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Studies on 1-*O*-acetylbritannilactone and its derivative, (2-*O*-butyloxime-3-phenyl)-propionyl-1-*O*-acetylbritannilactone ester

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Abstract—The crystal structures of 1-*O*-acetylbritannilactone **6** isolated from *Inula britannica,* and its derivative (2-*O*-butyloxime-3-phenyl) propionyl 1-*O*-acetylbritannilactone ester **7** are reported. The structure of synthesized derivative **7** was established by spectroscopic methods. Both compounds inhibited the growth of human HL-60, Bel-7402 cell lines and compound **7** had the stronger cytotoxic activity.

1. Introduction

Inula, from Compositae, has more than 100 species and is found mainly in Europe, Africa and Asia. *Inula britannica* is a wild plant found in Eastern Asia, including China, Korea and Japan. In traditional Chinese medicine, both *Inula britannica* and *Inula japonica* are called 'Xuanfuhua.' The flowers from these have been used for the treatment of digestive disorders, bronchitis, and inflammation. Its extracts have been reported to have anti-inflammatory, anti-bacterial, anti-hepatitic and anti-tumor activities.[1] Various sesquiterpene lactones have been isolated from Chinese Inula species such as *I. britannica*,[2] *I. salsoloides*, *I. hupehensis* and *I. helianthus-aquatica*,[3–5] and several sesquiterpene lactones have been shown to be cytotoxic. Several derivatives of 1-*O*-acetylbritannilactone **6**, the major component in *I. Britannica* have been synthesized, and one of them has displayed potent cytotoxicity in human HL-60 cells (ED_{50} = 4.6 μg/mL).[6] Recently, using a clonogenic assay, it was demonstrated that 1,6-*O*,*O*-diacetylbritannilactone decreased cell growth in breast, prostate and colorectal cell lines (IC_{50} 200 nM–2 μM) and G2-M cell cycle arrest. So it was been concluded that 1,6-*O*,*O*-diacetylbritannilactone is cytotoxic in multiple tumor cell lines.[7] The acetyl group in position 6 may be essential to the bioactivity, because in this assay 1,6-*O*,*O*-diacetylbritannilactone is ten times more active than 1-*O*-acetylbritannilactone **6**. This condition is similar to the bioassay results in our previously synthesized 1-*O*-acetylbritannilactone derivatives. In addition, four other cytotoxic sesquiterpene lactones were identified[8] from *I. britannica*. According to other reports,[9–14] acylation of the hydroxy group in the fragment is similar to that of 1-*O*-acetylbritannilactone, and both can ameliorate bioactivity. The present paper describes the crystal structure of 1-*O*-acetylbritannilactone **6** and its derivative, (2-*O*-butyloxime-3-phenyl) propionyl 1-*O*-acetylbritannilactone ester **7**. To date, numerous prodrug strategies that could improve the aqueous solubility and pharmacological functions have been tried. Some of them have reported the synthesis of prodrug esters with amino acid moieties.[15–17] We designed and synthesized a novel chain, (2-*O*-butyloxime-1-phenyl) propionic acid **4**, and introduced it into the structure of 1-*O*-acetylbritannilactone **6** to transform the 6-hydroxy group into its ester **7** and to try to influence its activity. The structure of synthesized derivative **7** was established by spectroscopic methods. Their cytotoxicity was also determined and compound **7** showed stronger cytotoxic activity.

