VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 158-161 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-037

TERPENOIDS FROM LEAVES OF *VIBURNUM SAMBUCINUM* REINW. ex. BLUME (CAPRIFOLIACEAE)

Nguyen Thanh Tra[1,2], Truong Bich Ngan[1], Vu Van Nam[1], Do Thi Thao[3], Marc Litaudon[4], Doan Thi Mai Huong[1], Nguyen Van Hung[1], Chau Van Minh[1], Pham Van Cuong[1*]

[1]*Institute of Marine Biochemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[2]*Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[3]*Institute of Biotechnology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[4]*Institute of Natural Product Chemistry, 91190 Gif-sur-Yvette Cedex, France*

Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

Six compounds were isolated from the leaves of *Viburnum sambucinum* Reinw. ex Blume (Caprifoliaceae). The structures of the isolates were determined by spectroscopic analysis including MS and NMR. Accordingly, the isolated compounds were identified as α-amyrin (**1**), ursolic acid (**2**), 3β,28-dihydroxy-urs-12-ene (**3**), oleanolic acid (**4**), 16β-hydroxylup-20(29)-ene-3-one (**5**) and *trans*-2-phyten-1-ol (**6**).

Keywords. *Viburnum sambucinum*, terpenoids, Caprifoliaceae.

1. INTRODUCTION

Viburnum genus (Caprifoliaceae) comprises about 200 species, which are widely distributed in Southern America and Asia [1]. Several species of this genus are employed as folk medicines to treat different diseases, such as cough, diarrhea, rheumatoid arthritis, and tumefaction. Recently, much attention has been paid to *Viburnum* genus and their chemical constituents because of their multifaceted activities. Previous studies of the *Viburnum* genus have led to the identification of secondary metabolites with various structures such as monoterpenes, diterpenes, sesquiterpenes, triterpenes, iridoids, flavonoids and lignans [2-5]. As part of our study in the search for new bioactive compounds from plants of Vietnam under the international cooperative program (LIA-NAPROCHEMLAB), a plant extract (VN1681, *Viburnum sambucinum*, Reinw. ex. Blume, Caprifoliaceae) collected from Sa Pa, Lao Cai was found to inhibit the growth of KB cell line (51 % inhibition at 1.0 μg/mL). Since a literature review showed that no chemical study of this plant had previously been reported, we selected this species for further studies. In this paper, we describe the isolation and structural characterizations of 6 terpenoids, **1**-**6** from the leaves of *Viburnum sambucinum*.

2. EXPERIMENTAL

2.1. General experimental procedures

Optical rotations were measured on a Polax-2 L polarimeter. Melting points were determined using a Buchi B-545 instrument. ESI-MS were obtained on an Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on Bruker 500.13 MHz spectrometer, operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C NMR, respectively.

2.2. Plant material

Leaves of *V. sambucinum* Reinw. ex. Blume. were collected in Sa Pa, Lao Cai province, Vietnam in July 2006. A voucher specimen (VN-1681) was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy Science and Technology (VAST).

2.3. Extraction and isolation

Dry powdered leaves of *V. sambucinum* (1.20 kg) was successively extracted with *n*-hexane, EtOAc and methanol. The *n*-hexane, EtOAc and methanol solutions were concentrated to dryness to obtain crude extracts.

EtOAc crude extract (65.0 g) was subjected to column chromatography (CC) on silica gel, eluted with *n*-hexane/EtOAc gradient to yield 12 fractions. Fraction 2 (0.9 g) was separated by CC on silica gel using *n*-hexane/EtOAc gradient to provide 5 subfractions. Subfractions 5 (300 mg) was subjected to CC on Sephadex LH-20 (CH_2Cl_2/MeOH: 2/8) following by CC on silica gel (*n*-hexane/acetone: 95/5) to give **1** (11 mg) and **6** (15 mg). Fraction 3 (2.1 g) was separated by silica gel CC (*n*-hexane/acetone gradient) to yield 3 subfrations. Subfraction 1 (150 mg) was recrystallized in *n*-hexane to afford compound **5** (7 mg). Subfraction 3 (130 mg) was subjected to silica gel CC, eluted with *n*-hexane/acetone to furnish compound **3** (8 mg). Fraction 4 (1.75 g) was chromatographed on a silica gel CC, using *n*-hexane/EtOAc gradient to give 3 subfractions. Subfraction 3 (210 mg) was purified by CC on silica gel (CH_2Cl_2/acetone gradient) to provide **4** (5 mg) and **2** (7 mg).

