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Vitexlactam A, a novel labdane diterpene lactam from the fruits of *Vitex agnus-castus*

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Abstract—A novel labdane diterpene alkaloid, vitexlactam A (**1**) was isolated as a prism from the *n*-hexane extract of the fruits of *Vitex agnus-castus* through normal and reverse phase column chromatography. Its structure was elucidated to be 6β-acetoxy-9α-hydroxy-13(14)-labden-16,15-amide, based on chemical and spectral evidences including 1D and 2D NMR spectra. The structure was confirmed by X-ray crystallographic analysis. Compound **1** is the first naturally occurring labdane diterpenoid containing an α,β-unsaturated γ-lactam moiety. © 2002 Elsevier Science Ltd. All rights reserved.

Vitex, a genus of the Verbenaceae family, contains about 250 species worldwide,[1] many of which have been used as traditional medicinal plants. One of them, *Vitex agnus-castus* Linn., commonly known as the chaste tree, grows to a height of 2–3 m, and is distributed in the Mediterranean region, Central Asia and Southern Europe.[2] It is cultivated in Jiangsu Province and Shanghai City of China. The fruit of *V. agnus-castus* is a popular phytomedicine in Europe for the treatment of the hormone imbalance syndromes in women.[3,4] In a search for the active principles in the fruits of *V. agnus-castus*, a novel labdane-type diterpene containing an α,β-unsaturated-γ-lactam, named vitexlactam A (**1**), was obtained for the first time from nature. The current paper presents the isolation and structure elucidation of this compound.

Dried fruits of *V. agnus-castus* (4077 g) purchased from Frontier Botanicals, Norway, Iowa, USA (Lot. No. 799. 0116), were milled and macerated in *n*-hexane (3×8 L) for 28 h. The extract was filtered and concentrated in vacuo to dryness to afford 200 g of a residue, which was absorbed on 200 g of silica gel and chromatographed on a prepacked (500 g) silica gel column, eluting stepwise with *n*-hexane, $CHCl_3$, $CHCl_3/Me_2CO$ (1:1) and Me_2CO. The $CHCl_3/Me_2CO$ (1:1) eluate was filtered (40 g) and subjected to further chromatography separation over Chromatorex ODS (using $MeOH/H_2O$ as eluents) and silical gel (using *n*-hexane/EtOAc as eluents) columns to yield 40 mg of compound **1**.

Vitexlactam A (**1**) showed a weak molecular ion peak at m/z 377 $[M]^+$ (3%) but a strong ion fragment at m/z 317 $[M–AcOH]^+$ (87% relative intensity) under EI MS (70 eV).[5] The odd numbered molecular weight suggested a nitrogen atom in the molecular formula of **1**. That **1** was an alkaloid or a nitrogen containing compound was supported by a positive reaction to the Dragendorff reagent.[6] The molecular formula of **1** was unequivocally established to be $C_{22}H_{35}NO_4$ by HREI MS[5] (found: m/z 377.2569, calcd 377.2566).

Keywords: *Vitex agnus-castus*; Verbenaceae; labdane diterpene; lactam; vitexlactam A; NMR; X-ray.

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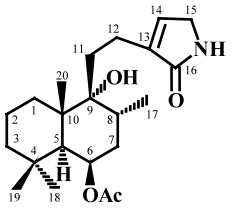

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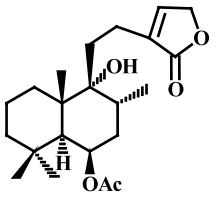

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The IR spectrum displayed diagnostic absorption bands of hydroxyl (3463 cm^{-1}), acetoxy (1731 cm^{-1}), and α,β-unsaturated γ-lactam (1684 cm^{-1}) groups.[5] In the ^1H NMR spectrum of **1** (Table 1), a secondary methyl group at δ_H 0.89 (3H, d, $J=6.7$ Hz) and three characteristic tertiary methyl groups at δ_H 0.94, 0.97 and 1.19 besides an acetyl methyl signal at δ_H 2.03 (3H, s) were observed. The ^{13}C NMR and DEPT spectra (Table 1) further showed that **1** has four methyls, seven methylenes, two methines, an oxy-methine, an olefinic methine, two quaternary carbons, an oxygenated quaternary carbon, an olefinic quaternary carbon and a carbonyl carbon. Based on these spectral data, **1** was determined to be a labdane diterpene. According to the literature, a known labdane diterpenoid, (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*)-6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**2**), isolated from the fruit of *V. rotundifolia* by Ono et al.,[7] was found to possess very similar NMR spectral features to **1**. Compound **1**, however, has one less atomic mass unit (amu) than **2**, and its NMR data were almost identical to except for the signals on the C-9 side chain. Major differences between the two compounds centered on H_2-15 and C-15, of which dramatic upfield shifts were observed from $\delta_{H/C}$ 4.79 (2H, ddd, $J=1.5$, 1.5, 1.5 Hz)/70.3 (t) in **2** to 3.91 (2H, br s)/46.6 (t) in **1**. These data, along with the above-described chemical and IR evidences, suggested the presence of an α, β-unsaturated γ-lactam moiety for **1** instead of the α,β-unsaturated γ-lactone found in **2**. This deduction was further supported by the analysis of 2D NMR data including ^1H–^1H COSY, HMQC and HMBC spectra (Table 1).

