Bioorganic & Medicinal Chemistry Letters 19 (2009) 2562–2565

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New Labdane diterpenes as intestinal α-glucosidase inhibitor from antihyperglycemic extract of *Hedychium spicatum* (Ham. Ex Smith) rhizomes

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ARTICLE INFO

Article history:
Received 13 November 2008
Revised 11 February 2009
Accepted 7 March 2009
Available online 16 March 2009

Keywords:
Hedychium spicatum
Labdane-type diterpenes
Spicatanol
Spicatanol methyl ether
Antihyperglycemic activity
α-Glucosidase inhibitory activity

ABSTRACT

Phytochemical investigation of antihyperglycemic extract of rhizomes of *Hedychium spicatum* led to the isolation of two new labdane type diterpenes **2, 3** along with seven known compounds (**1**, **4–9**). Their structures were established on the basis of NMR (1D and 2D) and mass spectroscopic analysis. The new compound **2** displayed strong intestinal α-glucosidase inhibitory activity. Other compounds also displayed varying degree of intestinal α-glucosidase inhibitory potential.

Hedychium spicatum Ham. Ex Smith (Zingiberaceae) is a native herb of South East Asian countries. It is a perennial rhizomatous herb growing at the altitude of 3500–7500 ft and is widely used as insect repellant and tobacco perfume.[1] Rhizomes of *H. spicatum* taste bitter camphor-like and possess strong aromatic odor. Therefore, in folklore, it is called *Kapura-Kachri* (Hindi). In Indian system of medicine Ayurveda, it is called *Sati*. The essential oil of rhizomes is used for the treatment of skin diseases, stomach ailments,[2] and is reported to possess analgesic,[3] anti-inflammatory,[4] antimicrobial,[5] pediculicidal,[6] mild tranquillizing[7] and anthelmintic activities.[8] In Ayurveda, *H. spicatum* has been advocated beneficial for the treatment of diarrhea, piles, bronchial asthma and semen disorders.[9]

Postprandial hyperglycemia (PPHG) has emerged as a prominent and early defect in type II diabetes[10] and as an important risk factor for cardiovascular disease.[11] Postprandial hyperglycemic excursions is the increase in glycemia immediately following a meal, particularly meals that contain starchy food. This rise in glycemia occurs 10–20 min following the beginning of meal ingestion and returns to the basal values within 2–5 h.[12] Recent observations suggest that prevention of PPHG by acarbose; an intestinal α-glucosidase inhibitor presents promising therapeutic strategy for reducing the increased risk for diabetes, hypertension, dyslipidemia, obesity as well as cardiovascular diseases.[13]

In the course of our recent efforts of identifying compounds that reduce diet induced postprandial hyperglycemic excursion,[14] we noticed antihyperglycemic activity in the hexane extract of rhizomes of *H. spicatum* with starch tolerance test in rats. When hexane extract was evaluated for inhibition of intestinal α-glucosidase in vitro, it displayed moderate enzyme inhibitory activity. Further fractionation of this extract led to the isolation and identification of two new labdane diterpenes with strong α-glucosidase inhibitory activity along with other known compounds possessing moderate enzyme inhibitory potentials. In this Letter, we report isolation, identification, and structure elucidation of two new labdane-type diterpenes, its α-glucosidase inhibitory activity and discuss the structure–activity relationship for this type of new family of natural α-glucosidase inhibitors. Structures of the new compounds were established using IR, MS, 1D and 2D NMR (HSQC, HMBC, COSY and NOESY) spectroscopic techniques.[15]

The dried rhizomes (1 kg) were ground and extracted three times with hexane in a soxhlet apparatus. Combined extracts were concentrated under vacuum. The portion of active hexane extract (8.5 g) was subjected to column chromatography (silica gel, 60–120 mesh) using step gradient of hexane/EtoAc to yield five major fractions (F1–F5). Fraction F1 was subjected to repeated silica gel (100–200 mesh) column chromatography (CC) by eluting with EtoAc–ether–Hexane (2:3:95) to yield compound **4** (2.15 g). Fraction F2 was subjected to silica gel column chromatography eluting with EtoAc–ether–hexane (7:3:90) to get compound **5**

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doi:10.1016/j.bmcl.2009.03.045

