Bioorganic & Medicinal Chemistry Letters 19 (2009) 192–195

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Two new cytotoxic diterpenes from the rhizomes of *Hedychium spicatum*

P. Prabhakar Reddy [a], R. Ranga Rao [a], K. Rekha [a], K. Suresh Babu [a], J. Shashidhar [b], G. Shashikiran [b], V. Vijaya Lakshmi [b], J. Madhusudana Rao [a,*]

[a] Natural Products Laboratory, Indian Institute of Chemical Technology, Uppal Road, Hyderabad 500 607, India
[b] Biochemistry Division, National Institute of Nutrition, Tarnaka, Hyderabad 500 607, India

ARTICLE INFO

Article history:
Received 14 May 2008
Revised 5 October 2008
Accepted 28 October 2008
Available online 5 November 2008

Keywords:
Hedychium spicatum
Gingiberaceae
Labdane diterpenes
Cytotoxic activity

ABSTRACT

Phytochemical investigation of $CHCl_3$ extract of the rhizomes of *Hedychium spicatum* led to the isolation of two new labdane-type diterpenes, compounds **1** and **2** along with five known compounds (**3–7**). Their structures were established on the basis of NMR (1D and 2D) and mass spectroscopic analysis. In addition, all the isolates were tested for their cytotoxicity against the Colo-205 (Colo-cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and Chinese hamster ovary cells (CHO). Two new compounds **1** and **2** were shown good cytotoxic activity.

Hedychium spicatum is a plant belonging to the family Gingiberaceae and commonly named as kapura kachri (Indian trade name). It is widely cultivated in India and South East Asian countries. It is perennial rhizomatous herb growing at altitude of 3500–7500 ft. It's rhizomes, which have a bitter camphor-like taste and a strong aromatic odour, have been used as an insect repellant and as a tobacco perfume.[1] Medicinally, the rhizome (essential oil of rhizomes) is used for the treatment of skin diseases, stomach ailments,[2] analgesic,[3] anti-inflammatory,[4] antimicrobial,[5] in vitro pediculicidal[6] and mild tranquillizing[7] action of short duration and anthelmintic activity.[8] Cytotoxic activity guided investigation of the rhizomes of *H.* spicatum led to the isolation of two new labdane-type diterpenes. The structures of the two new compounds were established using IR, MS, 1D and 2D NMR (HSQC, HMBC, COSY and NOESY) techniques.

As part of our continuing efforts directed towards the discovery of the structurally interesting and biologically active compounds from the Indian medicinal plants,[9] it was found that the $CHCl_3$ extract of the rhizomes of the *H. spicatum* showed the cytotoxic activity against the Colo-205 (Colo-cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and Chinese hamster ovary cells (CHO). The phytochemical analysis resulted in the isolation of two new labdane-type diterpenes (**1** and **2**) along with known compounds. Here in, we report the isolation and anticancer activity of the labdane diterpene constituents of the *H. spicatum*.

The dried rhizomes (1 kg) were ground and extracted three times with $CHCl_3$ in a soxhlet apparatus. The combined extracts were concentrated under vacuum. The portion of active $CHCl_3$ extract (8.5 g) was subjected to column chromatography (silica gel, 60–120 mesh) using step gradient of $CHCl_3$/MeOH to yield five major fractions (F1–F5). Fraction F1 was subjected to repeated silica gel (100–200 mesh) column chromatography (CC) by eluting with MeOH:$CHCl_3$ (4:96) to yield compound **4** (2.15 g). Fraction F2 was subjected to silica gel column chromatography eluting with MeOH:$CHCl_3$ (7:93) to get compound **6** (1.22 g). A portion of fraction F3 was subjected to silica gel column chromatography with MeOH:$CHCl_3$ (11:89) to yield 0.080 g of compound **1**, with MeOH:$CHCl_3$ (12:88) to yield 0.87 g of compound **3**. Similarly, Fractions F4 and F5 were subjected to repeated column chromatography eluting with MeOH:$CHCl_3$ (15:85) to yield 0.091 g of compound **2**, with MeOH:$CHCl_3$ (18:82) to yield 1.79 g of compound **5**, and **7** respectively.

