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New Diterpenoids of *Sigesbeckia pubescens* (Makino) Makino

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Abstract: The composition of diterpenoids isolated from the aerial parts of *Sigesbeckia pubescens* growing in Primorsky Territory was investigated. Diterpenoids β-glucopyranosyl-18-acetoxy-16α, 17-dihydroxykauran-19-oate (1), 15-*O*-malonylkirenol (2), and 16-*O*-malonylkirenol (3) were isolated by extraction and preparative column chromatography. The diterpenoids 1-3 were new compounds, the structures of which were established using NMR spectroscopy and mass spectrometry.

Keywords: *Sigesbeckia pubescens*; diterpenoids; NMR spectroscopy.

1. Introduction

Sigesbeckia pubescens (Makino) Makino – an annual plant of the Asteraceae family (Compositae), is widespread on the territory of China, Korea, Japan, and the Primorsky Region of the Russian Far East. An annual plant *Sigesbeckia pubescens* is used in traditional Chinese medicine to treat rheumatic arthritis, hypertension, malaria, and neurasthenia [1-4]. This plant has anti-inflammatory, anti-allergic, antithrombotic, antirheumatic, antihistamine, and anti-atherosclerotic properties [5-14]. The medicinal properties of *Sigesbeckia pubescens* are due to the presence in its composition of flavonoids, sesquiterpenoids, triterpenoids, and diterpenoids [5,7,15-19]. Pharmacological studies of this plant have shown that diterpenoids are the main biologically active compounds used to treat rheumatic arthritis [1]. It is necessary to conduct further studies of the composition of new diterpenoid compounds of this plant for further investigation of the pharmacological properties of *Sigesbeckia pubescens*.

2. Materials and Methods

2.1. General experimental procedures.

Mass spectrometric analysis used a Bruker MaXis (USA) with electrospray ionization (ESI) in positive-ion mode. NMR spectra were recorded in DMSO-d_6 on a Bruker AVANCE-400 spectrometer (USA) at an operating frequency 400 MHz.

2.2. Plant material.

The aerial parts of *Sigesbeckia pubescens* were collected in Primorsky Territory (in the vicinity of Shkotovo village, Shkotovo District, September 2020, during flowering) and identified by professor Peter G. Gorovoy (G. B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS). A voucher specimen (№ 103618) was deposited in the Pacific Institute of Bioorganic Chemistry FEB RAS.

2.3. Extraction and isolation

The aerial parts of *Sigesbeckia pubescens* (100 g) were extracted with refluxing EtOH (70 %, 1000 ml) on a boiling-water bath for 1.5 h. The resulting extract was concentrated in a rotary evaporator to an aqueous residue that was worked up successively with CCl_4, EtOAc, and *n*-BuOH.

The BuOH extract was dried over Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The dry residue (15 g) was treated with EtOH (96%, 10 ml) and mixed with silica gel (5g, 70-230 mesh fraction). The mixture of extract and silica gel was dried at room temperature, transferred to a column (1×15 cm) of silica gel (70-230 mesh), and eluted with CCl_4 – EtOH (EtOH content increasing from 0 to 100 %). As a result of the BuOH extract of *Sigesbeckia pubescens* identified the compounds **1** (12 mg), **2** (35 mg), and **3** (28 mg).

β-glucopyranosyl-18-acetoxy-16α,17-dihydroxykauran-19-oate (1)

HR-ESI-MS *m/z*: 579.2807 $[M+Na]^+$ (calcd. for $C_{28}H_{44}O_{11}Na^+$, 579.2776); ^1H NMR and ^{13}C NMR: Table 1.

15-O-malonylkirenol (2)

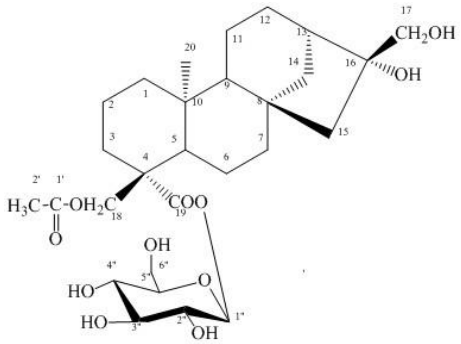
HR-ESI-MS *m/z*: 447.5169 $[M+Na]^+$ (calcd. for $C_{23}H_{36}O_7Na^+$, 447.5175); ^1H NMR and ^{13}C NMR: Table 1.

16-O-malonylkirenol (3)

HR-ESI-MS *m/z*: 447.5170 $[M+Na]^+$ (calcd. for $C_{23}H_{36}O_7Na^+$, 447.5175); ^1H NMR and ^{13}C NMR: Table 1.