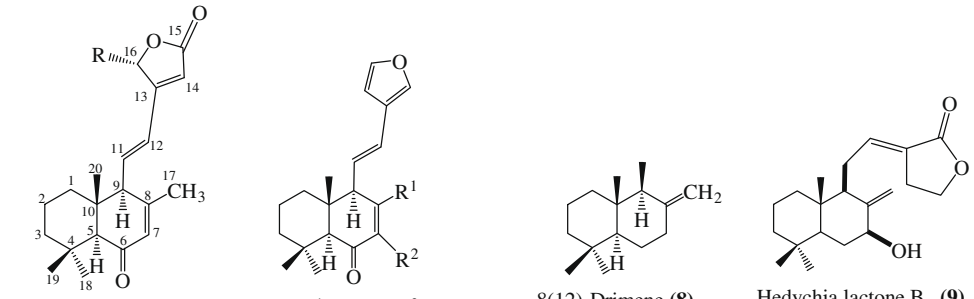

Figure 1. Isolated compounds from hexane extract of *H. spicatum*.

(1.22 g). A portion of fraction F3 was subjected to silica gel column chromatography with EtoAc–ether–hexane (11:9:80) to yield 0.080 g of compound **1**, with EtoAc–ether–hexane (15:9:76) to yield 0.87 g of compound **2**. Similarly, Fractions F4 and F5 were subjected to repeated column chromatography eluting with EtoAc–hexane (19:81) to yield 1.79 g of compound **7**, and 0.091 g, 0.098 g of compounds **8** and **9**, respectively (Fig. 1).

Compound **2** was obtained as an optically active pale yellow semi solid with $[\alpha]_D^{25} = +13.64$ (*c*1.24, $CHCl_3$). The molecular formula of **2** was determined as $C_{20}H_{26}O_4$ by HRESIMS, which provided a molecular ion peak at *m/z* 331.1942 $(M+H)^+$, in conjunction with its ^{13}C NMR spectrum displaying 20 resonances. A DEPT NMR experiment permitted differentiation of the 20 resonances into four methyls (one vinylic methyl), three methylenes, seven methines (one oxymethine) and six quaternary carbons (two carbonyls).The IR spectrum displayed absorption bands diagnostic of hydroxyl (3320 cm^{-1}), α,β-unsaturated ketone (1642 cm^{-1}) and α,β-unsaturated γ-lactone (1753 cm^{-1}) functionalities. The ^1H NMR data (recorded in $CDCl_3$) indicated the presence of four quaternary methyl singlets at δ 0.98, 1.11, 1.18 and 1.80. It has displayed a signals attributed to one oxygen-bearing methine at δ 6.28 (s, H-16) and *trans*-double bond at δ 6.42 (1H, dd, *J* = 15.8 Hz, 9.8 Hz,), δ 5.91 (1H, d, *J* = 15.8 Hz). It also had two singlets (each 1H) from a deshield shifted methine at δ 5.88 (H-7) and upshield shifted methine at δ 2.09 (H-5), and a partially overlapped multiplets due to the three methylenes between δ 1.26 and 1.61. Another sharp singlet integrating for one proton at δ 6.29 (1H, s) is assigned to the hydroxyl group attached methine (C-16) group in the lactone ring. The presence of a characteristic doublet at δ 3.01 (d, *J* = 7.0 Hz, H-9) associated with the data described above was indicative of labdane class diterpene. The ^{13}C NMR spectrum indicated the presence of α,β-unsaturated ketone (δ 199.84), trisubstituted olefin (δ 125.37 and 155.84) and four methyl signals (δ 22.01, 32.74, 16.27 and 23.09), which were supportive of a labdane skeleton. Further, it also displayed a signal at δ 173.11 due to C=O of lactone ring and at δ 97.91 assignable to oxymethine carbon.

The proton and protonated carbon signals in the NMR spectrum of **2** were unequivocally assigned by the HSQC experiments. The labdane diterpene skeleton was further confirmed, both by the ^1H-^1H COSY correlations (from H-1 through H-2 to H-3) and HMBC correlations (Fig. 2). The HMBC spectrum showed the following diagnostic correlations H-5/C-4, C-6, C-10; H-9/C-8, C-10, C-11; H-11/C-9, C-12; H-12/C-11; H-14/C-15; H-16/C-13, C-15; H-17/C-8 and H-18, H-3, H-19/C-4. These correlations clearly suggested that α,β-unsaturated γ-lactone ring (δ 141.20, 117.18, 128.53 and 155.36) was attached to the decalone nucleus through the double bond (δ 141.20, 117.18). Stereochemistry of double bond between C-11 and C-12 was established by coupling constant (*J* = 15.6 Hz). In addition, relative configuration of **2** was determined from the analysis of the 2D NOESY data. The configuration of the decalone portion of compound **2** was assumed to be the same as that of known diterpenes bearing the same skeleton such as yunnacoronarin D. Its NOESY spectrum showed the correlations between H_3-19/H_3-20; H-5/H-9, H_3-18 and H_3-20/H-16. These data were in accordance with the β-orientation of H_3-19, H_3-20, H-16 and α-orientation of H-5 and H-9. Based on these data, compound **2** was identified as 16-hydroxy, 6-oxo-7,11,13-labdatrien-16,15-olide (Fig. 1), a new labdane-type diterpenoid, trivially named as Spicatanol.

