Compounds from the Fruits of the Popular European Medicinal Plant *Vitex agnus-castus* in Chemoprevention via NADP(H):Quinone Oxidoreductase Type 1 Induction

Shenghong Li, Shengxiang Qiu, Ping Yao, Handong Sun, Harry H. S. Fong, and Hongjie Zhang

1 State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, Yunnan 650204, China
2 South China Botanical Garden, Chinese Academy of Sciences, 723 Xingke Road, Tianhe District, Guangzhou 510650, China
3 Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay Road, Hong Kong
4 Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612, USA
5 School of Chinese Medicine, Hong Kong Baptist University, 7 Baptist University Road, Kowloon Tong, Hong Kong

Correspondence should be addressed to Hongjie Zhang; zhanghj@hkbu.edu.hk

Received 30 January 2013; Accepted 5 March 2013

Academic Editor: Ludger Beerhues

Copyright © 2013 Shenghong Li et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

As part of our continuing efforts in the search for potential biologically active compounds from medicinal plants, we have isolated 18 compounds including two novel nitrogen containing diterpenes from extracts of the fruits of *Vitex agnus-castus*. These isolates, along with our previously obtained novel compound vitexlactam A (1), were evaluated for potential biological effects, including cancer chemoprevention. Chemically, the nitrogenous isolates were found to be two labdane diterpene alkaloids, each containing an \( \alpha, \beta \)-unsaturated \( \gamma \)-lactam moiety. Structurally, they were elucidated to be 9\( \alpha \)-hydroxy-13(14)-labden-16,15-amide (2) and 6\( \beta \)-acetoxy-9\( \alpha \)-hydroxy-13(14)-labden-15,16-amide (3), which were named vitexlactams B and C, respectively. The 15 known isolates were identified as vitexilactone (4), rotundifuran (5), 8-epi-manoyl oxide (6), vitetinol D (7), spathulenol (8), *cis*-dihydro-dehydro-diconiferylalcohol-9-O-\( \beta \)-D-glucoside (9), luteolin-7-O-glucoside (10), 5-hydroxy-3,6,7,4\( ^{1} \) -tetramethoxyflavone (11), casticin (12), artemetin (13), aucubin (14), agnuside (15), \( \beta \)-sitosterol (16), p-hydroxybenzoic acid (17), and p-hydroxybenzoic acid glucoside ester (18). All compound structures were determined/identified on the basis of 1D and/or 2D NMR and mass spectrometry techniques. Compounds 6, 8, 9, and 18 were reported from a *Vitex* species for the first time. The cancer chemopreventive potentials of these isolates were evaluated for NADP(H):quinone oxidoreductase type 1 (QRI) induction activity. Compound 7 demonstrated promising QRI induction effect, while the new compound vitexlactam (3) was only slightly active.

1. Introduction

Botanicals are widely used as either dietary supplements or herbal medicines throughout the world for the prevention and mitigation against various diseases or ailments. Among these botanicals are plants of the genus *Vitex* plants. Botanically, this genus was previously placed in the family of Verbenaceae but was recently revised as belonging to the family Lamiaceae, which itself was formerly known as the Labiatae. *Vitex* consists of about 250 species distributed worldwide, but mainly in the tropical and temperate zones [1]. A number of species (e.g., *V. agnus-castus*, *V. trifolia*, *V. negundo*, and *V. rotundifolia*) have been used as traditional medicinal plants. To date, more than 20 *Vitex* species have been investigated for chemical and biological properties, with approximately 200 compounds, mainly flavonoids, terpenoids, steroids, iridoids, and lignans, having been isolated and characterized [2].

*Vitex agnus-castus* Linn., is 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 [3]. It is also cultivated in the various regions including the United States [4]. The fruits of *V. agnus-castus* are popularly used as a phytomedicine in Europe for the treatment of female hormonal disorders [5–7]. The fruit extract is also used as an alternative phytotherapeutic agent in the treatment of mastalgia [8]. There has been extensive research conducted on this phytomedicine leading to a large library of published literature on the pharmacognosy, traditional uses, chemical constituents, biology/pharmacology, and clinical studies [9]. In a previous communication we reported the isolation, structure determination, and X-ray crystallographic analysis of a novel labdane diterpene lactam from the *n*-hexane extracts of the fruits of this plant [10]. Further phytochemical studies of both of the *n*-hexane and methanol extracts resulted in the isolation of two additional new labdane diterpene lactams (2–3) and fifteen known compounds (4–18). In this paper, we describe the isolation and structure characterization of the two new metabolites and the identification of the 15 known compounds, as well as evaluating their NADP(H):quinone oxidoreductase type I (QRI) induction activity potentials.