3. Results and Discussion

Preparative column chromatography on the butanol extract of *Sigesbeckia pubescens* afforded the three new diterpenoids (Figure 1).



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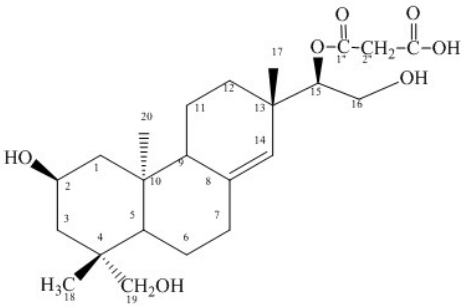

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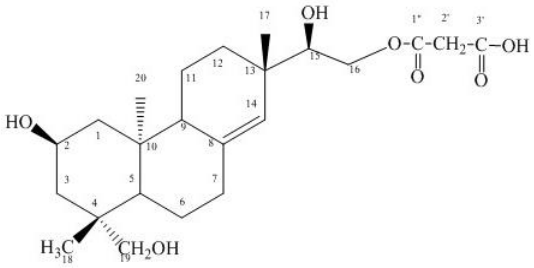

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Figure 1. Chemical structures of diterpenoids 1-3.

Table 1. ^{1}H and ^{13}C NMR data of 1 - 3 in d^{6}-DMSO (δ in ppm, J/Hz).

№		^{1}H	^{13}C	№		^{1}H	^{13}C	№		^{1}H	^{13}C
		1				2				3	
1	a b	0.68(m) 1.71(m)	40.1	**1**	a b	0.84(m) 1.78(m)	48.6	**1**	a b	0.86(m) 1.78(m)	48.7
2	a b	1.35(m) 1.83(m)	18.4	**2**		3.55(m)	62.9	**2**		3.56(m)	63.0
3	a b	1.04(m) 2.17(m)	32.5	**3**	a b	0.69(m) 2.04(m)	44.8	**3**	a b	0.69(m) 2.04(m)	45.0
4		-	47.7	**4**		-	40.3	**4**		-	40.3
5		1.25(m)	51.3	**5**		1.07(m)	55.0	**5**		1.07(m)	55.1
6	a b	1.55(m) 1.77(m)	21.8	**6**	a b	1.20(m) 1.58(m)	22.3	**6**	a b	1.19(m) 1.59(m)	22.3
7	a b	1.30(m) 1.43(m)	41.9	**7**	a b	1.90(m) 2.22(m)	36.2	**7**	a b	1.90(m) 2.20(m)	36.3
8		-	44.2	**8**		-	139.2	**8**		-	138.3
9		0.92(m)	55.8	**9**		1.69(m)	50.6	**9**		1.69(m)	50.9
10		-	39.4	**10**		-	39.4	**10**		-	39.4
11	a b	1.47(m) 1.79(m)	18.6	**11**	a b	1.41(m) 1.41(m)	18.6	**11**	a b	1.41(m) 1.41(m)	18.6
12	a b	1.26(m) 1.46(m)	26.2	**12**	a b	0.81(m) 1.91(m)	32.3	**12**	a b	0.81(m) 1.90(m)	32.1
13		1.82(m)	44.9	**13**		-	37.0	**13**		-	37.6
14	a b	1.46(m) 1.71(m)	37.0	**14**		5.13(s)	127.6	**14**		5.15(s)	128.8
15	a b	1.25(m) 1.39(m)	53.1	**15**		4.84(dd,6.6,2.1)	79.1	**15**		3.51(m)	71.9
16		-	81.0	**16**	a b	3.44(m) 3.56(m)	60.7	**16**	a b	4.09(dd,8.8,2.2) 4.11(dd,8.8,2.1)	67.1
17	a b	3.38(m) 3.46(d,11.6)	65.7	**17**		0.83(s,3H)	23.5	**17**		0.79(s,3H)	22.8
18	a b	3.82(d,10.4) 4.26(d,10.4)	70.6	**18**		0.87(s,3H)	28.1	**18**		0.87(s,3H)	28.1
19		-	173.4	**19**	a b	3.11(d,10) 3.43(d,10)	63.9	**19**	a b	3.12(d,10) 3.44(d,10)	63.9
20		0.84(s,3H)	15.6	**20**		0.72(s,3H)	16.7	**20**		0.67(s,3H)	16.8
1'		-	170.8	**1'**		-	167.3	**1'**		-	167.5
2'		1.94(s,3H)	20.9	**2'**		3.31(s,2H)	42.6	**2'**		3.31(s,2H)	42.2
1''		5.29(d,8.2)	94.5	**3'**		-	168.7	**3'**		-	168.5
2''		3.11(m)	72.9								
3''		3.15(m)	77.2								
4''		3.10(m)	70.1								
5''		3.21(m)	78.1								
6''	a b	3.40(m) 3.61(d,10.4)	61.2								

3.1. Compound 1.

The molecular formula was identified as $C_{28}H_{44}O_{11}$ by HR-ESI-MS data ($[M+Na]^{+}$ *m/z* 579.2807, calcd. 579.2776).

