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Flavonoids and other compound isolated from leaves of *Aconitum carmichaeli* Debx. growing in Viet Nam

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ABSTRACT

*Two flavonol glycosides: 5,7,3'-trimethoxyquercetin -3-O-β-D-fructofuranoside (**1**), 7,4'-O-dimethylluteolin 5-O-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside] (**2**) and one fatty acid ester (Z)-3-hydroxypentan-2-yl-10-aminooctacos-9-enoate (**3**) were isolated from the leaves of Aconitum carmichaeli Debx growing in Ha Giang province, Vietnam. Their structures were elucidated by spectroscopic methods, including IR, MS and NMR. This is first time these compounds were isolated from leaves of A. carmichaeli Debx.*

Keywords: *Aconitum carmichaeli*, flavonoids, trimethoxyquercetin, dimethylluteolin

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INTRODUCTION

The *Aconitum* L. genus which is mainly characterized by the presence of highly toxic diterpene alkaloids has been traditionally used in China as source of arrow poison for 2000 years. The alkaloid composition of different European *Aconitum* species has been analyzed in some reports. In Vietnam, Bui Hong Cuong has been reported to chemical compositions of *A. carmichaeli* Dexb. var. *carmichaeli* collected in Sa Pa, Lao Cai province including alkaloids, acid amin, free sugars, organic acids. Its rhizomes contain fatty acid, sterol and its stems contain carotenoids, its leaves and flowers present carotenoids, sterols, flavonoids. Several alkaloids named karacolin, benzoylmesaconin also reported in this study [1]. In the last years the study of *Aconitum* genus lead to the other secondary metabolites such as flavonoids. The flavonol composition of the aerial parts of *A. jaluense*, *A. pseudolaeve* and *A. chiisanense* have been reported [2]. Regarding the European species, the flowers of *A. paniculatum* have shown the presence of three flavonol glycosides [3]. However, there have been no reports of flavonoid compositions from *A. carmichaeli* collected in Ha Giang province, Viet Nam. In this study we describe the isolation and structure elucidation of compounds isolated from the leaves of *Aconitum carmichaeli* Dexb**.**

EXPERIMENTAL SECTION

Plant material

The leaves of *Aconitum Carmichaeli* Debx.were collected in Ha Giang province, Viet Nam during 2012 and authenticated by the National Institute of Medicinal Materials (NIMM). A voucher specimen has been deposited in the NIMM.

General experimental procedures

Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB Waegetechnik Rapido, Germany). The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). The NMR [^{1}H (500 MHz), ^{13}C (125 MHz), and DEPT-90 and 135 MHz)] spectrum were recorded on an AVANCE spectrometer AV 500 (Brucker, Germany) in the Institute of Chemistry, Viet Nam Academy of Science and Technology (VAST). Chemical shifts were reported in ppm downfield from TMS with *J* in Hz. Electrospray Ionization Mass Spectrum (ESI-MS) were recorded on a Varian Agilent 1100 LC-MSD mass spectrometer. Analytical TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F_{254} (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 254 and 365 nm) and by spraying with 10% H_2SO_4, followed by heating with a heat gun. Column chromatography was performed on silica gel (70–230 and 230–400 mesh, Merck). Organic solvents were of analytical grade.

Extraction and isolation

The dried and powdered leaves of *A. carmichaeli* (1.5 kg) were extracted with 96% ethanol (5L x 4 times) at room temperature. The ethanol extract were combined, filtrated, and evaporated to dryness *in vacuum* at 40°C. The residue (80 g) was suspended in water and then partitioned with EtOAc. The EtOAc extract (15 g) was subjected to column chromatography (CC) over silica gel with a gradient solvent system [*n*-hexane : EtOAc (EtOAc 0%-100%) and EtOAc : MeOH (0%-80%)] to give 5 fractions: A, B, C, D, E. The fraction B (0.8 g) was subjected to repeated silica gel CC and eluted with $CHCl_3$ - EtOAc (90:10) to give compound **3** (16 mg)**.** The fraction E (2.1 g) were subjected to repeat silica gel CC and eluted with MeOH - EtOAc (90:10) to yield compound **1** (12 mg) and compound **2** (22 mg).

Compound 1: 5,7,3'-trimethoxyquercetin -3-*O*-*β*-D-fructofuranoside

M= 506, R_f = 0.4 ($CHCl_3$- MeOH, 10:90). *IR* (KBr, v_{max} cm^{-1}): 3060 (OH), 2924 (CH), 1687 (C=O), 1073 (C–O–C). The ^{1}H-NMR (500 MHz, $CDCl_3$-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, $CDCl_3$-d6, δ, ppm, J/Hz) and DEPT data for compound **1** are presented in table 1.