α-Amyrin (1): white powder; ESI-MS: *m/z* 427 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 0.78 (3H, d, *J* = 7.0 Hz, CH_3), 0.79 (3H, s, CH_3), 0.80 (3H, d, *J* = 7.0 Hz), 0.91 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.07 (3H, s, CH_3), 3.22 (1H, dd, *J* = 5.0; 11.0 Hz), 5.13 (1H, t, *J* = 3.5 Hz).

Ursolic acid (2): white powder; ESI-MS: *m/z* 439 $[M-H_2O+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$+ CD_3OD): δ_H (ppm) 0.70 (3H, s, CH_3), 0.74 (3H, s, CH_3), 0.78 (3H, d, *J* = 6.5 Hz, CH_3), 0.83 (3H, d, *J* = 6.5 Hz, CH_3), 0.85 (3H, s, CH_3), 0.91 (3H, s, CH_3), 1.01 (3H, s, CH_3), 3.12 (1H, dd, *J* = 1.5; 11.0 Hz, H-3), 5.2 (1H, t, *J* = 3.0 Hz, H-12).

3*β*,28-dihydroxy-urs-12-ene (3): white crystals; ESI-MS: *m/z* 443 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 0.79 (3H, s, CH_3), 0.81 (3H, d, *J* = 4.5 Hz, CH_3), 0.93 (3H, d, *J* = 4.5 Hz, CH_3), 0.96 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.09 (3H, s, CH_3), 3.18 (1H, d, *J* = 11.0 Hz, H-28a), 3.24 (1H, d, *J* = 11.0 Hz, H-28b), 3.53 (1H, dd, *J* = 1.5; 11.0 Hz, H-3), 5.13 (1H, t, *J* = 3.0 Hz, H-12).

Oleanolic acid (4): white solid**;** ESI-MS: *m/z* 439 $[M-H_2O+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$ + CD_3OD): δ_H (ppm) 0.77 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.15 (3H, s, CH_3), 2.84 (1H, dd, *J* = 4.0; 13.5 Hz, H-18), 3.17 (1H, dd, *J* = 5.0; 11.0 Hz, H-3), 5.26 (1H, t, *J* = 3.5 Hz, H-12).

16β-hydroxylup-20(29)-ene-3-one (5): white needle crystals, m.p 170-171 ^{o}C; ESI-MS: *m/z* 439 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 0.80 (3H, s, CH_3), 0.93 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.68 (3H, s, CH_3), 3.61 (1H, dd, *J* = 4.5; 11.0 Hz, H-16), 4.60 (1H, dq, *J* = 1.5; 2.5 Hz, H-29a), 4.70 (1H, d, *J* = 2.0 Hz, H-29b).

***Trans*-2-phyten-1-ol (6):** colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 0.84 (3H, d, *J* = 6.5 Hz, CH_3), 0.85 (3H, d, *J* = 6.4 Hz, CH_3), 0.87 (3H, d, *J* = 6.4 Hz, CH_3), 1.67 (3H, s, CH_3), 1.96 (2H, m, CH_2), 4.15 (2H, d, *J* = 7.0 Hz, CH_2), 5.41 (1H, dt, *J* = 1.0, 7.0 Hz).