The ^{1}H NMR spectrum (Table 1) displayed signals for an anomeric proton at δ_H 5.29 (d, H-1″, J=8.2 Hz), two methyl groups at δ_H 0.84 (s, CH_3-20), 1.94 (s, CH_3-2′). The ^{13}C NMR spectrum revealed two methyls, twelve methylenes (three oxygenated, at δ_C 65.7 and 70.6 in the aglycone, one oxygenated carbon in a sugar moiety at δ_C 61.2), eight methines, and six quaternary carbons (two carbonyl carbon at δ_C 170.8 and 173.4). The HMBC correlations from CH_3-20 to C-1, C-6, and C-10; from CH_2-17 to C-16; from CH_2-18 to C-3, C-4, and C-19, as depicted in Figure 2. The relative configuration has established the correlations in the NOESY spectrum of CH_2-18/H-5/H-9 and CH_2-17/H-9 (Figure 2). The NMR spectra of **1** were similar to those of 16α,17,18-trihydroxykauran-19-oic acid [20] except for an additional acetyl moiety [δ_H 1.94 (3H, s); δ_C 170.8, 20.9] and glucopyranosyl moiety (δ_C 72.9, 77.2, 70.1, 78.1, and 61.2). The acetyl group was attached to C-18, as was supported by an HMBC cross-peaks between H_2-18 (δ_H 3.82 and 4.26) and the carboxyl carbon (173.4). The coupling constant of the anomeric proton at δ_H 5.29 (d, J=8.2 Hz) and C-1″ signal at δ_C 94.5 indicated a β-glucopyranose. In the HMBC spectrum (Figure 2), the correlation between the anomeric proton H-1″ (δ_H 5.29) and C-19 (δ_C 173.4) showed that sugar moiety in diterpenoid was located at C-19. Therefore, the structure of compound **3** was defined as β-glucopyranosyl-18-acetoxy-16α,17-dihydroxykauran-19-oate.

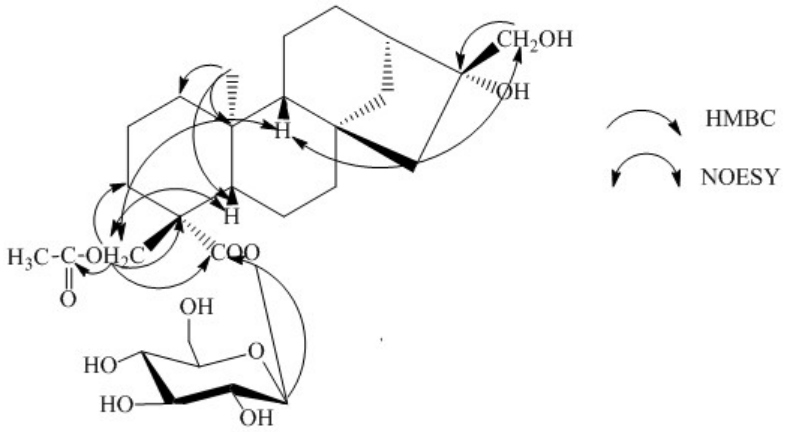

Figure 2. Key HMBC and NOESY correlations of compound 1.

3.2. Compound 2.

The molecular formula was identified as $C_{23}H_{36}O_7$ by HR-ESI-MS ($[M+Na]^+$ *m/z* 447.5169, calcd. 447.5175).