2. Chemistry

By comparing the structure and activity of **6**[18] with those of diacetylbritannilactone,[2] multiradiatin[9–11] and eupaformosanin,[12,13] which all contain a similar struc-

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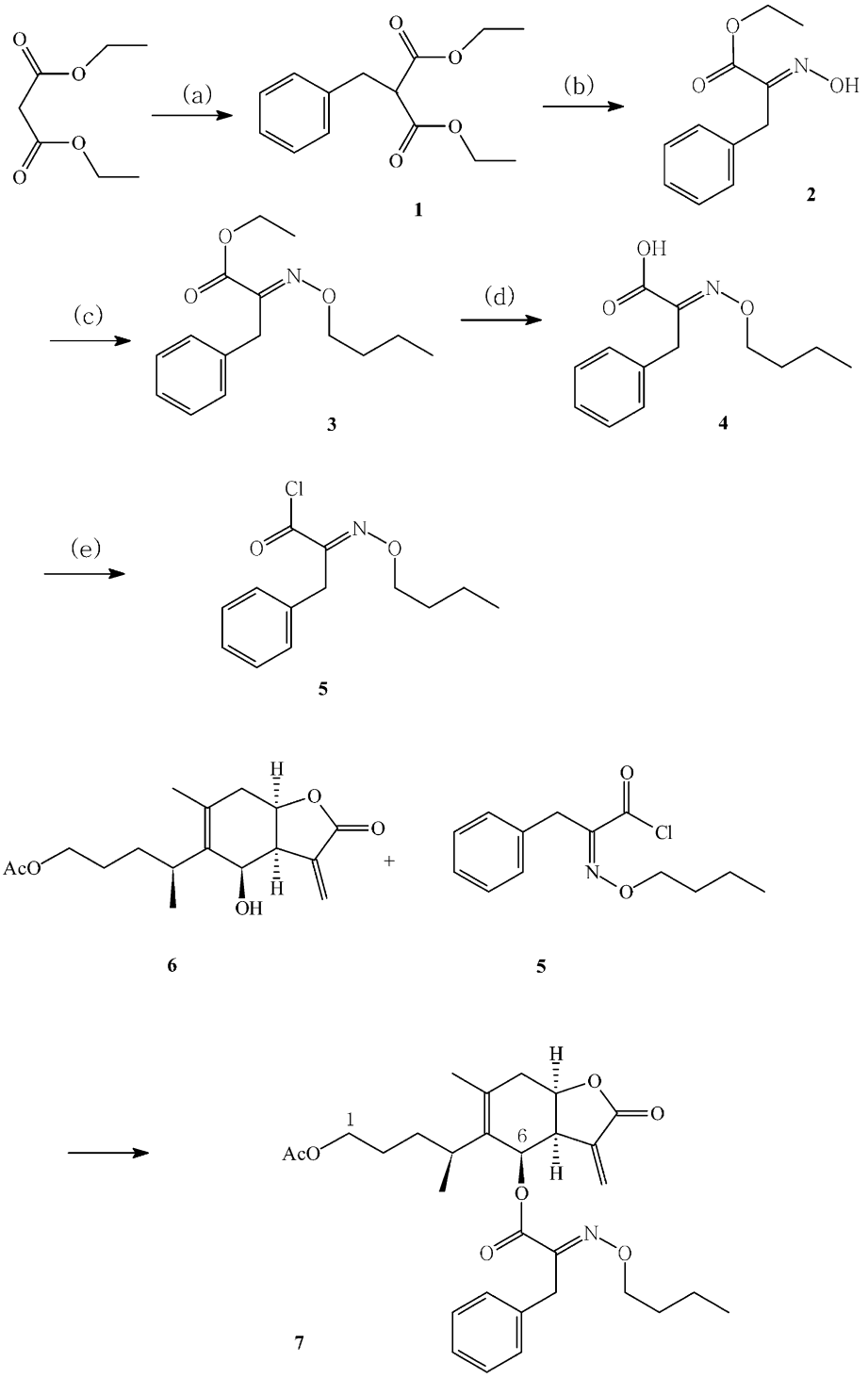
ture of sesquiterpene lactone but with different ester groups, it may be possible to determine that the acylation of the hydroxy group can improve bioactivity. Therefore, we synthesized (2-*O*-butyloxime-1-phenyl) propionic acid **4** to realize the acylation of the hydroxy group in **6**. In addition an oximno group is a common and stable functional group frequently employed in current drugs, so we introduced an *O*-butoxyloxime group into compound **4**, (2-*O*-butyloxime-1-phenyl) propionic acid. **4** was used for the synthesis of compound **7**. The general procedure for the preparation of target compound **7**,[19,20] (2-*O*-butyloxime-3-phenyl) propionyl 1-*O*-acetylbritannilactone ester, is shown in Scheme 1.