2. Materials and Methods

2.1. General Experimental Procedures. All melting points were measured on an XRC-1 micromelting point apparatus and are uncorrected. 1D (one-dimensional) and 2D (two-dimensional) NMR (nuclear magnetic resonance) experiments were performed either on a Bruker AM-400 or a Bruker DRX-500 spectrometer. Unless otherwise is specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. FABMS (fast atom bombardment mass spectrometry) and HRFABMS (high resolution fast atom bombardment mass spectrometry) were obtained spectrometry) and HRFABMS (high resolution fast atom bombardment mass spectrometry) were taken on a VG Auto Spec-3000 or a Finnigan MAT 90 instrument. IR (infrared) spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV (ultraviolet) spectral data were obtained on a U 210A spectrometer. Optical rotations were carried out on a HORIBA SEPA-300 High Sensitive Polarimeter or a Perkin-Elmer model 241 Polarimeter. Column chromatography was performed either on Si gel (silica gel) (200–300 mesh, Qingdao Marine Chemical Inc., China), Si gel H (10–40 μ, Qingdao Marine Chemical Inc., China), Diaion HP-20 (Shandong Lukang Pharmaceutical Co., Ltd., China), Chromatorex ODS (Fuji Silysia Chemical Corporation, Ltd., Japan), or Lichroprep RP_{18} gel (40–63 μm, Merck, Darmstadt, Germany). Fractions were monitored by silica gel TLC (thin layer chromatography) (CHCl_{3}-Me_{2}CO (chloroform-acetone) 9:1, 8:2, 7:3), and spots were visualized by heating silica gel plates sprayed with 10% H_{2}SO_{4} in EtOH (ethanol).

2.2. Plant Material. The fruits of *V. agnus-castus* were purchased from Frontier Botanicals, Norway, IA, USA (Lot No. 799, 0116).

2.3. Extraction and Isolation. Dried fruits of *V. agnus-castus* (4077 g) were milled and sequentially extracted with *n*-hexane (3 × 8 L) for 28 h and MeOH (methanol) (4 × 9 L) for 24 h. The *n*-hexane extract was filtered and concentrated in vacuo to dryness to afford 200 g of a residue (part I). The MeOH extract was filtered, concentrated, and diluted with water (2 L), followed by partitioning with EtOAc (ethyl acetate) (4 × 3 L). The organic layer was evaporated in vacuo to dryness to give 60 g of a residue (part II). The water-soluble fraction was chromatographed on a column of Diaion HP-20 eluting with aqueous MeOH (30% → 80% → 100%). The 80% MeOH-H_{2}O fraction was concentrated in vacuo to yield 48 g of a dry residue (part III).

2.3.1. Isolation. Part I (200 g) was absorbed on 200 g of silica gel and chromatographed on a packed (500 g) silica gel column, eluting stepwise with *n*-hexane, CHCl_{3}, CHCl_{3}-Me_{2}CO/1:1, and Me_{2}CO. Compound 16 (27 mg) was crystallized from the CHCl_{3} fraction and compound 11 (336 mg) was crystallized from the CHCl_{3}-Me_{2}CO/1:1 fraction. The remaining CHCl_{3}-Me_{2}CO/1:1 eluate was filtered (40 g, net weight) and subjected to further chromatographic separation over a Chromatorex ODS column (eluent: 80% MeOH-H_{2}O as eluents) and silica gel columns (using *n*-hexane-CHCl_{3}/1:2, *n*-hexane-EtOAc/3:2, and *n*-hexane-Me_{2}CO/2:1 as eluents) to provide compounds 1 (40 mg), 2 (4 mg), 3 (11 mg), 4 (25 mg), 5 (67 mg), 6 (6 mg), 7 (14 mg), 8 (14 mg), and 13 (9 mg).

Part II (60 g) was absorbed on 100 g of silica gel and chromatographed on a packed (300 g) silica gel column, eluting stepwise with CHCl_{3}-Me_{2}CO (1:0, 9:1, 8:2, 7:3, 0:1). Compound 12 (1.635 g) was crystallized from the CHCl_{3}-Me_{2}CO/1:0–1:9 fraction. Part of the CHCl_{3}-Me_{2}CO/8:2 fraction (0.810 g) was further chromatographed on RP_{18} gel (100 g) with 40% aqueous MeOH as eluents to give compound 17 (125 mg).