Compound **1** was isolated as a yellow semi solid with the positive optical rotation $[\alpha]_D^{25}$ +98.64 (*c* 1.64, $CHCl_3$). The HRESI-MS of compound **1** revealed a molecular ion peak corresponding to $(M+H)^+$ at *m/z* 331.142 indicating the molecular formula $C_{20}H_{26}O_4$. The ^{1}H NMR spectrum of compound **1** showed all the features of labdane diterpene.The IR spectrum displayed absorption bands at 3320 cm^{-1} (OH), 1642 cm^{-1} (α,β-unsaturated C=O) and 1753 cm^{-1} (α,β-unsaturated γ-lactone). The ^{1}H NMR spectrum displayed four quaternary methyl signals each integrating for three protons as singlets at δ 0.97, δ 1.16, δ *1.19* and δ 1.81. It has displayed a singlet at δ 2.09 for one proton (H-5) indicating the presence of one methine adjacent to the carbonyl (C-6) carbon

* Corresponding author. Tel.: +91 40 27193166; fax: +91 40 27160512.
E-mail address: janaswamy@iict.res.in (J.M. Rao).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.121

atom. A sharp *singlet* integrated for 1H at δ 5.88 is due to methine proton (H-14) in the lactone ring. A characteristic doublet for one proton at δ 2.94 (d, J = 7.0 Hz) indicating the presence of methine (H-9) group adjacent to olefinic double bond. The presence of one *trans* double bond at δ 6.73 (1H, dd, J = 15.8 Hz, 9.8 Hz,) and δ 6.28 (1H, d, J = 15.8 Hz) was suggested by the ^{1}H NMR, and NOESY spectrum. And comparison of NMR data with that of yunnacoronarin D[10] indicates the presence of *trans* double bond at C-11/C-12 position. Another sharp *singlet* integrated for two protons at δ 4.83 (2H, s) is assigned to CH_2 group in the lactone ring. Furthermore, the ^{1}H NMR spectrum also revealing that the *trans* double bond (C-11/C-12) is conjugated with lactone ring. The ^{13}C NMR spectrum of compound **1** showed the presence of 20 C-atoms. The DEPT experiment indicated the presence of four methyls, four CH_2, five CH groups, and seven quaternary Carbons. The^{13}C NMR spectrum of compound **1** also showed all the features of labdane diterpene. The ^{13}C NMR spectrum indicated the presence of α,β-unsaturated ketone (δ 199.65), trisubstituted olefin (δ 143.86 and 156.10) and four methyl signals (δ 22.90, 33.57, 15.79 and 21.65).Further, it also displayed signal at δ 172.11 is due to C=O of lactone ring, δ 128.83, 128.27 are corresponding to disubstituted olefin, and δ 69.71 is assignable to methylene carbon in the lactone ring. A complete assignment of protons and carbons was assisted by HMBC, COSY and HSQC experiments. The carbon skeleton suggested by several 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; H-5, H-18, H-3, H-19/C-4) and ^{1}H–^{1}H COSY (H-11/H-12; H-14/H-15; H-1/H-2; H-2/H-3). The HMBC correlations also suggested that α,β-unsaturated γ-lactone ring (δ 128.83, 128.27, 69.71 and 172.11) is attached to the decalin nucleus through the *trans* double bond (δ 134.62, 123.24). All the key HMBC correlations are depicted in Figure 2. In addition, the relative configuration of **1** was proposed on biogenetic basis and by inspection of NOESY spectrum, which showed the correlation between the following proton pairs (19-H_3 and 20-H_3; 5-H and 9-H, 18-H_3). Based on these data, compound **1** was identified as 7-hydroxy,6-oxo-7,11,13-labdatrien-16,15-olide (Fig. 1), a new labdane-type named hedychilactone D.