Compound **3** was isolated as an optically active colorless oil with positive optical rotation $[\alpha]_D^{25} = +15.41$. Its molecular formula was determined as $C_{21}H_{28}O_4$ by HRESIMS (*m/z* 345.2052 $(M+H)^+$. The IR spectrum revealed similar absorption bands as for the compound **2**. Comparison of the NMR data of **3** with that of **2** revealed that both compounds were based on the same carbon skeleton. Typical difference in 1D NMR spectra of compound **3** was that the hydroxyl group of compound **2** was replaced by the methoxyl group in the lactone ring. The position of methoxyl group was confirmed by HMBC spectrum (Fig. 3) showing correlations δ 3.86 with oxymethine carbon (C-16) at δ 63.09. Hence, this compound was identified as a simple methylated product of **2**. All the NMR data of **3** were assigned by DQF-COSY, HMQC and HMBC experiments. The NOESY correlations are very similar to that of **2** which determined the relative stereochemistry of the chiral centers and con-

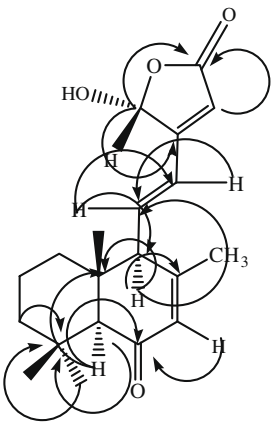

Figure 2. Key HMBC correlations of compound **2**.

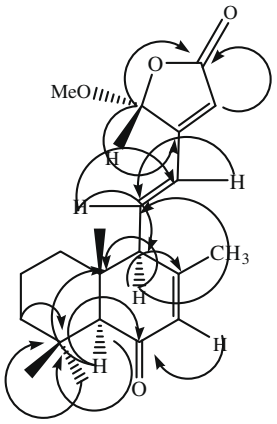

Figure 3. Key HMBC correlations of compound **3**.

firmed the structure of **3** as 16-methoxy, 6-oxo-7,11,13-labdatrien-16,15-olide, and assigned with trivial name of spicatanol methyl ether.[17]

In addition to the above new compounds, eight known compounds were also isolated from hexane extract. By comparison of their physical and spectroscopic data with literature, they were characterized as 6-oxo-7,11,13-labdatrien-16,15-olide (**1**),[18] spicatanol (**2**), spicatanol methyl ether (**3**), hedychenone (**4**),[19] 7-hydroxy hedychenone (**5**),[20] yunnacoronarin D (**6**),[16] 7-acetoxy hedychenone (**7**),[20] 8 (12) Drimene (**8**)[21] and hedychia lactone B (**9**)[22] (Fig. 1). To the best of our knowledge, this is the first report on chemical analysis of isolation of compounds **8** and **9** from *H. spicatum*.

Antihyperglycemic activity of the extract was evaluated by starch tolerance test.[14a] Pretreatment of rats with hexane extract significantly reduced the sharp rise in blood glucose level for the first 30 min of post starch feeding (Fig. 4). In order to explore the mechanism of action of antihyperglycemic activity, we analyzed