Part III (48 g) was again chromatographed on a Chromatorex ODS column eluting with aqueous MeOH (30%) and over a silica gel column eluting with CHCl_{3}-Me_{2}CO (3:1), CHCl_{3}-Me_{2}CO/H_{2}O (4:1:0.1), and EtOAc-MeOH (12:1) to yield compounds 9 (108 mg), 10 (23 mg), 14 (55 mg), 15 (60 mg), and 18 (15 mg).

2.4. Structural Characterization of Novel Isolates

2.4.1. Vitexlactam B (2). White crystals, m.p. 162°C, C_{20}H_{33}NO_{2}; [α]_{D}^{23} +18.75° (c 0.2, CHCl_{3}); IR (KBr) δ_{max}: 3473, 3187, 3055, 2924, 2682, 1684, 1648, 1442, 1379, 1296, 1254, 1228, 1140, 1085, 1057, 1041, 1018, 972, 962, 943, 909, 832, 791, 777, 698 cm\(^{-1}\). \(^{1}\)H NMR (500 MHz, CDCl_{3}) δ_{1.50} (1H, dd, J = 11.0, 2.0 Hz, H-5), 1.75 (1H, m, H-8), 1.78 (1H, m, H-11a), 1.67 (1H, m, H-11b), 2.36 (2H, br t, J = 8.2 Hz, H-12), 6.69 (1H, br s, H-14), 3.89 (2H, br s, H-3), 0.88 (3H, d, J = 6.6 Hz, H_{3}-17), 0.85 (3H, s, H_{3}-18), 0.80 (3H, s, H_{3}-19), 0.90 (3H, s, H_{3}-20), 6.61 (1H, br s, NH); \(^{13}\)C NMR data, see Table I; EIMS (electron impact mass spectrum) m/z 319 [M]+ (81), 304 (7), 286 (8), 206 (7), 194 (19), 180 (100), 167 (75), 152 (11), 138 (47), 123 (17), 110 (81), 96 (86), 82 (58), 69 (72), 55 (97); HREIMS m/z found 319.2509 [M]+, calcld. (calculated) 319.2511.
Table 1: $^{13}$C NMR data of compounds 1–7 (CDCl$_3$, δ in ppm).

