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SOME DI- AND TRITERPENES OF *WEDELIA URTICAEFOLIA* (BL.) (ASTERACEAE)

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NGUYEN THANH HOANG, VO THI LINH CHI, NGUYEN KIM PHI PHUNG

Department of Chemistry, UNS, National University Ho Chi Minh City

ABSTRACT

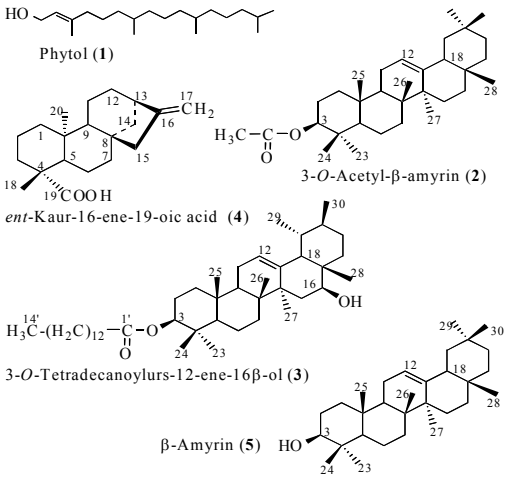
*Wedelia urticaefolia Bl. DC. (Vietnamese name Sơn cúc nhám) has not yet been chemically studied. From leaves of this plant, four compounds were isolated: phytol (**1**), 3β-O-acetyl-β-amyrin (**2**), 3-O-tetradecanoylurs-12-ene-16β-ol (**3**), ent-kaur-16-ene-19-oic acid (**4**) and from the flowers, three compounds were isolated: β-amyrin (**5**), stigmasterol (**6**) and 3-O-tetradecanoylurs-12-ene-16β-ol (**3**). Their chemical structures were established by spectroscopic analysis.*

I - INTRODUCTION

In the continuation of a chemical study on the genus *Wedelia* we now report the isolation and characterization of some di- and triterpenoids from *Wedelia urticaefolia* (Bl.) DC, the plant grows widely in the urban of Ho Chi Minh city. As far as we know no chemical research has been reported on this plant.

A review on the chemical constituents of the genus *Wedelia* showed that this genus contained derivatives of *ent*-kaur-16-ene-19-oic acid, ursolic acid and derivatives of oleanolic acid. The last one often contained long side chain ester at C-3 [1 - 4]. Our research showed that *Wedelia urticaefolia* (Bl.) DC also agrees with the phytochemistry of the genus *Wedelia* so far investigated but this plant contained ursane-type-triterpene with aliphatic long chain ester at C-3.

Part of the plant *Wedelia urticaefolia*



II - EXPERIMETAL

1. General

Melting points were determined on a block maquene apparatus and uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer FT-IR 2000 spectrometer. ^{1}H- and ^{13}C-NMR were recorded on Bruker Avance 500 MHz and 125 MHz, respectively. MS spectra were carried out on Agilent-MSD-Trap-SL.

2. Plant material

Leaves and flowers of *Wedelia urticaefolia* were separatedly collected in Ho Chi Minh City in April 2006. A voucher specimen was prepared and deposited by Mr. Phan Duc Binh, University of Medicine—Ho Chi Minh City.

3. Extraction and isolation

Dried and powdered leave (1100 g) of *Wedelia urticaefolia* was exhaustively extracted with ethanol at room temperature to yield the ean cethanolic crude extract (79g). The crude extract was subjected successively to silica gel solid phase extraction to obtain petroleum ether, chloroform, ethyl acetate and methanol fractions. The chloroform fraction of leave (12.5g) was chromatographed on silica gel column eluting with increasing amount of ethyl acetate in petroleum ether to yield seven fractions. Fraction 3 was carried out column chromatography with $CHCl_3$-EtOAc (9: 1) to yield (**1**, 22 mg) and (**2**, 22 mg). Fraction 5 was carried out column chromatography with $CHCl_3$-EtOAc (8:2) to yield (**3**) (11 mg). The ethyl acetate fraction of leave (7.7 g) was chromatographed on silica gel column eluting with increasing amount of ethyl acetate in chloroform to yield six fractions. Fraction 3 was carried out column chromatography with EtOAc: methanol (9:1) to yield (**4**) (8.2 mg).