3. RESULTS AND DISCUSION

Compound **1** was isolated as white powder. Its ^{1}H-NMR presented signals of 8 methyl groups (6 singlets and two doublets), an oxygenated methine at δ_H 3.22 (dd, *J* = 5.0, 11.0 Hz, H-3), an olefinic proton at δ_H 5.13 (t, *J* = 3.5 Hz, H-12) and a complex pattern of aliphatic protons. The ^{13}C-NMR and DEPT spectra of **1** presented signals of 30 carbons, including 8 methyls, 9 methylenes, 6 sp^3 methines (one of which was bonded to oxygen as suggested by its chemical shifts: δ_C 79.1, δ_H 3.22), a double bond, and five sp^3 quaternary carbons. This observation suggested that compound **1** was a triterpene belonging to ursane skeleton. The H-3 was determined to be axial disposition by its strong coupling constant (*J* = 11.0 Hz). Careful analyses of NMR spectra and comparison with reported values in the literature allowed establishing the structure of **1** as α-amyrin [6].

^{1}H-NMR spectrum of **2** exhibited signals close to those of **1**, except for the absence of one singlet methyl group in comparison with **1**. In addition, the signal of a carboxylic group at δ_C 180.6 was noted in the ^{13}C-NMR spectrum of **2**. This analysis strongly suggested that one methyl group of **1** was oxidized into carboxylic functionality to afford compound **2**. Comparison of NMR data of **2** with those reported for ursolic acid [7] revealed that they were identical.

Compound **3** was obtained as a white crystal. Comparison of the 1D-NMR spectra of **3** with those of **1** indicated that compound **3** had the same skeleton with **1**, except for the presence of an oxymethylene (δ_C 69.9, δ_H 3.18 and 3.24) instead of a singlet methyl. Detailed analyses of NMR spectra revealed the structure of **3** as 3*β*,28-dihydroxy-urs-12-ene. The NMR data of **3** were in agreement with those reported in the literature [8, 9].

Compound **4** was isolated as a white solid. The ^{1}H NMR of **4** indicated signals of 7 singlet methyl groups, an olefinic proton [δ_H 5.26 (1H, t, *J* = 3.5 Hz, H-12)], an oxygenated methine [δ_H 3.17 (1H, dd, *J* = 5.0 and 11.0 Hz, H-3)] and signals of overlapping aliphatic protons. The ^{13}C NMR and DEPT spectra of **4** displayed signals of 30 carbons, including 7 methyls, 10 methylenes, 4 sp^3 methines

(one of which was attached to oxygen as suggested by its chemical shifts: δ_C 79.3, δ_H 3.17), a double bond, 6 sp^3 quaternary carbons and a carboxylic functionality (δ_C 181.6). These NMR signals suggested that **4** should be a triterpene of olean skeleton with a methyl group oxidizing into carboxylic functionality. Careful analysis of NMR spectra and comparison with the literature allowed determining the structure of **4** as oleanolic acid [7].

Compound **5** was isolated as a white needle crystal**,** mp. 170-171 ^oC. Its ^1H-NMR spectrum exhibited signals of 7 singlet methyl group, an oxymethine [δ_H 3.61 (dd, J = 4.5, 11.0 Hz, H-16)], two olefinic protons [δ_H 4.60 (dd, J = 1.5, 2.5 Hz, H_a-29), 4.70 (br. d, J = 2.5 Hz, H_b-29)] and overlapping aliphatic proton signals. Analysis of ^{13}C NMR and DEPT spectra suggested **5** was a lupane-type triterpenoid. This was confirmed by interpretation of 2D NMR spectra. The ketone function at C-3 and hydroxyl group at C-16 were established from HMBC spectrum analysis. Thus, compound **5** was 16*β*-hydroxylup-20(29)-ene-3-one [10].