<table>
<thead>
<tr>
<th>Carbon</th>
<th>1$^a$</th>
<th>2$^b$</th>
<th>3$^a$</th>
<th>4$^a$</th>
<th>5$^a$</th>
<th>6$^a$</th>
<th>7$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>33.7t</td>
<td>32.5t</td>
<td>33.7t</td>
<td>33.8t</td>
<td>33.9t</td>
<td>36.5t</td>
<td>25.9t</td>
</tr>
<tr>
<td>C-2</td>
<td>18.8t</td>
<td>18.7t</td>
<td>18.6t</td>
<td>18.9t</td>
<td>18.7t</td>
<td>20.7t</td>
<td>19.4t</td>
</tr>
<tr>
<td>C-3</td>
<td>43.8t</td>
<td>41.7t</td>
<td>43.6t</td>
<td>43.9t</td>
<td>43.7t</td>
<td>42.3t</td>
<td>39.4t</td>
</tr>
<tr>
<td>C-4</td>
<td>33.9s</td>
<td>33.3s</td>
<td>34.0s</td>
<td>34.3s</td>
<td>34.8s</td>
<td>32.9s</td>
<td>34.6s</td>
</tr>
<tr>
<td>C-5</td>
<td>47.5d</td>
<td>46.2d</td>
<td>47.6d</td>
<td>48.0d</td>
<td>47.5d</td>
<td>46.3d</td>
<td>132.5s</td>
</tr>
<tr>
<td>C-6</td>
<td>70.6d</td>
<td>21.7t</td>
<td>69.9d</td>
<td>70.1d</td>
<td>70.3d</td>
<td>21.0t</td>
<td>66.2d</td>
</tr>
<tr>
<td>C-7</td>
<td>36.3t</td>
<td>31.4t</td>
<td>36.1t</td>
<td>36.4t</td>
<td>36.1t</td>
<td>37.9t</td>
<td>72.7d</td>
</tr>
<tr>
<td>C-8</td>
<td>76.4s</td>
<td>76.8s</td>
<td>76.7s</td>
<td>76.8s</td>
<td>76.8s</td>
<td>61.2d</td>
<td>42.9s</td>
</tr>
<tr>
<td>C-9</td>
<td>76.4s</td>
<td>76.8s</td>
<td>76.7s</td>
<td>76.8s</td>
<td>76.8s</td>
<td>61.2d</td>
<td>42.9s</td>
</tr>
<tr>
<td>C-10</td>
<td>44.0s</td>
<td>43.3s</td>
<td>43.8s</td>
<td>44.1s</td>
<td>43.7s</td>
<td>38.9s</td>
<td>141.5s</td>
</tr>
<tr>
<td>C-11</td>
<td>32.3t</td>
<td>32.0t</td>
<td>32.3t</td>
<td>31.9t</td>
<td>31.8t</td>
<td>18.6t</td>
<td>29.3t</td>
</tr>
<tr>
<td>C-12</td>
<td>21.7t</td>
<td>22.0t</td>
<td>26.5t</td>
<td>25.7t</td>
<td>25.7t</td>
<td>21.5t</td>
<td>38.6t</td>
</tr>
<tr>
<td>C-13</td>
<td>140.6s</td>
<td>140.8s</td>
<td>163.6s</td>
<td>171.3s</td>
<td>125.5s</td>
<td>73.6s</td>
<td>73.0s</td>
</tr>
<tr>
<td>C-14</td>
<td>137.1d</td>
<td>136.9d</td>
<td>121.2d</td>
<td>115.3d</td>
<td>110.8d</td>
<td>146.1d</td>
<td>144.5d</td>
</tr>
<tr>
<td>C-15</td>
<td>46.6t</td>
<td>46.4t</td>
<td>175.3s</td>
<td>171.3s</td>
<td>142.9d</td>
<td>111.1t</td>
<td>112.1t</td>
</tr>
<tr>
<td>C-16</td>
<td>175.3s</td>
<td>175.8s</td>
<td>50.5t</td>
<td>73.4t</td>
<td>124.9d</td>
<td>111.1t</td>
<td>112.1t</td>
</tr>
<tr>
<td>C-17</td>
<td>16.4q</td>
<td>16.6q</td>
<td>16.0q</td>
<td>16.3q</td>
<td>16.1q</td>
<td>21.3q</td>
<td>28.1q</td>
</tr>
<tr>
<td>C-18</td>
<td>33.6q</td>
<td>33.7q</td>
<td>33.6q</td>
<td>33.8q</td>
<td>33.6q</td>
<td>21.3q</td>
<td>28.1q</td>
</tr>
<tr>
<td>C-19</td>
<td>23.7q</td>
<td>22.1q</td>
<td>23.6q</td>
<td>23.9q</td>
<td>23.7q</td>
<td>21.3q</td>
<td>28.1q</td>
</tr>
<tr>
<td>C-20</td>
<td>18.9q</td>
<td>16.2q</td>
<td>19.0q</td>
<td>19.2q</td>
<td>19.0q</td>
<td>24.7q</td>
<td>28.0q</td>
</tr>
<tr>
<td>OAc</td>
<td>170.5s</td>
<td>170.3s</td>
<td>170.6s</td>
<td>170.7s</td>
<td>170.8s</td>
<td>21.9q</td>
<td>21.8q</td>
</tr>
<tr>
<td>(C=O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20.9q</td>
</tr>
</tbody>
</table>

$^a$Recorded at 100 MHz.

$^b$Recorded at 125 MHz.

2.4.2. Vitex lactam C (3). White crystals, m.p. 178°C, C$_{22}$H$_{35}$NO$_4$; [α]$^D$$_{20}$ = 12.73° (c 0.55, CHCl$_3$); IR (KBr) $\nu_{\text{max}}$: 3364, 3297, 2925, 2867, 1711, 1670, 1465, 1426, 1383, 1362, 1271, 1256, 1283, 1203, 1152, 1097, 1039, 1024, 977, 953, 916, 849, 819 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 1.31 (1H, br d, J = 13.2 Hz, H-3a), 1.13 (1H, dt, J = 2.7, 13.2 Hz, H-3b), 1.58 (1H, d, J = 2.0 Hz, H-5), 5.35 (1H, br d, J = 2.2 Hz, H-6), 2.10 (1H, m, H-8), 1.90 (1H, m, H-11a), 1.72 (1H, m, H-11b), 2.43 (2H, m, H$_3$-12), 5.82 (1H, br s, H-14), 3.91 (2H, br s, H$_2$-16), 0.87 (3H, d, J = 6.7 Hz, H$_3$-17), 0.93 (3H, s, H$_3$-18), 0.97 (3H, s, H$_3$-19), 1.22 (3H, s, H$_3$-20), 6.92 (1H, br s, NH), 2.03 (3H, s, 6-OAc); $^{13}$C NMR data, see Table I; EIMS m/z 377 [M]$^+$ (3), 317 (76), 302 (15), 284 (6), 260 (29), 242 (8), 222 (21), 202 (23), 187 (48), 167 (60), 150 (28), 133 (41), 119 (64), 110 (68), 96 (97), 83 (72), 69 (77), 55 (100); HREIMS m/z found 377.2547 [M]$^+$, calcd. 377.2566.