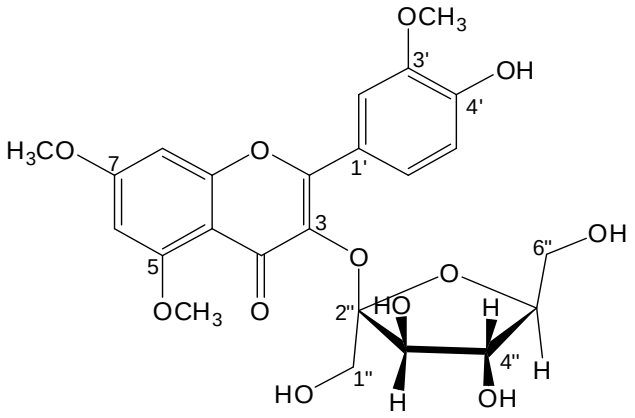

Figure 1. Structure of compound 1

Table 1. ^{1}H-NMR, ^{13}C-NMR and DEPT Data for compound 1

Position C	DEPT	δ_C ppm	δ_H ppm	HMBC (C→H)
2	C	156,2	-	
3	C	130,0	-	
4	C	177,47	-	
5	C	161,9	-	
6	CH	110,6	6,19 *s*	5; 7; 8; 10
7	C	162,2	-	
8	CH	110,9	6,79 *s*	6; 7; 9; 10
9	C	148,4	-	
10	C	111,3	-	
5-OCH_3	CH_3	55,8	3,42 *s*	5
7-OCH_3	CH_3	53,7	3,61 *s*	7
1’	C	129,1	-	
2’	CH	128,8	8,25 *s*	2; 3’; 4’; 6’
3’	C	145,1	-	
4’	C	147,5	-	
5’	CH	124,5	8,12 *d* (7,0)	1’
6’	CH	127,9	8,13 *d* (7,0)	2; 2’; 4’
3’-OCH_3 4’-OCH_3	CH_3	55,9	3,86 s	3’
1’’	CH_2	60,3	3,83 *dd* (2,5; 13,0); 3,43 *dd* (3,0; 12,5)	1; 2’; 3’
2’’	C	111,7	-	
3’’	CH	73,1	3,17 *dd* (4,0; 15,0)	
4’’	CH	67,9	4,46 *dd* (4,5; 14,5)	
5’’	CH	75,8	4,93 *dd* (4,0; 14)	
6’’	CH_2	60,2	3,09 *m*	4’; 5’

Compound 2: 7,4′-*O*-dimethylluteolin 5-*O*-[*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranoside]
M = 608, R_f = 0,3 ($CHCl_3$- MeOH, 15:85), ESI-MS: m/z 609,2 $[M+H]^+$, IR (KBr, ν_{max} cm^{-1}): 3414 (OH), 2936 (CH), 1725 (C=O), 1100 (C–O–C). The ^{1}H-NMR (500 MHz, $CDCl_3$-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, $CDCl_3$-d6, δ, ppm, J/Hz*)* and DEPT data for compound **2** are presented in table 2.

