81 (H-5) and 7.00 (H-9). These findings demonstrated the N(b)-formylated spiro oxindole part structure shown in Fig. 1.

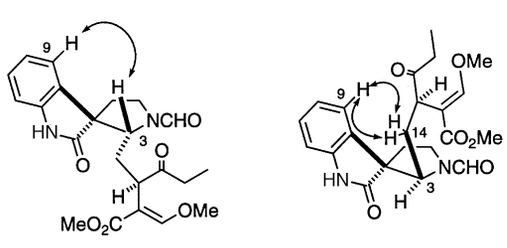


Fig. 1

The UV spectrum of Us-8 **2** is superimposable on that of **1** and the high resolution mass spectrum demonstrated the same molecular formula, $C_{22}H_{26}N_2O_6$, as **1**. The NMR spectra showed the presence of an *N*-formyl group [δ 161.8 (C-21) and 8.32 (H-21)] and a ketone carbonyl group [δ 208.3 (C-20)] besides the other fundamental structural units *i.e.* the oxindole and β-methoxyacrylic ester moieties. These facts indicated that **1** and **2** were stereochemical isomers, and ^{1}H,^{1}H COSY, FG-HMQC and FG-HMBC spectra of **2** showed the expected pattern of correlation to support the identical plane structures of **1** and **2**.

The C-3 stereochemistries of **1** and **2** were elucidated *via* NOESY spectroscopy. The NOESY spectrum of **1** showed a cross-peak between H-3 (δ 4.02) and H-9 (δ 7.00). In the case of **2**, no NOE was observed between H-3 (δ 3.89) and H-9 (δ 7.03), and instead a marked NOE was observed between H-9 (δ 7.03) and H-14 (δ 1.65, 2.42). These findings demonstrate that the relative configurations at C-3 and C-7 are 3*R**,7*S** for **1** and 3*S**,7*S** for **2** respectively.

The absolute configuration at C-7 was clarified by the well-established circular dichroism (CD) criteria of natural oxindole alkaloids.[5] The CD spectrum of **1** showed a negative Cotton effect at the longest wave length absorption band (280 nm), while that of **2** showed a band of the same sign at 285 nm. This observation indicated that the spiro carbon (C-7) of both molecules has *S* configuration. The stereochemistry at C-15 is most likely to be *R* on the basis of previous findings. An attempt to determine the stereochemistry at C-15 is now under way.

Experimental

Isolation of US-7 1 and US-8 2

The bark and hooks (800 g) was extracted with MeOH (10.6 l) at room temperature. The MeOH extract were concentrated under reduced pressure and the residue (23.4 g) was subjected to chromatography on a DIAION HP-20 column. The column was eluted with H_2O–MeOH (4:1 to 1:4). This chromatographic purification gave 6 fractions and fraction 3 (6.0 g), eluted with H_2O–MeOH (3:2 to 1:4), was submitted to normal phase HPLC (Kusano Pack Si-5) [$CHCl_3$–MeOH (99:1)] to afford pure **1** (15.0 mg) and **2** (40.5 mg).

Us-7 1. Pale yellow oil; $[\alpha]_D^{25}$ −12.9 (*c* 1.10, MeOH); λ_{max}(MeOH)/nm 208, 244; EI-MS *m*/*z* 414 (M^+, 8%) (HRMS: found M^+, 414.1788. Calc. for $C_{22}H_{26}N_2O_6$, 414.1788); δ_H(600 MHz; $CDCl_3$) 0.84 (3 H, t, *J* 7.3,† H-18), 1.86 (1 H, dq, *J* 17.4 and 7.3, H-19), 1.94 (1 H, dt, *J* 12.9 and 8.61, H-14), 2.14 (1 H, ddd, *J* 12.9, 8.3 and 8.3, H-6), 2.19 (1 H, dq, *J* 17.4 and 7.3, H-19), 2.43 (1 H, ddd, *J* 12.9, 8.0 and 4.3, H-6), 2.62 (1 H, dt, *J* 14.4 and 7.3, H-14), 3.26 (1 H, dd, *J* 7.3 and 6.5, H-15), 3.62 (3 H, s, H-24), 3.78 (3 H, s, H-23), 3.81 (1 H, m, H-5), 3.98 (1 H, ddd, *J* 12.3, 8.2 and 3.6, H-5), 4.02 (1 H, dd, *J* 6.9 and 6.9, H-3), 6.93 (1 H, br d, *J* 7.2, H-12), 7.00 (1 H, dd, *J* 7.5 and 1.5, H-9), 7.01 (1 H, dt, *J* 1.5 and 7.5, H-11), 7.24 (1 H, dt, *J* 7.2 and 1.2, H-10), 7.26 (1 H, s, H-17), 8.20 (1 H, s, H-1) and 8.37 (1 H, s, H-21); δ_C(150 MHz; $CDCl_3$) 7.9 (C-18), 30.0 (C-14), 32.9 (C-18), 34.3 (C-6), 42.2 (C-5), 44.7 (C-15), 51.6 (C-24), 56.3 (C-7), 62.0 (C-23), 62.6 (C-3), 108.6 (C-16), 110.0 (C-12), 122.7 (C-9), 122.8 (C-11), 128.6 (C-10), 131.5 (C-13), 140.2 (C-8), 160.9 (C-17), 161.3 (C-21), 167.3 (C-2), 177.9 (C-22) and 208.4 (C-20).

Us-8 2. Yellow oil; $[\alpha]_D^{25}$ −9.6 (*c* 1.25, MeOH); λ_{max}(MeOH)/nm 208, 244; EI-MS *m*/*z* 414 (M^+, 8%) (HRMS: found M^+, 414.1754. Calc. for $C_{22}H_{26}N_2O_6$, 414.1788); δ_H(600 MHz; $CDCl_3$) 0.96 (3 H, t, *J* 7.3, H-18), 1.65 (1 H, ddd, *J* 13.2, 8.3 and 4.9, H-14), 2.17 (1 H, ddd, *J* 12.7, 8.3 and 4.2, H-6), 2.24 (1 H, dq, *J* 14.4 and 7.3, H-19), 2.31 (1 H, dq, *J* 14.4 and 7.3, H-19), 2.35 (1 H, m, H-6), 2.42 (1 H, m, H-14), 3.47 (1 H, dd, *J* 8.3 and 6.1, H-15), 3.58 (3 H, s, H-24), 3.66 (1 H, m, H-5), 3.78 (3 H, s, H-23), 3.89 (1 H, dd, *J* 8.3 and 4.9, H-3), 3.99 (1 H, ddd, *J* 12.9, 9.5 and 3.7, H-5), 6.92 (1 H, d, *J* 7.3, H-12), 7.03 (1 H, d, *J* 7.3, H-9), 7.14 (1 H, dt, *J* 1.0 and 7.6, H-11), 7.24 (1 H, dt, *J* 1.2 and 7.8, H-10), 7.37 (1 H, s, H-17), 8.24 (1 H, s, H-1), 8.32 (1 H, s, H-21); δ_C(150 MHz; $CDCl_3$) 8.0 (C-18), 29.3 (C-14), 33.0 (C-6), 33.8 (C-19), 42.2 (C-5), 44.8 (C-15), 51.5 (C-24), 56.3 (C-7), 61.6 (C-3), 62.1 (C-23), 107.8 (C-16), 110.1 (C-12), 122.7 (C-9), 124.9 (C-11), 128.6 (C-10), 128.6 (C-13), 140.6 (C-8), 161.4 (C-17), 161.8 (C-21), 167.1 (C-2), 179.0 (C-22) and 208.3 (C-20).

† *J* Values are given in Hz.

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