Scheme 1. Reagents and conditions: (a) NaOEt, $PhCH_2Cl$; (b) EtONO, H_2SO_4, 0 °C; (c) Bromobutane, K_2CO_3/acetone, 30–35 °C; (d) NaOH, H_2O/EtOH; (e) $SOCl_2$, CH_2Cl_2.

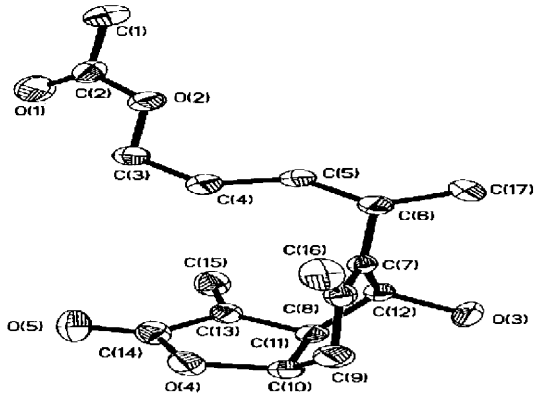


Figure 1. The structure of 1-*O*-acetylbritannilactone **6**. Selected bond distances (Å) and bond angles (°): O(3)–C(12) 1.441(2), O(4)–C(14) 1.334(2), O(5)–C(14) 1.207(3), C(7)–C(8) 1.329(2), C(8)–C(16) 1.503(3), C(13)–C(15) 1.319(3), C(13)–C(14) 1.471(3), C(14)–O(4)–C(10) 112.15(15), C(7)–C(6)–C(5) 112.60(15), C(7)–C(6)–C(17) 111.90(16), C(7)–C(8)–C(16) 126.03(18), C(16)–C(8)–C(9) 114.82(18), C(8)–C(9)–C(10) 113.31(16), O(4)–C(10)–C(9) 107.82(16), O(4)–C(10)–C(11) 106.31(15), C(13)–C(11)–C(12) 113.52(15), O(3)–C(12)–C(7) 111.45(14), O(3)–C(12)–C(11) 108.03(14), C(7)–C(12)–C(11) 113.21(14), C(15)–C(13)–C(14) 122.1(2), C(15)–C(13)–C(11) 129.4(2), C(14)–C(13)–C(11) 108.51(16), O(5)–C(14)–O(4) 121.6(2), O(5)–C(14)–C(13) 128.9(2) O(4)–C(14)–C(13) 109.52(17).

3. X-ray crystal structures of 1-*O*-acetylbritannilactone 6 and (2-*O*-butyloxime-1-phenyl) propionyl 1-*O*-acetylbritannilactone ester 7

Single crystals of the 1-*O*-acetylbritannilactone **6**[21] suitable for X-ray analysis were obtained from a CH_2Cl_2 solution. The structure of **6** is depicted in Figure 1. As anticipated, the structure of **6** is in accord with the results obtained from other methods (NMR, MS, IR). It is an α,β-unsaturated sesquiterpene lactone containing a six-membered ring, which adopted a slightly twisted boat conformation and was fused by a planar five-membered ring. The dihedral angle between two rings is 58.9°. The length of double bond $C_7=C_8$ in the six-membered ring is 1.329(2) Å, while the bond distances of $C_{13}=C_{15}$ and $C_{14}=O_5$, which are both connected to the five-membered ring, are 1.319 (3) Å and 1.207(3) Å, respectively. The bond of C (12)–*O* (3) is at the opposite of the planar five-membered ring and the long alkyl chain connected to C_7 atom, and can reduce the steric obstacle.