Dried and powdered flower (850 g) was exhaustively extracted with ethanol at room temperature. During the removal under reduced pressure the ethanolic solution gave a prepicitate (2.4 g). This precipitate was chromatographed on silica gel 60H using mixture of petroleum ether: ethyl acetate as eluant and eight main fractions were collected. Fraction 3 (0.5g, eluted by chloroform: ethyl acetate 8:2) was rechromatographed on silica gel eluting with the same eluant, then preparative TLC and finally recrystallized in appropriate solvent, afforded (**5**, 11.8 mg) and (**3**, 21.4 mg), respectively.

Phytol (1): White powder. M.p. 206 - 209°C. EI-MS: *m/z* = 284 $[M]^+$. IR (KBr), ν_{max} cm^{-1}: 3613 (O-H), 1638 (C=C), 1095 (C-O). ^{1}H-NMR ($CDCl_3$), δ ppm = 5.40 (1H, t, =CH), 4.16 (2H, d, *J*=7.0 Hz, =CH-C<u>H</u>$_2$-OH), 1.9 - 0.8 (-CH, -CH_2, -CH_3). ^{13}C-NMR ($CDCl_3$), δ ppm = 59.4 (-CH_2OH, C1), 123.1 (=CH, C2), 140.33 (C=, C3), 39.9 (-CH_2-, C4), 25.2 (-CH_2-, C5), 36.7 (-CH_2-, C6), 32.7 (-CH, C7), 37.4 (-CH_2-, C8), 24.5 (-CH_2-, C9), 37.4 (-CH_2-, C10), 32.8 (-CH, C11), 37.3 (-CH_2-, C12), 24.8 (-CH_2-, C13), 39.4 (-CH_2-, C14), 27.9 (-CH, C15), 22.6 (C16), 22.7 (-CH_3, C17), 19.7 (-CH_3, C18), 19.7 (-CH_3, C19) and 16.2 (-CH_3, C20).

β-Amyrin acetate (2): White powder. IR(KBr), ν_{max} cm^{-1}: 3505 (O-H), 1734 (C=O), 1248 (C-O). ^{1}H-NMR ($CDCl_3$), δ ppm = 5.18 (1H, t, =CH, H12), 4.50 (2H, dd, *J*=3.5, 7.0 Hz, H-3), 2.08 (3H, s, H2'), 1.11 (3H, s, H-27), 0.97 (3H, s, H-26), 0.94 (3H, s, H-26), 0.94 (3H, s, H-25), 0.88 (6H, s, H-29; H-30), 0.84 (3H, s, H-28), 0.72 (3H, s, H-24). ^{13}C-NMR, $CDCl_3$, δppm: 38.3 (C1), 26.7 (C2), 81.0 (C3), 37.7 (C4), 55.3 (C5), 18.3 (C6), 32.6 (C7), 39.9 (C8), 47.6 (C9), 37.0 (C10), 23.6 (C11), 121.7 (C12), 145.2 (C13), 41.7 (C14), 27.0 (C15), 26.2 (C16), 32.5 (C17), 47.3 (C18), 46.8 (C19), 31.1 (C20), 34.8 (C21), 37.2 (C22), 28.4 (C23), 16.8 (C24), 15.6 (C25), 16.7 (C26), 25.9 (C27), 28.1 (C28), 33.3 (C29), 23.6 (C30), 171.02 (C-1') and 21.3 (C-2').