3. Results and Discussion

3.1. Plant Extracts and Isolation of Compounds. The purchased fruits of V. agnus-castus were milled and sequentially
extracted with n-hexane and methanol. The n-hexane extract was successively chromatographed on silica gel and Chromatorex ODS to afford compounds 1–8, 11, 13, and 16. The methanol extract was partitioned between EtOAc and water. The EtOAc layer was chromatographed on silica gel to give compounds 12 and 17. The water-soluble fraction was chromatographed on columns of Diaion HP-20, Chromatorex ODS, and silica gel to yield compounds 9, 10, 14, 15, and 18 (Scheme 1).

3.2. Structure Elucidation and Identification of Isolated Compounds

3.2.1. Vitexlactam B (2). Vitexlactam B (2) was obtained as white crystals. EI mass spectrum showed strong molecular ion peak at m/z 319 [M]⁺ (81% relative intensity), corresponding to a molecular formula of C₁₉H₂₁N₂O₃, which was confirmed by high resolution EI mass spectrum (found: m/z 319.2509, calcd. 319.2511). The existence of a nitrogen atom was supported by its odd numbered molecular weight and a positive reaction to the Dragneendorf reagent.

The ¹H and ¹³C NMR (Table 1) spectra of 2, being very similar to those of 1 [10], suggested that 2 is a closely related labdane diterpene alkaloid (Table 1), with a α, β-unsaturated γ-lactam moiety at the C-9 side chain. 2 differed from 1 only by the absence of the signals for an acetyl group and the replacement of an oxygen-bearing methine at δC 70.6 by a methylene signal at δC 21.7, indicating that 2 is the 6-deacetoxy derivative of 1. The result was further supported by the facts that 1 was 58 atomic mass units less than 2 and the lack of an acetoxy group being observed in the IR spectrum of 2. Full assignments of 2 using 2D NMR (including ¹H-¹H COSY (correlation spectroscopy), HMQC (heteronuclear multiple-quadrum correlation spectroscopy), HMBC (heteronuclear multiple bond correlation spectroscopy), and ROESY (rotating-frame Overhauser spectroscopy)) techniques established the structure of 2 to be the expected 9α-hydroxy-13(14)-labden-16,15-amide. Compound 2 was accordingly identified as the deacetoxy derivative of 1 and was given the trivial name of vitexlactam B.

3.2.2. Vitexlactam C (3). Vitexlactam C (3) was also isolated as white crystals. EI mass spectrum under 70 eV displayed a weak [M]⁺ ion peak at m/z 377 (3%) identical with that of 1 in both the mass charge ratio and the relative intensity [11]. In addition, a strong fragment ion peak at m/z 317 (76%) due to [M-AcOH]⁺ and a series of fragment ions similar to those for 1 were also observed. High resolution EI mass spectrum (found: m/z 377.2547, calcd. 377.2566) established that both compounds have the same molecular formula of C₂₂H₂₅NO₄. Therefore 3 was tentatively identified as an isomer of 1. Comparison of the ¹H and ¹³C NMR (Table 1) spectra of 3 with those of 1 (Table 1) indicated that the two compounds were equivalent not only in their skeletons but also in their oxygenation patterns. NMR spectral differences between these two compounds are mainly due to the α, β-unsaturated γ-lactam moieties in their C-9 side chains. The conjugate functionality occurred in 3 was deduced to be type (a) in contrast to type (b) in 1 (Figure 1). In the former conjugating system, C-13 is in a deshielded position while C-14 and H-14 are in a shielded position. On the contrary, in the latter (type (b)), C-13 is in a shielded position while C-14 and H-14 are in a deshielded position. Accordingly, C-13 of 3 moved downfield from δC 140.6 (s) in 1 to δC 163.6 (s), and C-14/H-14 of 3 shifted upfield from δC/H 137.1 (d)/6.71 (IH, br s) in 1 to δC/H 121.2 (d)/5.82 (IH, br s). 2D NMR analysis of 3 revealed that, unlike in 1, the ¹H-¹H COSY correlation between H-14 and the nitrogen-bearing methine at δH 3.91 (2H, br s) and the ¹H-¹³C interaction (Figure 2) between H₃-12 [δH 2.44 (2H, m)] and the lactam carbonyl carbon at δC 175.3 (s) disappeared while ¹H-¹³C interaction between H₃-12 and the nitrogen-occurring methylene at δC 50.5 (t) were observed, thus confirming the presence of a type (a) conjugate functionality in 3. Other structural correlations, including key NOEs (nuclear Overhauser effects) (Figure 3) in 3, were identical with those in 1.