Table 1. The activity of compounds **6** and **7** against different cell bioassays (ID_{50} μg/mL)

Cell lines	6	7
HL-60[a]	18.4	4.3
Bel-7402[b]	15.6	7.2

[a] HL-60: Promyelocytic leukemia.
[b] Bel-7402: Hepatocellular carcinoma.

The crystals of (2-*O*-butyloxime-3-phenyl) propionyl 1-*O*-acetylbritannilactone ester **7**[21] were obtained from a CH_2Cl_2 solution. The structure is depicted in Figure 2. The oxime ether group is in *E*-configuration to separate it from the fragment that is similar to **6**.

The bond lengths and angles of bicyclic structure in both compounds are nearly equal, as seen by comparing the X-ray diffraction data of **6** with those of **7**. On this account it can be concluded that the influence of the introduced side chain is very slight. The steric structure in the fragment similar to that of **6** varies little, probably because the introduced group is at the opposite end, far away from the other big groups.

4. Cytotoxicity assay

The cytotoxicities[22] of compounds **6** and **7** against two cell lines are compared in Table 1. From the results, we can see that the cytostatic activity of compound **7** is much higher than that of **6**. The data of crystal structure shows that the cyclic difference between compound **6** and **7** is very small. The improvement of bioactivities is

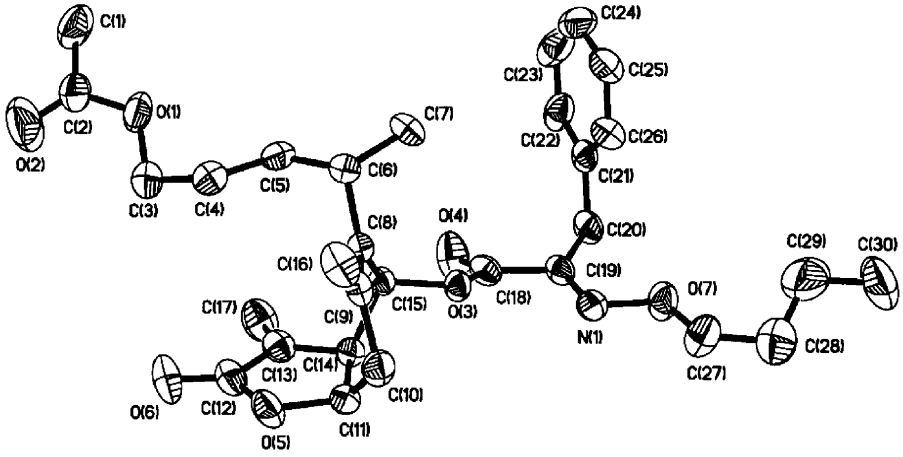


Figure 2. The structure of (2-*O*-butyloxime-1-phenyl) propionyl 1-*O*-acetylbritannilactone ester **2**. Selected bond distances (Å) and bond angles (°): C(8)–C(9) 1.334(6), C(9)–C(16) 1.495(6), C(11)–O(5) 1.483(6), C(12)–O(6) 1.219(6), C(12)–O(5) 1.350(7), C(12)–C(13) 1.467(7), C(13)–C(17) 1.324(8), C(15)–O(3) 1.473(5), C(18)–O(4) 1.203(6), C(18)–O(3) 1.360(5), C(19)–N(1) 1.279(6), C(27)–O(7) 1.438(7), N(1)–O(7) 1.411(5), C(9)–C(8)–C(6) 126.0(4), C(8)–C(9)–C(16) 126.7(4), C(11)–C(10)–C(9) 112.1(3), O(5)–C(11)–C(10) 107.3(4), O(5)–C(11)–C(14) 105.5(4), C(13)–C(14)–C(15) 110.8(3), O(3)–C(15)–C(8) 107.5(3), O(3)–C(15)–C(14) 109.6(3), C(8)–C(15)–C(14) 13.4(3), O(4)–C(18)–O(3) 123.8(4), O(4)–C(18)–C(19) 122.2(4), O(3)–C(18)–C(19) 114.0(4), N(1)–C(19)–C(18) 117.1(4), N(1)–C(19)–C(20) 127.9(5), C(18)–C(19)–C(20) 114.9(4), C(19)–N(1)–O(7) 110.8(4), C(18)–O(3)–C(15) 15.6(3).