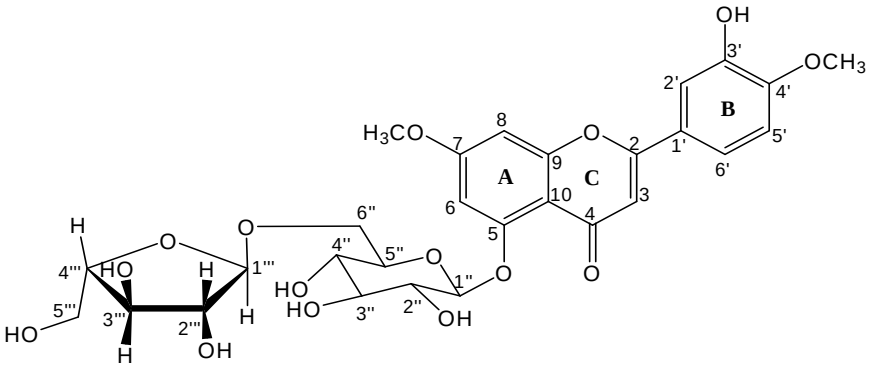

Figure 2. Structure of compound 2

Table 2. ^{1}H-NMR, ^{13}C-NMR and DEPT Data for compound 2

Position C	DEPT	δ_C ppm	δ_H ppm	HMBC (C→H)
2	C	165,0	-	
3	CH	106,3	6,61 *s*	2; 4; 1'; 2'
4	C	180,2	-	
5	C	159,5	-	
6	CH	104,3	6,90 *d* (2,0)	5; 7; 8; 10
7	C	165,9	-	
8	CH	97,5	6,95 *d* (2,0)	6; 7; 9; 10
9	C	160,6	-	
10	C	110,4	-	
7-OCH_3	CH_3	56,8	3,7 *s*	7
1'	C	121,3	-	
2'	CH	110,1	7,45 *d* (2,0)	2; 3'; 4'; 6'
3'	C	150,4	-	
4'	C	155,0	-	
5'	CH	117,6	6,89 *d* (7,5)	1'
6'	CH	122,0	7,51 *dd* (2,0, 8,0)	2; 2'; 4'
4'–OCH_3	CH_3	56,7	3,96 *s*	4'
1"	CH	104,8	4,86	5
2"	CH	74,8	3,62	
3"	CH	77,3	3,70	
4"	CH	71,8	3,42	
5"	CH	77,3	3,53 *t* (7,5)	
6"	CH_2	68,4	3,65	
1"'	CH	110,5	4,97 *s*	6"
2"'	CH	83,3	4,05	
3"'	CH	78,8	3,87 *dd* (3,5; 6,0)	
4"'	CH	85,7	4,01 *m*	
5"'	CH_2	63,0	3,61 3,75 *dd* (3,5; 12,0)	

Compound 3: (Z)-3-hydroxypentan-2-yl-10-aminooctacos-9-enoate

M=524, R_f = 0,3 (CH_2Cl_2 - EtOAc, 90:10), ESI-MS: m/z 547 $[M+Na]^+$, IR: (KBr, ν cm^{-1}): 3426, 3334 (NH_2); 2923, 2853 (CH); 1658 (C=O); 1625 (C=C); 1548 (C-N); 1089 (C-O-C). The ^{1}H-NMR (500 MHz, $CDCl_3$-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, $CDCl_3$-d6, δ, ppm, J/Hz) and DEPT data for compound **3** are presented in table 3.

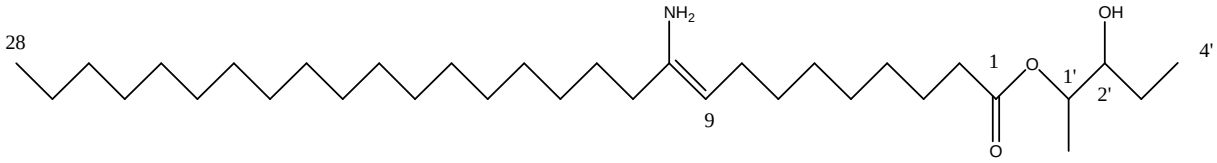

Figure 3. Structure of compound 3

Table 3.^{1}H-NMR, ^{13}C-NMR and DEPT Data for compound 3

Position C	DEPT	δ_C ppm	δ_H ppm
1	C=O	173,1	
2	CH_2	36,9	H-2a :2,28 d, (6,0) H-2b: 1,62 d, (7,0)
3	CH_2	26,0	1,44-1,45 m
4	CH_2	29,5	1,25 m
5	CH_2	29,5	1,25 m
6	CH_2	29,5	1,25 m
7	CH_2	29,5	1,25 m
8	CH_2	22,6	2,15-2,19 m
9	CH	118,2	5,69 d, (7,5)
10	C	162,1	
11	CH_2	49,4	1,50-1,60 m
12	CH_2	29,5	1,25 m
13	CH_2	29,5	1,25 m
14	CH_2	29,5	1,25 m
15	CH_2	29,5	1,25 m
16	CH_2	29,5	1,25 m
17	CH_2	29,5	1,25 m
18	CH_2	29,5	1,25 m
19	CH_2	29,5	1,25 m
20	CH_2	29,5	1,25 m
21	CH_2	29,5	1,25 m
22	CH_2	29,5	1,25 m
23	CH_2	29,5	1,25 m
24	CH_2	29,5	1,25 m
25	CH_2	29,5	1,25 m
26	CH_2	33,6	1,25 m
27	CH_2	25,8	1,25 m
28	CH_3	14,2	1,09 d, (7,0)
1'	CH	76,7	4,00-4,03 m
2'	CH	74,4	3,62 d, (5,0)
3'	CH_2	31,9	1,37-1,40 m
4'	CH_3	14,1	0,88 t, (14,0)