![Figure 1: Electronic clouds movements of two different conjugated systems in compounds 3 (a) and 1 (b).](image-url)
Scheme 1
3.2.3. Identification of Known Compounds. Along with the new compounds, fifteen known compounds were also isolated in the course of the current study. Through comparison of their $^1$H and $^{13}$C NMR and MS data with those values reported in the literature, they were identified as three labdane-type diterpenoids, vitexlactone (4) [11]; rotundifuran (5) [11], and 8-epi-manoyloxide (6) [12] (α$^{19.5}$ – 11.8°; c = 0.55, CHCl$_3$); a rearranged labdane (halimane) diterpenoid, vitetrifolin D (7) [13]; an aromadendrene-type sesquiterpenoid, spathulenol (8) [14, 15]; a lignan glucoside, cis-dihydro-dehydro-diconiferylalcohol-9-O-$\beta$-D-glucoside (9) [16]; four flavonoids, luteolin-7-O-glucoside (10) [17], 5-hydroxy-3,6,7,4'$\prime$-tetramethoxyflavone (11) [18], casticin (12) [19], and artemetin (13) [20]; two iridoid glycosides, aucubin (14) [21] and augsidene (15) [22]; a sterol, $\beta$-sitosteryl (16) (comparison with an authentic sample); and two simple phenolics, p-hydroxybenzoic acid (17) [22] and p-hydroxybenzoic acid glucose ester (18) [22]. The occurrence of compounds 7–9 and 18 in the genus Vitex is being reported for the first time.

3.3. Activity Evaluation of the Isolated Compounds on QR1 Induction. These compounds have been evaluated for their potential chemopreventive activity by induction of the ubiquitous flavoenzyme NADP(H):quinone oxidoreductase type I (QR1) with cultured Hepa 1c1c7 cells. QR1 has been determined as an important phase II detoxification enzyme that can protect cells against the harmful effects caused by free radicals and reactive oxygen species by catalyzing the reduction of quinones to hydroquinones [23]. Hence, enhanced activity of the enzyme provides protection of cells from potential carcinogenicity. Vitetrifolin D (7) was shown to induce QR1 activity with a CD value of 23.2 $\mu$M. Although vitexlactam C (3) induced QR1 by 1.5 times that of the vehicle control at a concentration of 5.3 $\mu$M, it was toxic to Hepa 1c1c7 cells with 57% inhibition of the cells at 26.5 $\mu$M. None of the other compounds demonstrated QR1 induction activity.

4. Conclusion

The fruits of Vitex agnus-castus have been popularly used as a phytomedicine in Europe, especially Germany, for the treatment of premenstrual stress syndrome. However, the evaluation of this herb or its phytochemical constituents for cancer chemoprevention activity has not been reported. Thus, we undertook a study of the 18 compounds we isolated from the fruits of this plant in a bioassay, which have been used for assessing chemoprevention potentials. The isolates, including several novel nitrogen containing labdane diterpenes, were thus evaluated for their potentials in the induction of the phase II detoxification enzyme QR1. Results showed that only the labdane compounds 3 and 7 demonstrated QR1 induction effect. We have demonstrated that compounds possessing potential chemopreventive action do exist in V. agnus-castus and that further phytochemical and biological investigations of this plant material coupled with structure modification studies are needed in order to discover additional/modified labdanes possessing more potent QR1 induction activity and chemopreventive potential.

Conflict of Interests

The authors have no conflict of interests with the trademarks included in the paper.

Acknowledgments

The authors thank the members of the analytical group of the State Key Laboratory of Phytochemistry Laboratory and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sinica, for measurements of the NMR, MS, IR, UV, and ORD spectral data. This work was in part supported by the “Hundred Talents Program” of the Chinese Academy of Sciences (awarded to Dr. S. Li).

References