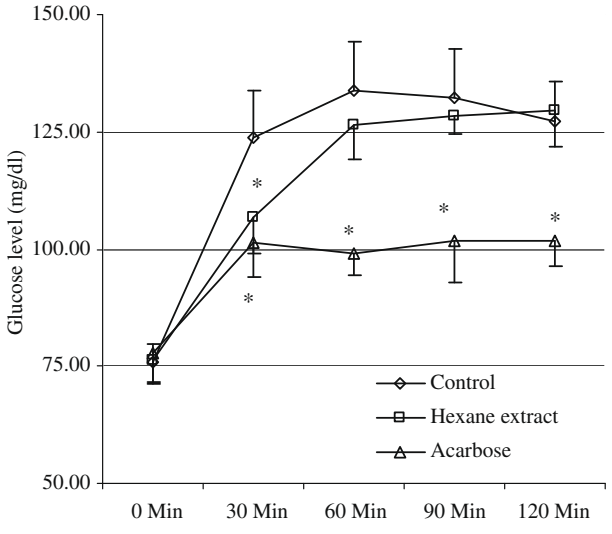

Figure 4. Plasma glucose level of rats at different time intervals after oral starch feeding. Hexane extract (250 mg/kg body weight) and standard drug Acarbose (10 mg/kg body weight) was administered *p.o.*15 min before feeding of soluble starch (2-g/kg body weight in normal saline).[14a] Male Wistar rats (weight 195 ± 10) were used in this study. ANOVA analysis followed by Dunnett's Multiple Comparison test was applied to find the changes in glucose level at different time points in different group of rats after starch feeding. $^{*}p < 0.01$ when compared with respective control time points. Data presents mean ± SD of six animals in each group.

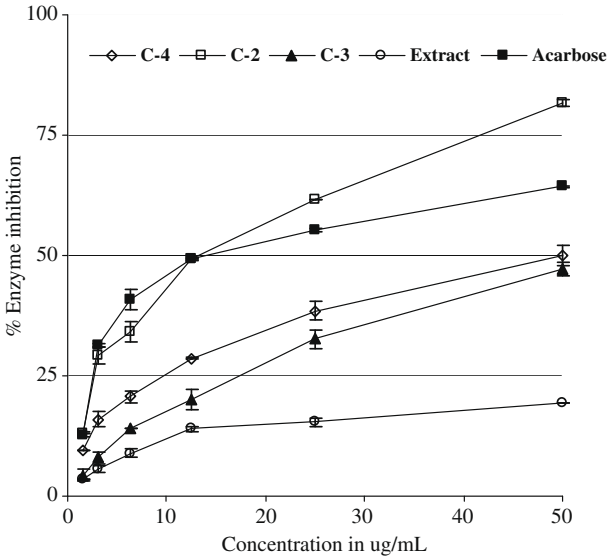

Figure 5. Concentration dependent α-glucosidase inhibitory potential of compounds isolated from *H. spicatum*. The α-glucosidase inhibitory assay was performed as reported earlier.[14b] Briefly, 10 μL of test samples were incubated for 5 minutes with 50 μL of crude enzyme before reacting with 50 μL of substrate (5 mM, ρ-nitrophenyl-α-D-glucopyranoside prepared in 100 mM phosphate buffer, pH 6.8) for 5 min. Absorbance of released nitrophenol was monitored spectrophotometrically at 405 nm. **C-2** represents spicatanol; **C-3**, spicatanol methyl ether; **C-4**, hedychenone; and extract represents hexane extract. Acarbose served as standard α-glucosidase inhibitor (IC_{50}, 23.8 μM). IC_{50} represents concentration of the test sample required to inhibit 50% enzyme activity. IC_{50} values were calculated applying logarithmic regression analysis to the data.

in vitro rat intestinal α-glucosidase inhibitory potential of this extract using ρ-nitrophenyl-α-D-glucopyranoside as substrate.[14b] It was found that hexane extract displayed 21.2% inhibition of the enzyme at primary screening concentration (100 μg/mL). Further fractionation of this extract led to the isolation of several new families of labdane diterpenoids possessing different degrees of intestinal α-glucosidase inhibitory activity (Table 1). Compounds **2**–**4** displayed more than 50% enzyme inhibitory activity in primary screening (Table 1), hence were further studied for their concentration-dependent enzyme inhibitory potential (Fig. 5). The new compound **2** displayed most potent α-glucosidase inhibitory activity (IC_{50}, 34.1 μM) than other compounds.

This study reveals that presence of α,β-unsaturated γ-lactone (**1–3**) and furan (**4–7**) ring in labdane diterpenes is required for α-glucosidase inhibition because compound **8** could not display enzyme inhibitory activity. Presence of –OH at C-16 in lactone ring (**2**) strongly increases enzyme inhibitory potential as against –OMe

Table 1
α-Glucosidase inhibitory activity of compounds isolated from *H. spicatum*

Compound	% Enzyme inhibition
Hexane extract	21.2 ± 4.2
1	35.1 ± 0.0
2	89.5 ± 2.6
3	54.1 ± 0.1
4	55.6 ± 1.0
5	13.5 ± 0.0
6	15.1 ± 0.0
7	25.7 ± 0.0
8	NA
9	11.3 ± 2.8

Percentage of enzyme inhibition by the compounds at a primary screening concentration of 100 μg/mL. The assay was performed as reported earlier.[14a,14b] NA: no inhibition. Data represents mean ± SD of triplicate samples.