Compound **2** was isolated as a pale yellow viscous liquid, with positive optical rotation $[\alpha]_D$ +96.9 (c 1.28, $CHCl_3$). The HRESI-MS of compound **2** revealed a molecular ion peak corresponding to (M^++H) at m/z 315.121 indicating the molecular formula $C_{20}H_{26}O_3$. The IR spectrum showed absorption bands at 3425 cm^{-1} (OH), and 1691 cm^{-1} (C=O). The ^{1}H NMR and ^{13}C NMR spectroscopic data of the compound **2** (Table 2) indicated labdane skeleton, which is similar to that of compound **1**; both have α,β-unsaturated ketone (199.65 in **1**,199.88 in **2**) and double bond (δ 134.62,123.24 in **1**; δ 121.25, 128.39 in **2**) directly attached to the dacalin nucleus. The principal difference in 1D NMR spectra of compound **2** was that with the replacement of lactone ring with furon. In ^{13}C NMR δ 78.17 indicates hydroxy bearing carbon atom at C-9. The structure was further confirmed by HMBC correlations (Fig. 3) between H-5/C-4, C-6, C-10; H-7/C-6, C-8; H-11/C-9, C-12; H-12/C-11; H-14/C-15; H-15/C-14; H-16/C-15, C-13; H-17/C-8; H-5, H-18, H-3, H-19/C-4) and ^{1}H–^{1}H COSY (H-11/H-12; H-1/H-2; H-2/H-3), respectively. Similarly, the doublet attributed to H-12 showed J = 15.8 Hz, which is an indicative of *trans* configuration at C-11/C-12. Thus, compound **2** was identified as 9-hydroxy-15,16-epoxy-7,11,13(16)14-labdatetraen-6-one, another new labdane-type diterpenoid named 9-hydroxy hedychenone (Fig. 1).

In addition to the above two new compounds described, four known compounds were isolated. By comparing their physical

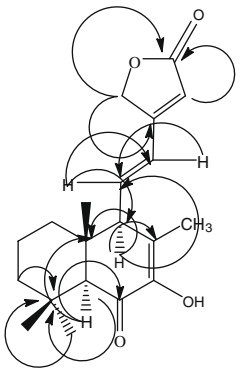

Figure 2. Key HMBC correlations of compound **1**.

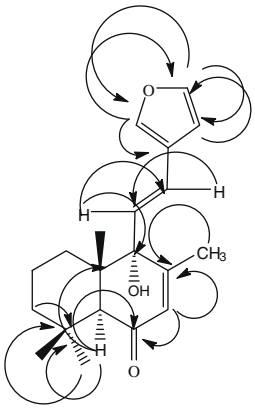

Figure 3. Key HMBC correlations of compound **2**.