3-*O*-Tetradecanoylurs-12-ene-16β-ol (3): Yellow oil. ESI-MS (Positive mode): *m/z* = 635 $[M+H-H_2O]^+$. ($C_{44}H_{76}O_3$). IR (KBr), ν_{max} cm^{-1}: 3449 (O-H), 1729 (C=O), 1049 (C-O). The ^{1}H-, ^{13}C and HMBC-NMR were presented in table 1 and figure 1.

***ent*-Kaur-16-ene-19-oic acid (4)**: mp. 196ºC. $[\alpha]_D$ = - 91 (C=2, CH_2Cl_2). IR(KBr) ν_{max} cm^{-1}: 3444 (O-H), 1690 (C=O of COOH), 1654 (C=C), 1261 (C-O). LC-MS-ESI: *m/z* = 303 $[M+H]^+$, 256 [M-HCOOH]. ^{1}H-, ^{13}C and

HMBC-NMR were presented in table 2.

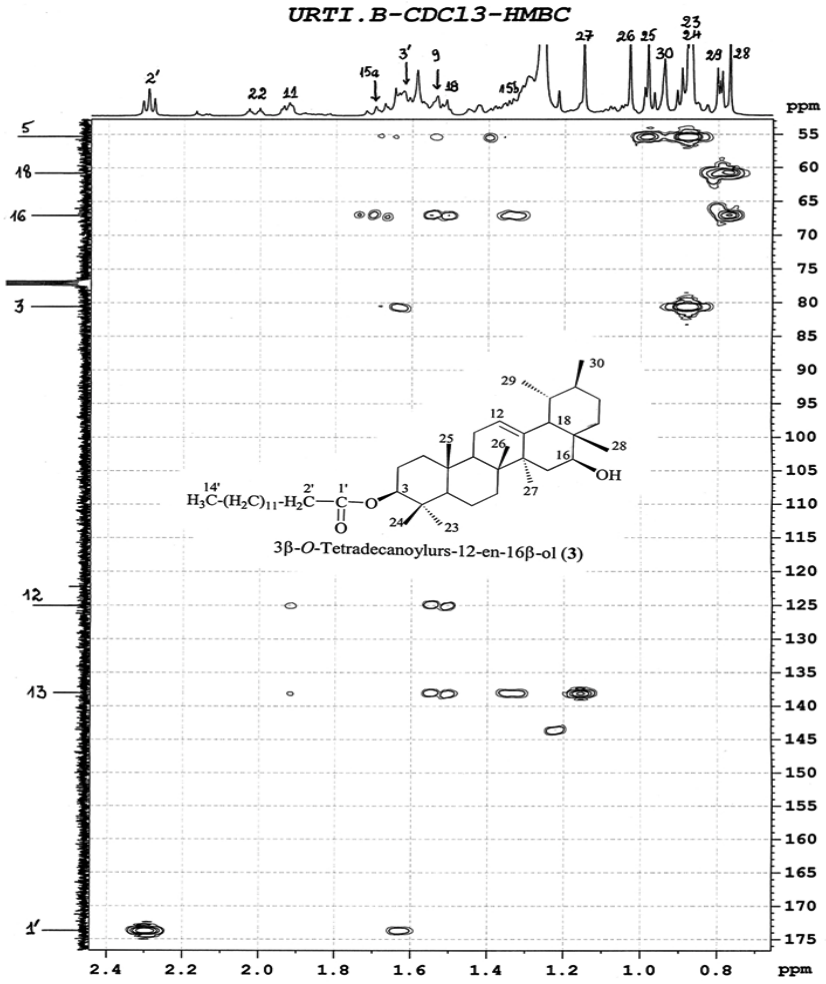


Figure 1: Part of HMBC-NMR of (**3**)