Compound **6** was obtained as oil. In the ^1H-NMR spectrum, signals of 4 doublet methyl groups, a singlet methyl, an olefinic proton (δ_H 5.41), an oxygenated methylene [δ_H 4.15 (d, J = 7.0 Hz, CH_2-1)] and a number of overlapping aliphatic protons were observed. Its ^{13}C-NMR and DEPT spectra indicated signals of groups observed in the ^1H NMR spectrum with additional signals of an olefinic quaternary carbon (δ_C 140.3) and 9 methylene groups. The chemical shifts of the methyl CH_3-17 (δ_H 19.7, δ_H 1.67) suggested its linkage to a double bond. Complete analysis of NMR spectra established the structure of **6** as *trans*-2-phyten-1-ol [11].

Table 1: ^{13}C NMR data of compounds **1-6**

position	**1**	**2**	**3**	**4**	**5**	**6**
1	38.8	38.1	38.7	39.3	39.6	59.4
2	28.1	27.9	26.0	27.4	34.1	123.1
3	79.1	78.8	79.1	79.3	218.0	140.3
4	38.9	39.4	38.8	39.4	47.3	39.9
5	55.2	52.7	55.2	56.2	54.9	25.2
6	18.2	18.2	18.3	19.1	19.7	36.7
7	32.9	32.9	32.8	33.3	33.6	32.7
8	40.0	39.4	40.0	40.0	40.9	37.4
9	48.7	47.7	47.7	48.6	49.4	24.5
10	36.9	36.7	36.9	37.7	36.8	37.5
11	23.3	23.2	23.4	23.7	21.4	32.8
12	124.4	125.4	125.1	123.1	24.8	37.3
13	139.6	138.1	138.7	144.7	37.4	24.8
14	42.1	41.9	42.1	42.1	44.1	39.4
15	27.3	26.7	27.3	28.4	36.8	28.0
16	26.6	24.2	23.1	24.1	76.9	16.2
17	33.8	47.5	38.7	47.2	48.6	19.7
18	59.3	55.1	54.0	42.5	47.7	22.7
19	39.7	38.9	39.3	46.7	47.6	-
20	39.6	38.7	39.4	30.4	149.9	-
21	31.3	30.6	30.6	34.6	29.9	-
22	41.5	36.8	35.2	33.5	37.7	-
23	28.1	23.4	28.2	28.5	26.6	-
24	15.7	16.5	15.7	15.7	21.0	-
25	15.6	15.4	15.6	16.1	16.1	-
26	16.8	16.8	16.8	17.4	15.8	-
27	23.4	23.4	23.3	26.4	15.9	-
28	28.7	180.6	69.9	181.6	11.7	-
29	17.5	16.8	17.4	33.4	109.9	-
30	22.1	21.0	21.3	23.9	19.3	-