Table 1. NMR data and HMBC correlations of vitexlactam A (**1**) ($CDCl_3$, δ in ppm and J in Hz)

Position	δ_C[a]	δ_H[a]	HMBC (^1H–^{13}C)[b]
1a	33.7 t	1.52 (1H, m)	C-2
1b		1.40 (1H, m)	C-3, 5, 10
2a	18.8 t	1.55 (1H, m)	
2b		1.48 (1H, m)	C-3
3a	43.8 t	1.28 (1H, d, $J=13.2$ Hz)	C-4, 5, 18
3b		1.16 (1H, t, $J=13.2$ Hz)	
4	33.9 s		
5	47.5 d	1.72 (1H, d, $J=2.1$ Hz)	C-3, 4, 18, 19, 20
6	70.6 d	5.37 (1H, br d, $J=2.3$ Hz)	C-8, 10, OAc-C=O
7a	36.3 t	1.68 (1H, m)	C-8
7b		1.50 (1H, m)	
8	32.1 d	2.04 (1H, m)	C-17
9	76.4 s		
10	44.0 s		
11	32.3 t	1.78 (2H, t, $J=7.4$ Hz)	C-8, 9, 10, 12, 13
12	21.7 t	2.44 (2H, m)	C-9, 11, 13, 14, 16
13	140.6 s		
14	137.1 d	6.71 (1H, br s)	C-12, 13, 15, 16
15	46.6 t	3.91 (2H, br s)	
16	175.3 s		
17	16.4 q	0.89 (3H, d, $J=6.7$ Hz)	C-7, 8, 9
18	33.6 q	0.94 (3H, s)	C-3, 4, 5, 19
19	23.7 q	0.97 (3H, s)	C-3, 4, 5, 18
20	18.9 q	1.19 (3H, s)	C-1, 5, 9, 10
NH		7.68 (1H, br s)	
OAc	21.9 q 170.5 s	2.03 (3H, s)	OAc-C=O

[a] Data recorded on a Bruker AM-400 MHz spectrometer with reference to the solvent signals (δ_H 7.24 ppm/δ_C 77.0 ppm).
[b] Data recorded on a Bruker DRX-500 MHz spectrometer.

A relative stereochemistry of **1** was determined by a 2D ROESY (Fig. 1) experiment. The C-6 acetoxy group was assigned to be in the β-orientation due to the presence of the ROESY correlation between H-6 and Me-18, as well as a coupling constant between H-6 and H-5 of 2.2 Hz. The fact that both H-8 and H_2-11 correlated to Me-20 indicated that the Me-17 and the C-9 hydroxyl groups were positioned in the same direction (α-oriented). The chiral centers in **1** were thus proved to be identical with those found in **2**. Accordingly, **1** was elucidated to be 6β-acetoxy-9α-hydroxy-13(14)-labden-16,15-amide, and was given the trivial name of vitexlactam A. The proposed structure of **1** was further confirmed by a single-crystal X-ray analysis (Fig. 2).[8]

According to Aphaijitt et al.,[9] diterpenoids are not widely distributed among *Vitex* species. However, recent studies by Ono et al.[10–12] on *V. rotundifolia* and by Li et al.[13] have resulted in more than 35 diterpenoids having been isolated from two *Vitex* species. The isolates included 29 labdane- and 6 abietane-type diter-

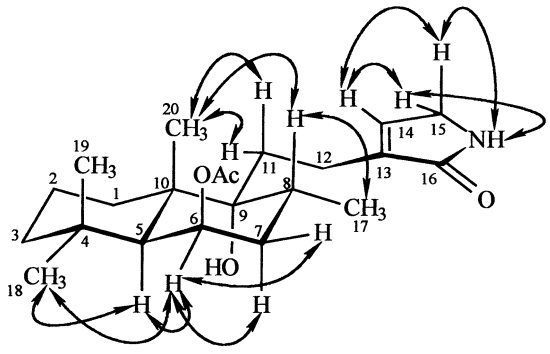

Figure 1. Selected 2D NOESY correlations of vitexlactam A (**1**).