a comprehensive result of many factors, but in this case perhaps the most important reason is the introduction of the modifying group (2-*O*-butyloxime-1-phenyl) propionyl into the target compound. Apparently, this side chain has the effect of increasing bioactivity of the parent compound.

Acknowledgements

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References and notes

1. Jiangsu, New Medical College. *Dictionary of Traditional Chinese Materia Medica*; Shanghai People's Press, 1977; Vol. 2, p 2216.
2. Zhou, B. N.; Bai, N. S.; Lin, L. Z.; Cordell, G. A. *Phytochem.* **1993**, *34*, 249.
3. Zhou, B. N.; Bai, N. S.; Lin, L. Z.; Cordell, G. A. *Phytochem.* **1994**, *36*, 721.
4. Wang, Q.; Zhou, B. N.; Zhang, R. W.; Lin, Y. Y.; Lin, L. Z.; Gil, R. R.; Cordell, G. A. *Planta Med.* **1996**, *62*, 166.
5. Shao, Y.; Bai, N. S.; Zhou, B. N. *Phytochem.* **1996**, *42*, 783.
6. Cheng, Y. H.; Liu, S. X.; Bai, N. S. *Yingyong Huaxue* **2000**, *17*, 550.
7. Ho, C.-T.; Rafi, M.; DiPaola, R. S.; Rosen, R. T.; Ghai, G.; Bai, N. United States Patent Number: 6,627,623, 2003.
8. Park, E. J.; Kim, J. *Planta Med.* **1998**, *64*, 752.
9. Pettit, G. R.; Herald, C. L.; Gust, D.; Herald, D. L.; Vanell, L. D. *J. Org. Chem.* **1978**, *43*, 1092.
10. Pettit, G. R.; Herald, C. L.; Judd, G. F.; Bolliger, G.; Thayer, P. S. *J. Pharm. Sci.* **1975**, *64*, 2023.
11. Pettit, G. R.; Herald, C. L.; Judd, G. F.; Bolliger, G.; Vanell, L. D.; Lehto, E.; Pase, C. P. *Lloydia* **1978**, *41*, 29.
12. Lee, K. H.; Kimura, T.; Haruna, M.; McPhail, A. T.; Onan, K. D.; Huang, H. C. *Phytochem.* **1977**, *16*, 1068.
13. Hall, I. H.; Lee, K. H.; Starnes, C. O.; Eigebaly, S. A.; Ibuka, T.; Wu, Y. S.; Kimura, T.; Haruna, M. *J. Pharm. Sci.* **1978**, *67*, 1235.
14. Hall, I. H.; Lee, K. H.; Williams, W. L., Jr.; Kimura, T.; Hirayama, T. *J. Pharm. Sci.* **1980**, *69*, 294.
15. Feng, X.; Yuan, Y. J.; Wu, J. C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3301.
16. Deutsh, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. *J. Med. Chem.* **1989**, *32*, 788.
17. Zhao, Z.; Kingston, D. G. I.; Crosswell, A. R. *J. Natl. Prod.* **1991**, *54*, 1607.
18. Compound **6** (1-*O*-acetylbritannilactone) was isolated from the flowers of *Inula britannica var. Chinensis*.[2] The flowers were percolated with 95% EtOH at room temperature. The $CHCl_3$ soluble part of the EtOH extract was subject to silica gel column chromatography using $CHCl_3$–Me_2CO to yield product **6**. It was previously characterized by elemental analysis, IR, MS and NMR.
19. Armarego, W. L. F.; Perrin, D. D. *Purification of Laboratory Chemicals*, 4th ed.; Butterworth-Heinemann: Oxford, UK, 1999.