β-Amyrin (5): mp. 178°C. ^{1}H-NMR, $CDCl_3$, δppm: 5.18 (1H, t, *J* = 3.5 Hz, H-12), 3.22 (1H, t, *J*= 6.0 Hz, H-3), 1.13 (3H, s, H-27), 0.97 (3H, s, H-26), 0.94 (3H, s, H-26), 0.94 (3H, s, H-25), 0.87 (6H, s, H-29; H-30), 0.83 (3H, s, H-28), 0.79 (3H, s, H-24). ^{13}C-NMR, $CDCl_3$, δppm: 38.6 (C1), 26.9 (C2), 79.1 (C3), 38.8 (C4), 55.2 (C5), 18.4 (C6), 32.7 (C7), 39.8 (C8), 47.7 (C9), 36.9 (C10), 23.6 (C11), 121.7 (C12), 145.2 (C13), 41.8 (C14), 26.2 (C15), 27.3 (C16), 32.5 (C17), 47.3 (C18), 46.8 (C19), 31.1 (C20), 34.7 (C21), 37.2 (C22), 28.1 (C23), 15.5 (C24), 15.6 (C25), 16.8 (C26), 26.0 (C27), 28.4 (C28), 33.4 (C29) and 23.7 (C30).

III - RESULTS AND DISCUSSION

The determination of the chemical structure of the oleanane type of this plant was easy by recognizing the characteristic resonances at low field with δppm 171.0, 145.2, 121.6, 80.9, 55.3 (for β-amyrin acetate), 145.2, 121.7, 79.1, 55.2 (for β-amyrin). Their spectroscopic data well suited to the ones of authentic samples.

Plants of the genus *Wedelia* contained *ent*-kaur-16-ene-19-oic acid and its derivatives. In these derivatives, one of the carbons at C-2, 3, 9, 13, 15 of *ent*-kaur-16-ene-19-oic acid was oxygenated [1 - 6]. *Wedelia urticaefolia* also

contained *ent*-kaur-16-ene-19-oic acid and its structure was determined by spectroscopic method and the comparison with the one in *Wedelia glauca* [6]. Owing to the 1 and 2D-NMR, some chemical shifts of protons and carbons thirteen were corrected comparing to the data presented in the literature [6].

(**3**) was quickly recognized as an ursane type triterpene by the typical resonances at δppm 138.1 (quaternary =C) and 125.0 (=CH) of the double bond at C-12. A resonance at δppm 80.5 (CH-O) was oxygenated carbon C-3 as normal. The appearance of the second oxygenated carbon (CH-O) at δppm 67.0 caused

Table 1: Spectroscopic data of (**3**)

N	δ_C	δ_H (*J* in Hertz)	HMBC (H to C)
1	38.5		
2	25.2		
3	80.5	4.50 (1H, dd, 4.5,10.5)	C1, C1', C4
4	37.8		
5	55.3		
6	18.2		
7	32.8		
8	39.6		
9	46.9		
10	36.8		
11	23.4		C12, C13
12	125.1	5.19 (1H, t, 3.5)	C14
13	138.0		
14	44.1		
15	35.9		C13, C16
16	67.1	4.22 (1H, dd, 5.5, 11.5)	C28
17	38.6		
18	60.7	1.51 (1H, d, 6,0)	C12, C13, C16
19	40.1		
20	39.5		
21	30.5		
22	35.2		
23	28.1	1.03 (3H, s)	C3, C4, C5
24	16.8	0.94 (3H, s)	C3, C4, C5
25	15.7	0.77 (3H, s)	C1, C5, C9, C10
26	16.9	1.15 (3H, s)	C7, C8, C9, C14
27	24.5	1.25 (3H, s)	C4, C8, C13, C15
28	21.9	0.98 (3H, s)	C16, C17, C18
29	21.4	0.89 (3H, d, 3.0)	C18, C19, C20
30	17.6	0.87 (3H, d, 2.5)	C19, C20, C21
1'	173.7		
2'	34.8	2.29 (2H, t, 7.5)	C1', C2'
3'	31.9		C1'
4'-11'	29.8, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 29.2		
12'	23.6		
13'	22.7		
14'	14.1	0.90 (3H, t, 6.5)	C12', C13'

the difficulty in the determination the position of this carbon in the ursane skeleton. The HSQC, HMBC-NMR (figure 1) showed the correlation of the protons H-15, H-18, H-28 to C-16 so the second hydroxyl group was at C-16. The presence at the same time of the hydroxyl group at C-16 and the double bond at C-12 caused carbon C-18 down field to δppm 60.7. Proton H-18 had correlations to C-12, C13 and C16.