RESULTS AND DISCUSSION

Compound 1: ESI-MS spectrum gave the molecular ion peak at m/z 507,3 $[M+H]^+$, corresponded to molecular mass M= 506. The IR spectrum revealed the presence of hydroxyl (O-H) and conjugated carbonyl groups (C=O) at 3060 cm^{-1} and 1687 cm^{-1}, respectively. It also present the absorption bands at 2944 cm^{-1} (C-H) and 1073 cm^{-1} (C-O-C).

^{13}C-NMR, DEPT 90 and DEPT 135 spectrum of compound **1** showed signals for all 24 carbons. Among them, one signal belonged to carbonyl group at δ 177.4 ppm; fours signals belonged to aromatic carbons bearing an oxygen atom at δ 162,2; 148,9; 145,1; 147,5 ppm and three signals belonged to methoxy group at δ 55,9; 55,8; 53,7 ppm. Furthermore, ^{13}C-NMR spectrum showed six conjugated signals from δ 60,2 to δ 111,7 ppm indicating the presence of one sugar moiety. The J values (7.51 Hz) of the anomeric proton indicated β-orientations of the glycosidic linkages. Two signals at δ 60,3 and δ 60,2 ppm data permitted the identification of the sugar moiety as fructose.

^{1}H-NMR spectrum of compound **1** displayed signals for three aromatic protons at δ 8,25 (1H, s, H-2",), δ 8,13 (1H, d, J =7,0 Hz, H-5") and δ 8,12 (1H, d, J = 7,0 Hz, H-6"), indicating the presence of a 1,3,4-trisubstituted benzene ring. The remaining aromatic protons at δ 6.79 (1H, d, J=2.0 Hz, H-6) and δ6.19 (1H, d, J=2.0 Hz, H-8) showed the presence of a 1,2,3,5-tetrasubstituted benzene ring.^{1}H-NMR spectrum displayed the signal of one anomeric protons at δ 4,93 ppm (1H, dd, J=4,0; 14 Hz, H-5") suggested the presence of beta-fructose sugar moiety. Therefore, based on the above data and compared with previous published [4], the structure of compound **1** was established as 5,7,3'-trimethoxyquercetin-3-*O*-*β*-D-fructofuranosid (Fig. 1).

Compound 2 was obtained as a yellow powder. ESI-MS spectrum gave the molecular ion peak at m/z 609,2 $[M+H]^+$, corresponded to molecular mass M = 608. The IR spectrum revealed the presence of hydroxyl (O-H) and

conjugated carbonyl groups (C=O) at 3414 cm^{-1} and 1725 cm^{-1}, respectively. It also present the absorption bands at 2936 cm^{-1} (C-H) and 1100 cm^{-1}(C-O-C).