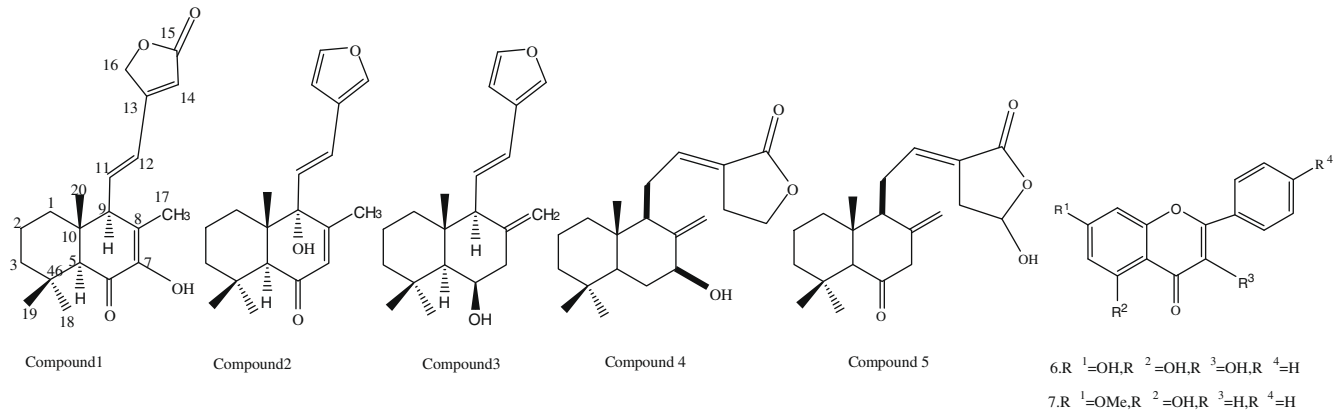

Figure 1. Isolated compounds from $CHCl_3$ extract of *H. spicatum*.

Table 1
Cytotoxicity effects of $CHCl_3$ extract and constituents from *H. spicatum.*

Compound	Cell lines (IC_{50}, μg/mL)				
	Colo-205	A-431	MCF-7	A-549	CHO
$CHCl_3$ extract	43.14 ± 1.12	37.45 ± 0.9	58.08 ± 0.15	63.21 ± 1.19	39.52 ± 0.06
1	12.03 ± 0.03	16.01 ± 0.05	21.05 ± 0.04	37.98 ± 0.91	7.69 ± 1.12
2	24.02 ± 0.01	26.09 ± 0.09	30.94 ± 0.06	41.21 ± 1.09	15.68 ± 0.09
3	27.14 ± 0.03	29.02 ± 0.05	28.05 ± 0.08	46.56 ± 1.01	17.89 ± 1.18
4	29.81 ± 0.06	31.53 ± 0.07	33.49 ± 0.02	NA	21.34 ± 1.11
5	27.56 ± 0.02	30.51 ± 0.04	49.29 ± 0.80	NA	20.36 ± 0.08
6	33.14 ± 1.10	39.45 ± 0.80	53.08 ± 0.05	54.21 ± 1.09	26.96 ± 0.04
7	37.14 ± 0.03	39.02 ± 0.05	51.05 ± 0.08	49.56 ± 1.01	23.54 ± 0.08

NA = not active, each value represents the mean ± standard deviation.

and spectroscopic data with the literature they were characterized as 7-hydroxy,6-oxo-7,11,13-labdatrien-16,15-olide (**1**), 9-hydroxy,15,16-epoxy-7,11,13(16)14-labdatetraen-6-one (**2**), yunnacoronarin A (**3**),[11] hedychilactone B,[12] hedychilactone C,[12] chrysin (**4**),[13] and teptochrysin (**6**),[14] respectively. To the best of our knowledge, this is the first report on chemical analysis of isolation of compounds **3,** and **6** from *H. spicatum.*

In vitro cytotoxicity evaluation. All the isolates obtained in this investigation of *H. spicatum* were tested for their in vitro cytotoxicity against Colo-205 (colon cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and CHO (Chinese hamster ovary cells) cell lines.[15] The cytotoxicity data and IC_{50} values in μg/mL are shown in Table 1. According to results obtained IC_{50} values ranged between 7.69 and 49.29 μg/mL for labdane diterpenes, and between 20.36 and 54.21 μg/mL for isoflavanoids. The small structural differences of labdane diterpenes influenced the cytotoxic activity. As evident from cytotoxic activity results (Table 1) compound **1** exhibited significant activity on Colo-205, A-431 and MCF-7 and CHO cell lines and moderate activity on A-549 cell lines. Compound **2** also shown moderate activity Colo-205, A-431 and significant activity on CHO cell lines. It is interesting to note that compound 4 and 5 are inactive against A-549 cell lines.