The hydroxyl group at C-3 was esterified to be —O-CO-R because the HMBC spectrum showed that H-3 (δppm 4.50) had the correlation with the resonant peak at δppm 173.7 (C=O, C-1'). This spectrum also showed the correlation of proton H-2' with C-1'. Protons H-2' (2H, triplet) confirmed the presence of the acyl group —O-CO-C**H**$_2$-CH$_2$-R at C-3. The long side chain ester at C-3 was determined by MS.

Table 2: Spectroscopic data of (**4**)

N	δ_C			DEPT -NMR	δ_H (*J* in Hertz)	HMBC (H to C)
	(*)	(**)	(**4**)			
1	40.8	39.5	40.7	-CH$_2$-	1.89 (1H, brs), 0.82 (1H, m)	2, 20
2	18.8	24.0	18.5	-CH$_2$-	1.60 (2H, m)	
3	37.8	78.7	37.9	-CH$_2$-	2.16 (1H, dbr, 14), 1.01 (1H, m)	
4	43.9	48.0	43.9	Quater C		
5	57.2	56.4	57.1	CH	1.07 (1H, dbr)	6, 19
6	21.9	21.5	21.8	-CH$_2$-	1.85 (2H, m)	
7	33.1	40.9	39.7	-CH$_2$-	2.04 (1H, dd, 2.5, 10.0) 1.12 (1H, dd, 5.0, 11.5)	9, 15
8	64.9	43.8	43.7	Quater C		
9	55.2	55.1	55.1	CH	1.03 (1H, dbr)	8, 10, 12
10	39.7	39.4	39.7	Quater C		
11	19.1	18.5	19.1	-CH$_2$-	1.86 (1H, brs), 1.40 (1H, m)	10
12	29.7	33.0	33.1	-CH$_2$-	1.53 (2H, m), 1.42 (1H, m)	
13	41.7	43.7	44.2	CH	2.63 (1H, brs)	
14	44.3	38.7	41.3	-CH$_2$-	1.42 (2H, m)	7, 8
15	49.1	48.7	48.9	-CH$_2$-	2.06 (2H, m)	7, 9, 13, 16, 17
16	155.8	155.4	155.9	Quater C=		
17	103.0	103.3	103.0	=CH$_2$	4.79 (1H, brs); 4.74 (1H, brs)	13, 15
18	28.9	23.9	28.9	CH$_3$	1.24 (3H, s)	3, 4, 5, 19
19	184.5	180.6	183.3	COOH		
20	15.6	15.3	15.6	CH$_3$	0.95 (3H, s)	5, 9, 10

Note: (*): ^{13}C-NMR data of *ent*-kaur-16-ene-19-oic acid [5]
(**):^{13}C-NMR data of 3α-tigloyloxykaur-16-ene-19-oic acid [6].

The ESI-MS (Positive mode) showed a molecular ion peak at *m/z* = 635 [M+H–H$_2$O]$^+$ corresponding to the formular of C$_{44}$H$_{76}$O$_3$. The aglycone moiety with two hydroxyl groups had the mass of 441 amu (C$_{30}$H$_{49}$O$_2$) so the side chain moiety had the mass of 211 amu. This mass well suited to the alcanoyl group of CH$_3$—(CH$_2$)$_{12}$-CO-. So the compound was determined as 3-*O*-tetradecanoylurs-12-ene-16β-ol. This compound was also found in the flowers of

Chrysanthemum morifolium (CAS registry number: 357419-19-3).

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