^{1}H-NMR spectrum displayed the signal corresponding to two aromatic rings of flavonol skeleton were observed at δ 6,62 (1H, s, H-3), and δ 6,90 (1H, d, J= 2,0; H-6) và δ 6,95 (1H, d, J= 2,0; H-8). The ^{1}H-NMR spectrum of **2** showed three signals assignable to ABX coupled aromatic protons at 1,3,4 positions at δ 7,45 (1H, d, J=2,0, H-2'); δ 6,89 (1H, d, J= 8,0; H-5'); δ 7,51 (1H, dd, J=2,0, 8,0; H-6'). Moreover, two methoxyl group δ 3,96 (3H, s, OCH_3) và δ 3,97 (3H, s, OCH_3) at were also presented. The J values (7.53 Hz) of the two anomeric protons indicated β-orientations of the glycosidic linkages. Furthermore, ^{1}H-NMR spectrum displayed the signal of two anomeric protons at δ 4,86 (1H, H-1") and δ 4,97 (H, s, H-1'") indicating the presence of two sugar moieties. The positions of the H-1" and H-1'" were confirmed by HSQC correlations between H-1" with δ 104,8 (C-1") and H-1'" with δ 110,5 (C-1'"). Four methylene protons of two groups oxymethylene in the two sugar moieties were revealed at δ 3,65 (2H, H-6", overlap) and δ 3,61 (1H, H_a-5'"), δ 3,75 (1H, dd, J=3,5; 12,0; H_b-5'"). The oxymethine proton signals were revealed at δ 3,62 (1H, H-2"); δ 3,70 (1H, H-3"); δ 3,42 (1H, H-4"); δ 3,53 (1H, H-5"); δ 4,05 (1H, H-2'"); δ 3,87 (1H, dd, J=3,5; 6,0; H-3'"); δ 4,01 (1H, m, H-4'"). The sugar moieties were confirmed through correlations in HSQC spectrum. ^{13}C-NMR spectrum showed six conjugated signals at δ 104,8 (C-1"); δ 74,8 (C-2"); δ 77,3 (C-3"); δ 71,8 (C-4"); δ 77,3 (C-5"); δ 68,4 (C-6") indicating the molecular of glucose. In the ^{13}C NMR spectrum, the downfield signal at δ 68,4 (C-6") indicated a ether group. Beside the presence of 17 carbon atoms of aglycon moiety (including two signals of methoxyl groups) there were 5 signals at δ 110,5 (CH, C-1'"); δ 83,3 (CH, C-2'"); δ 78,8 (CH, C-3'"); δ 85,7 (CH, C-4'"); δ 63,0 (CH_2, C-5'"). From this data, associated with the ^{13}C-NMR data, it was obvious that the sugar unit was *O*-[-L-arabinofuranosyl-(16)-*β*-D-glucopyranosid]. The positions of substituted groups were confirmed by ^{1}H-^{13}C correlation in HMBC. The correlation of proton H-1" and C-5; group methoxyl and C-7 indicated position of sugar moiety at C-5 and group methoxyl at C-7 in ring A. Also the correlations of proton H-1"' and C-6" was further confirmed the positions linked of two molecular sugar.

In the ring B, position of methoxyl group at C-4' was confirmed through the correlation between protons in methoxyl group and C-4'; H-6' and C-4'. This indicated that C-6' and carbon (-hydroxy) are in para positions. Based upon these evidences and compared with previous published [5], compound **2** was established as **7,4'-*O*-dimethylluteolin 5-*O*-[*α*-L-arabinofuranosyl-(16)-*β*-D-glucopyranosid]** (Fig. 2).

Compound 3: ESI-MS spectrum gave the molecular ion peak at m/z 547547 $[M+Na]^+$, corresponded to molecular mass M= 524 and molecular formula $C_{33}H_{65}NO_3$. The compound has absorption bands at 3426, 3334 cm^{-1} (NH_2); 2923, 2853 cm^{-1} (C-H); 1658 cm^{-1} (C=O); 1625 (C=C, *cis*); 1548 cm^{-1} (C-N); 1089 cm^{-1} (C-O-C) in its IR spectrum. The ^{1}H-NMR spectrum of **3** showed signal doublet with *J* = 7,5 Hz at δ 5,69 indicated a *cis* double bond.

The ^{1}H-NMR spectrum of **3** displayed signals corresponding to methyl group bonds to methine at δ 1,09 ppm *(J* =7,0 Hz), two methyl groups bond to methylen groups (*J* = 14 Hz), two signals of methinegroups at δ 4,00-4,03 and δ 3,62 (d, *J* = 5 Hz) and 25 signals of methylen groups. The ^{13}C-NMR, DEPT 90 and DEPT 135 spectrum of compound **3** showed signals for all 33 carbons. Among them, one signal belonged to carbonyl group at δ 173,1 ppm; one quaternary carbon signal belonged to the olefinic carbon bond to N at δ 162.1 ppm; two signals of methine group bearing an oxygen atom at δ 76,7 ppm and δ 74,4 ppm; three signals of methyl group and 25 signals of methylene group. Based upon these evidences and compared with previous published [6, 7], compound **3** was established as **(Z)-3-hydroxypentan-2-yl 10-aminooctacos-9-enoate** (Fig. 3).

CONCLUSION

Our phytochemical study of the 96% ethanol extracts of the leaves of *A. carmichaeli* Dexb. collected in Ha Giang province, Vietnam has resulted in the isolation of three compounds: 5,7,3'-trimethoxyquercetin-3-*O*-*β*-D-fructofuranoside (**1**), 7,4′-*O*-dimethylluteolin 5-*O*-[*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranoside] (**2**) and (Z)-3-hydroxypentan-2-yl-10-aminooctacos-9-enoate (**3**). These were isolated for the first timefrom leaves of *A. carmichaeli* Dexb. Our results could be beneficial for the search of new chemical agents from Vietnamese plants.

Acknowledgments
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