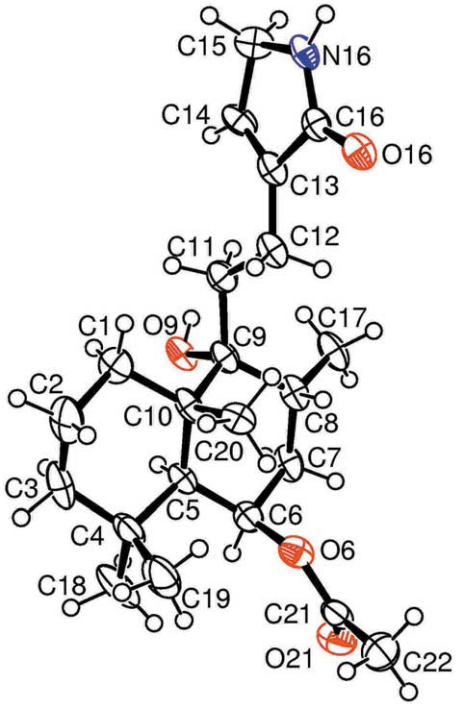

Figure 2. ORTEP drawing of one molecule of vitexlactam A (**1**).

penoids.[10–13] Compound **1** is the first naturally occurring labdane diterpenoid with an α,β-unsaturated γ-lactam moiety, and is structurally unique among the diterpenes previously found in *Vitex* species. Biogenetically, **1** may be regarded as an amination product of **2**. The natural occurrence of **1** is further supported by its extraction and isolation procedures, which were under mild conditions without the use of any nitrogen-containing solvents and chromatographic materials. Recent investigations have shown that some labdane-type diterpenoids possessed dopamine-D_2-receptor affinity and may possibly be partially responsible for the biological activities of *V. agnus-castus*.[14–16] As part of the UIC/NIH Center for Botanical Dietary Supplements Research Project, the biological activity profile of **1** is under investigation.

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5. Vitexlactam A (**1**): colorless crystals, mp 207°C, $C_{22}H_{35}NO_4$; $[\alpha]_D^{23.7}$ −10.71 (*c* 0.42, $CHCl_3$); UV ($CHCl_3$) λ_{max}: no absorption; IR (KBr) ν_{max}: 3463, 3188, 3054, 2942, 2923, 1731, 1684, 1460, 1442, 1381, 1361, 1250, 1226, 1142, 1087, 1039, 1020, 973, 962, 952, 913, 832, 781, 749, 645, 610, 598 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EI MS *m*/*z* 377 $[M]^+$ (3), 317 (87), 302 (5), 284 (17), 206 (13), 192 (15), 180 (72), 167 (100), 149 (18), 138 (74), 123 (22), 110 (70), 96 (56), 82 (40), 69 (50), 55 (67); HREI MS *m*/*z* found 377.2569 $[M]^+$, calcd 377.2566.
6. Preparation of Dragendorff reagent: (a) 0.85 g basic bismuth nitrate dissolved in the pre-mixed solvents (10 ml glacial acetic acid+40 ml distilled water) to afford solution I; (b) 2.0 g potassium iodide dissolved in 5 ml distilled water to afford solution II; (c) 5 ml of solution I was added to 5 ml of solution II and fully mixed to yield solution III; (d) 10 ml of solution III was added to 20 ml glacial acetic acid and 100 ml distilled water, which was subjected to TLC detection directly.
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8. X-Ray crystal structure of vitexlactam A (**1**): vitexlactam A (**1**) was crystallized from MeOH as colorless transparent plates. A colorless crystal, measuring 0.05×0.35×0.38 mm, was selected for data collection. The structure is triclinic, space group $P\bar{1}$, and two independent molecules in the asymmetric unit. The unit cell parameters are a=7.4942(6) Å, b=7.5210(6) Å, c=20.0151(19) Å, α=79.954(4)°, β=85.188(3)°, γ=68.463(5)°. The volume of the unit cell is V=1033.03(15) $Å^3$ with a calculated density of 1.214 mg/m^3. The diffraction data were collected on an Enraf–Nonius Kappa CCD area detector equipped with a rotating anode and Mo Kα radiation. An Ewald sphere of diffraction intensities were collected and averaged, resulting in 5007 observations. The integration of intensities and refinement of unit cell parameters and orientation matrix were carried out with HKL Denzo-SMN (Ref.: Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *A276*, 307–326). The WinGX package

(Ref.: Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837–838) was used for completing the structure determination. Direct methods, SIR-92 (Ref.: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343–350), were used to locate the non-hydrogen atoms. Repeated cycling with least-squares refinement on F^2 with SHELX-97 (Ref.: Sheldrick, G. SHELXTL, version 5.1, Bruker Analytical X-ray Systems: Madison, WI) and difference Fourier maps were used to identify hydrogen atom positions. All non-hydrogen atoms were refined with anisotropic Gaussian displacement parameters, and hydrogen atoms were restrained to ride on the adjacent non-hydrogen atoms. The ORTEP (Ref.: Johnson, C. K. ORTEP-I, ORNL-3794, Oak Ridge National Laboratory: Oak Ridge, TB, 1965; Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565) diagrams were drawn with 50% probability ellipsoids. The intensities are very weak, and the two independent molecules were restrained to be chemically similar with the SAME option; the anisotropic Gaussian displacement parameters were also restrained with the DELU and SIMU options in refinement. All methyl group hydrogen atoms were found to be disordered. Final agreement: R (obs, $I>2\sigma I$) = 0.097, R(all) = 0.103, goodness-of-fit = 1.05. Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC. Copies of the data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44(0)223 336033, e-mail: deposit@ccdc.cam.ac.uk).

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