On comparing the activity of compound **1** with remaining isolates, it can be concluded that the presence of an α,β-unsaturated γ-lactone ring and α,β-unsaturated ketone (C-7/C-8, C-6) enhances the resultant cytotoxicity for the tested cell lines. Even with α,β-unsaturated ketone (C-7/C-8, C-6) cytotoxicity is slightly decreased due to lack of α,β-unsaturated γ-lactone in compound **2**. It is interesting to note that all the isolates are significantly active on CHO cell lines. Due to lack of α,β-unsaturated γ-lactone and α,β-unsaturated ketone cytotoxicity is reduced in compounds **3**, **4** and **5**. Compounds **6** and **7** also shown considerable activity on tested cell lines. Hence, the compound **1** was found to possess significant cytotoxic activity among all the isolates. In summary, *H. Spicatum*, of the compositae family has been used as a traditional medicine for the treatment of cancer. The compounds responsible for this activity have yet to be determined. The cytotoxic experiments were repeated in triplicate, and the IC_{50} values were expressed as mean ± standard deviation.

These results encourage us to continue our research of this series by synthesizing additional labdane-type diterpenoid derivatives with the aim of obtaining cytotoxic compounds that are more potent and selective toward cancer cells.

Table 2
^1H NMR and ^{13}C NMR data of compounds **1** and **2** in $CDCl_3$.

Position	Compound **1**		Compound **2**	
	δc	δ_H multiplicity	δc	δ_H multiplicity
1	40.24	1.59 (2H, m)	39.57	1.51 (2H, m)
2	18.08	1.48 (2H, m)	17.67	1.31 (2H, m)
3	43.21	1.25 (2H, m)	42.79	1.38 (2H, m)
4	32.50	–	32.36	–
5	63.29	2.09(1H, s)	55.67	2.78 (1H, s)
6	199.65	–	199.88	–
7	143.86	–	128.89	5.82 (1H, s)
8	156.10	–	154.78	–
9	61.73	2.94 (1H, d, *J* = 7.0 Hz)	78.17	–
10	42.80	–	45.93	–
11	134.62	6.73 (1H, dd, *J* = 15.8, 9.8 Hz)	121.25	6.71 (1H, d, *J* = 15.8, 9.8 Hz)
12	123.24	6.28 (H, d, *J* = 15.8 Hz)	128.39	6.02 (1H, d, *J* = 15.8 Hz)
13	128.83	–	128.44	–
14	128.27	5.88 (1H, s)	143.72	7.39 (1H, s)
15	172.11	–	140.71	7.48 (1H, s)
16	69.71	4.83 (2H, s)	107.37	6.52 (1H, s)
17	21.65	1.81 (3H, s)	20.16	1.86 (3H, s)
18	33.57	1.16 (3H, s)	33.89	1.19 (3H, s)
19	22.91	1.19 (3H, s)	21.77	1.22 (3H, s)
20	15.79	0.97 (3H, s)	19.47	1.09 (3H, s)

Acknowledgements

The authors thank DST and IMPCL projects for their financial support.

Appendix

In vitro cytotoxicity evaluation. All the isolates were tested for in vitro cytotoxicity on different cancer cell lines. The cell lines used in this study were Colo-205 (Colo-cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer) and Chinese harmer ovary cells (CHO). All the cells were obtained from National Center for Cellular Sciences (NCCS)—Pune, India. Cells were maintained in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, at 37° C with 5% CO_2. The cells were seeded at 1×10^4 cells/well. After 24 h, cells were treated with the test compound and IC_{50} values were calculated in μg/mL.

Supporting information. Hedychium Spicatum plant is collected from Talakona forest, Tirupati, Chittor district, Andhra Pradesh, India. Rhizomes of this plant are shade dried, powdered and extracted using soxhlet apparatus. One kilogram of plant powder is loaded in the soxhlet apparatus and extraction is done using 5 l of hexane and chloroform. As a result hexane extract was 10 g and chloroform extract was 8.5 g. Hexane extract had many oils and fats, whereas the chloroform extract was promising and hence the isolation of chloroform extract was done. Five fractions (F1–F5) were collected from the column chromatography.