The ^{1}H NMR spectrum (Table 1) displayed signals for an olefinic proton at δ_H 5.13 (s, 14-H), three methyl groups at δ_H 0.83 (s, CH_3-17), 0.87 (s, CH_3-18) and 0.72 (s, CH_3-20). The ^{13}C NMR spectrum revealed three methyls, nine methylenes, four methines (including one olefinic carbon at δ_c 127.6), and four quaternary carbons (including one olefinic carbon at δ_c 139.2). The HMBC correlations from CH_3-17 to C-12, C-13, and C-15; from CH_3-18 to C-3, C-4, and C-5; from CH_3-20 to C-1, C-5, C-9, and C-10; from H-14 to C-12 and C-13, as depicted in Figure 3. The relative configuration has established the correlations in the NOESY spectrum of CH_3-18/H-5/H-9, CH_3-17/H-9, and CH_3-20/H-2 (Figure 3). The NMR data were very similar to those of kirenol [21-23] except for a set of resonances due to a malonyl moiety [δ_H 3.31 (2H, s); δ_c 167.3, 168.7, 42.3]. Availability of correlation between H-15 (δ_H 4.84) and C-1' (δ_c 167.3) in spectrum HMBC states that malonic acid moiety in diterpenoid is linked at C-15 (Figure 3). Alkaline hydrolysis of compound **2** (5 % KOH/CH_3OH, 100 °C, 60 min)

formed the kirenol, and spectrum ^{13}C NMR displayed a diagnostic resonance at δ_c 76.4 (C-15) defined the configuration of kirenol as the *R*. There is an empirical data for the chemical shift of C-15 in *ent*-pimarane skeleton. The chemical shift of C-15 for *R*-configuration is 77 ppm, while a change to 79 ppm for *S*-configuration [21,24,25]. Thus, the structure of compound **2** was determined to be 15*R*-malonyl-2α,16,19-trihydroxypimar-8(14)ene (15-*O*-malonylkirenol).

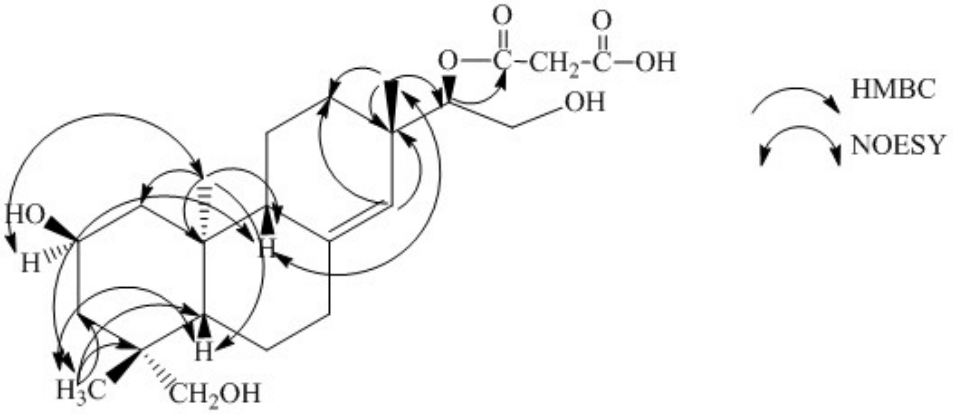

Figure 3. Key HMBC and NOESY correlations of compound 2.

3.3. Compound 3.

The molecular formula was established as $C_{23}H_{36}O_7$ by HR-ESI-MS data ($[M+Na]^+$ *m/z* 447.5170, calcd. 447.5175).

The ^1H and ^{13}C NMR spectra of compound **3** were nearly superimposable with those of compound **2** revealed a downfield shift of the signal of C-16 (+4.6 ppm) and upfield shift of the signals of C-15 (-7.2 ppm), showing that OH group has been attached to C-16 in diterpenoid by malonic acid [δ_H 3.31 (s, 2H); δ_c 167.5, 168.5, 42.2]. It was also supported by an HMBC cross-peak between the H_2-16 (δ_H 4.09 and 4.11) and the carbonyl carbon (δ_C 167.5). Alkaline hydrolysis of compound **3** (5 % KOH/CH_3OH, 100 °C, 60 min) formed the kirenol. Based on the empirical formula for 15,16-dihydroxy *ent*-pimarane skeletons [21,24,25], the chemical shift of C-15 in kirenol was 76.5 ppm. Therefore, the structure of compound **3** was defined as 16-malonyl-2α,15*R*,19-trihydroxypimar-8(14)ene (16-*O*-malonylkirenol).

Diterpenoids 1-3 were not previously described in the literature and were first isolated from *Sigesbeckia pubescens*.

4. Conclusions

Diterpenoids β-glucopyranosyl-18-acetoxy-16α,17-dihydroxykauran-19-oate (1), 15-*O*-malonylkirenol (2), and 16-*O*-malonylkirenol (3) were isolated for the first time from the aerial parts of *Sigesbeckia pubescens* by extraction and preparative column chromatography. The structures of the isolated compounds were elucidated and proved by NMR spectroscopy and mass spectrometry. The diterpenoids 1-3 were new and not previously described in the literature.

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Conflicts of Interest

The authors declared no potential conflict of interest.

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