(**3**) (Fig. 5). In case of furan containing labdanes, substitutions at C-7 (**5, 7**) in the presence of $-CH_3$ at C-8 drastically reduces α-glucosidase inhibitory potential.

Glycosidase inhibition has become important therapeutic target not only for diabetes but also in cancer, viral infections including HIV and influenza, and in lysosomal storage diseases, with a number of drugs in current clinical use.[23] To the best of our knowledge, *H. spicatum* is not reported yet to possess antihyperglycemic activity and indicated in diabetes. Therefore, this report observes for the first time antihyperglycemic activity in *H. spicatum* and assigns α-glucosidase inhibitory activity to labdane-type diterpenes. The potent α-glucosidase inhibitory activity of new compound spicatanol (**2**) along with other active compounds present in hexane extract of the rhizome of *H. spicatum* may be responsible for antihyperglycemic activity in starch induced hyperglycemia. This report provides for the first time labdane diterpenoids as a novel family of intestinal α-glucosidase inhibitors that may be useful for development of new therapies in the field of diabetes, cancer, and viral infections.

Acknowledgment

The authors thank Director IICT for encouragement and DST-IMPCL project for financial support.

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15. *Spectral data: Compound (**2**)*: Yellow semi solid, $[\alpha]_D^{25} = +13.64$ (*c*1.24, $CDCl_3$). IR (KBr): 3329, 1755, 1647 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.98, 1.11, 1.18, 1.80 (3H each, all s, 20, 18, 19, 17-H_3), 1.26 (2H, m, H_2-3), 1.41 (2H, m, H_2-2), 1.61 (2H, m, H_2-1), 2.10 (1H, s, H-5), 3.01 (1H, d, *J* = 7.0 Hz, H-9), 5.88 (1H, s, H-7), 5.91 (1H, d, *J* = 15.6 Hz, H-12), 6.28 (1H, s, H-16), 6.42 (1H, dd, *J* = 9.8,15.6 Hz, H-11), 6.48 (1H, s, H-14). ^{13}C NMR ($CDCl_3$): δc 16.27 (C-20), 18.32 (C-2), 22.01 (C-19), 23.09(C-17), 29.92 (C-4), 32.74 (C-18), 33.71 (C-10), 40.56(C-1), 43.27 (C-3), 61.77 (C-9), 63.26 (C-5), 97.91 (C-16), 117.18 (C-12), 125.37 (C-7),128.53 (C-14),141.20 (C-11), 155.87 (C-8), 160.42 (C-13), 171.33 (C-15), 199.84 (C-6). EIMS: *m/z* 331 $(M+H)^+$, HREIMS *m/z* 331.1942 (calcd for $C_{20}H_{27}O_4$, 331.1909).
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17. *Compound (**3**)*: Colorless oil, $[\alpha]_D^{25} = +15.41$ (*c* 1.42,$CHCl_3$). IR (KBr): 1751, 1641 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.99, 1.16, 1.19, 1.82 (3H each, all s, 20, 18, 19, 17-H_3), 1.24 (2H, m, H_2-3), 1.40 (2H, m, H_2-2), 1.59 (2H, m, H_2-1), 2.12 (1H, s, H-5), 3.03 (1H, d, *J* = 7.0 Hz,H-9), 3.86 (3H, s, OMe), 5.93 (1H, s, H- 7), 5.98 (1H, d, *J* = 15.6 Hz, H-12), 6.29 (1H, s, H-16), 6.44 (1H, dd, *J* = 9.8 Hz, 15.6 Hz, H-11), 6.51 (1H, s, H-14). ^{13}C NMR ($CDCl_3$): δ_c 15.98 (C-20), 18.09 (C-2), 21.93 (C-19), 22.25 (C-7), 28.81 (C-4), 31.75 (C-18), 32.69 (C-10), 41.52 (C-1), 44.29 (C-3), 57.79 (OMe), 59.63 (C-9), 60.99 (C-5), 63.08 (C-16), 119.14 (C-12), 125.53 (C-7), 128.29 (C-14), 139.84 (C-11), 155.04 (C-8), 161.39 (C-13), 170.93 (C-15), 199.82 (C-6). EIMS: *m/z* 345 $(M+H)^+$, HREIMS *m/z* 345.2052 (calcd for $C_{20}H_{29}O_4$, 345.2066).
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