20. (2-*O*-Butyloxime-3-phenyl) propionyl 1-*O*-acetylbritannilactone ester **7**: a bright yellow amorphous powder, mp 53–55 °C. $[\alpha]_D^{20}$: −59.5 (*c* 0.002 in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 5H), 6.35 (d, J = 2.7 Hz, 1H), 5.91 (d, J = 2.1 Hz, 1H), 5.29 (d, J = 1.8 Hz, 2H), 4.75 (m, 1H), 4.30 (t, 2H), 3.95 (m, 2H), 3.90 (m, 2H), 3.81 (m, 2H), 3.38 (m, 1H), 2.60 (m, 1H), 2.42 (m, 2H), 2.02 (m. 3H), 1.77 (s, 3H), 1.66 (m, 2H), 1.36 (m, 4H), 0.95 (m, 2H), 0.73 (d, J = 3.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.35, 17.84, 18.85, 19.95, 20.01, 26.02, 30.63 (2 C), 32.52, 34.04 (2 C), 42.11, 63.62, 69.63, 74.15, 75.21, 75.24, 78.42, 124.29, 126.08, 128.14 (2 C), 131.01, 133.94 (2 C), 135.78, 149.38, 162.60, 168.77, 170.40. Found: C, 68.59; H, 7.51; N, 2.61%. Calc. for $C_{30}H_{39}NO_7$: C, 68.55; H, 7.48; N, 2.66%. IR (KBr pellet): V_{max}/cm^{-1} 3029 (alkyl), 1767 (CO), 1734 (CO), 1663 (C=N), *m*/*z* 290, 272, 215, 202, 189 (100%), 143, 117, 91, 43.
21. X-ray crystal structure analysis: Single crystals of 1-*O*-acetylbritannilactone **6** and (2-*O*-butyloxime-3-phenyl) propionyl 1-*O*-acetylbritannilactone ester **7**, were obtained in the form of prisms needle and triclinic needle crystals, respectively. All diffraction measurements were performed at temperature of 298°K using a Bruker Smart 1000 diffractometer and graphite mono chromated Mo$K\alpha$ radiation (λ = 0.71073 Å). The structure was elucidated using direct methods and refined by full matrix least-squares on F^2. Crystal data of 1-*O*-acetylbritannilactone **6**: $C_{17}H_{24}O_5$, 308.36, Orthorhombic, space group $P_{2(1)2(1)2(1)}$, a = 8.0017 (7), b = 12.3653 (10), c = 16.8794 (14) Å, V = 1670.1 (2) Å^3, T = 298 (2) K, Z = 4, D_C = 1.226 Mg m^{-3}, F (000) = 664, absorption coefficient = 0.089 mm^{-1}, crystal size = 0.20×0.25×0.30 mm, 6995 reflection measured, 2949 unique (R_{int} = 0.0241) which were used in all calculations. The final $wR(F^2)$ was 0.0837 (all data).
Crystal data of (2-*O*-butyloxime-1-phenyl) propionyl 1-*O*-acetylbritannilactone ester **7**: $C_{30}H_{39}NO_7$, 525.62, Triclinic, space group P_1, a = 9.185(4), b = 9.470(4), c = 9.840(4) Å, V = 759.4(5) Å^3, T = 298(2) K, Z = 1, D_C = 1.149 Mg m^{-3}, F(000) = 282, absorption coefficient = 0.081 mitym^{-1}, crystal size = 0.20×0.25×0.30 mm, 2686 reflection measured, 2686 unique which were used in all calculations. The final $wR(F^2)$ was 0.1584 (all data).
22. The in vitro cytotoxicity was evaluated using a system based on tetrazolium salt (MTT), which was reduced by living cells to yield a soluble formazan product that could be assayed colorimetrically