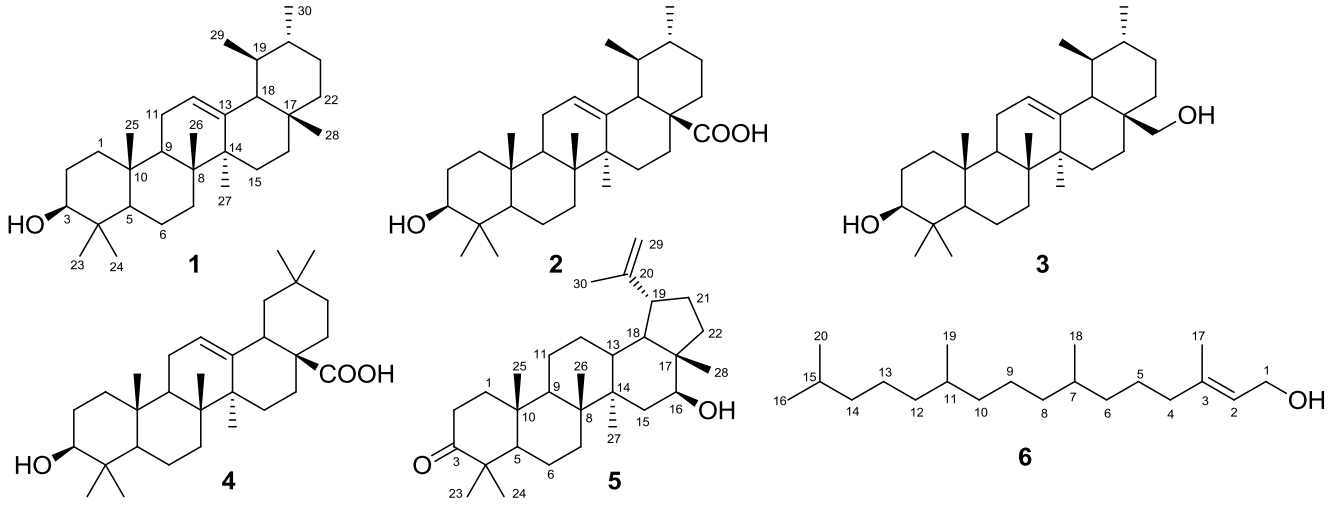

Figure 1: Structures of compounds **1-6** from *V. sambucinum*

Acknowledgements. *The authors thank Dr Nguyen Quoc Binh and MSc Dao Dinh Cuong for plant collection and botanical determination. The Vietnam Academy of Science and Technology (VAST), Vietnam and the National Center for Scientific Research, France (CNRS) are gratefully acknowledged for support of the LIA project NATPROCHEMLAB.*

REFERENCES

1. X. Y. Wang, H. M. Shi, X. B. Li. *Chemical Constituents of Plants from the Genus Viburnum*, Chem. Biodivers., **7**, 567-593 (2010).
2. K. E. Bae, H. S. Chong, D. S. Kim, Y. W. Choi, Y. S. Kim, Y. K. Kim. *Compounds from Viburnum sargentii Koehne and evaluation of their cytotoxic effects on human cancer cell lines*, Molecules, **15**, 4599-4609 (2010).
3. Mona A. Mohamed, Mohamed S. A. Marzouk, Fatma A. Moharram, Mortada M. El-Sayed, Ayman R. Baiuomy. *Phytochemical constituents and hepatoprotective activity of Viburnum tinus.* Phytochemistry, **66**, 2780-2786 (2005).
4. Xuan-Qin Chen et al. *Triterpenoids and diterpenoids from Viburnum chingii,* Chem. Pharm. Bull, **59(4)**, 496-498 (2011).
5. A. A. El-Gamal, S. K. Wang, C. Y. Duh. *New diterpenoids from Viburnum awabuki*, J. Nat. Prod., **67(3)**, 333-336 (2004).
6. S. P. C. Venkata, P. Indra. *Ursane and olenane triterpenes from Astragalus propinquus*, International Journal of Research and Development in Pharmacy and Life Science, **2(4)**, 503-506 (2013).
7. G. Zuhal, O. Hilal, K. Ayse, K. Cavit, L. Demirezer. *Secondary metabolites from Nepeta heliotropiflolia*, Turk. J. Chem, **33**, 667-675 (2009).
8. Q. Y. Liu, Y. S. Chen, F. Wang, S. W. Chen, Y. H. Zhang. *Chemical of Vitex trifolia*, Journal of Chinese Materia Medica, **39(11)**, 2024-2028 (2014).
9. Siddiqui S, Hafeez F, Begur S, Siddiqui BS. *Kaneric Acid, a New Triterpene from the Leaves of Nerium oleander*, J. Nat. Prod., **49**, 1086 (1986).
10. G. P. Gwendoli, F. B. Wendy, J. M. Gonzalo, Q. Leovigildo. *Constituents of Acacia cedilloi and Acacia gaumeri. Revised structure and complete NMR assignments of resione,* Z. Naurforsch, **57c**, 773-776 (2002).
11. W. Ho-Fai, D. B. Geoffrey. *Photo-oxygenation of phytol and the structure revision of phytene-1,2-diol from Atemisia annua to phytene-1-ol-2-hydroperoxide*, J. Chem. Research, **2002(1)**, 30-33 (2002).

Corresponding author: **Pham Van Cuong**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: phamvc@imbc.vast.vn.