Sixteen grams of 60–120 mesh silica gel was added to 8.5 g of chloroform extract and was adsorbed until fine dry powder was seen in the round bottom flask, and was loaded on the column (length of column 80 cm, width 16 mm) and eluted with plane chloroform. The flow rate of the column was 3 mL/min. Accordingly polarity was increased by adding methanol. Initially with 300 mL of plane $CHCl_3$ 3 g of fraction 1 (F1) was collected from the column. Later polarity of MeOH was increased. With 250 mL of MeOH:$CHCl_3$ (5:95), 1.8 g of fraction 2 (F2) collected. With 300 mL of MeOH:$CHCl_3$ (10:90), 1.0 g of fraction 3 (F3) collected.

Similarly with 350 mL of MeOH:$CHCl_3$ (15:85), 0.5 g of fraction 4 (F4) and With MeOH:$CHCl_3$ (20:80), 1.9 g of fraction 5 (F5) collected. This type of column is known as step gradient type of column. Each fraction is again loaded in another column (60 cm length, 12 mm of width) until single pure compound was isolated.

References and notes

1. *The Wealth of India*, **1959**, Vol. V, 13. C.S.I.R., New Delhi.
2. Chopra, R. N.; Nayar, S. L.; Chopra, I. C. *Glossary of Indian Medicinal Plant*; C.S.I.R.: New Delhi, 1956.
3. Tandon, S. K.; Chandra, S.; Gupta, S.; Lal, J. *Ind. J. Pharm. Sci.* **1997**, *59*, 148.
4. Srimal, R. C.; Sharma, S. C.; Tandon, J. S. *Ind. J. Pharm.* **1984**, *16*, 143.
5. Jinga, P.; Nehal, K.; Sumitra, C. *Ind. J. Pharm. Sci.* **2006**, *68*, 882.
6. Jadhav, V.; Kore, A.; Kadam, V. J. *Fitoterapia* **2007**, *78*, 470.
7. Dixit, V. K.; Varma, K. C. *Ind. J. Pharmacol.* **1979**, *2*, 147.
8. Akhtar, M. S.; Iqbal, Z.; Khan, M. N.; Lateef, M. *Small Ruminant Res.* **2000**, *38*, 99.
9. (a) Suresh Babu, K.; Tiwari, A. K.; Srinivas, P. V.; Ali, A. Z.; China Raju, B.; Rao, J. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3841; (b) Rao, A. S.; Srinivas, P. V.; Tiwari, K.; Vanka, U. M. S.; Rao, V. R. S.; Dasari, K. R.; Rao, J. M. *J. Chromatogr. B* **2007**, *855*, 166; (c) Anuradha, V.; Srinivas, P. V.; Rao, R. R.; Manjulatha, K.; Purohit, M. J.; Rao, J. M. *Bioorg. Med. Chem.* **2006**, *14*, 6820.
10. Zou, C.; Zhao, Q.; Hao, X.; Chen, Y.; Hong, X. *Acta Bot. Yunnica* **1999**, *21*, 253.
11. Zhao, Q.; Hao, X.-J.; Chen, Y.-Z.; Zou, C. *Chem. J. Chin. Univ.* **1995**, *16*, 64.
12. Matsuda, H.; Morikawa, T.; Sakamoto, Y.; Toguchida, I. *Bioorg. Med. Chem.* **2002**, *10*, 2527.
13. Benerji, A.; Das, R. *Ind. J. Chem. B* **1971**, *15B*, 495.
14. Subramanian, S. S.; Nair, A. G. R. *Curr. Sci.* **1972**, *4*, 62.
15. http://www.atcc.org/pdf/30-1010k.pdf.