Floccosic Acid, a New Triterpenic Acid from *Nepeta floccosa*

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The isolation and structure elucidation of a new triterpenic acid named floccosic acid (**1**) is reported on the basis of the 1D- and 2D-NMR assignments. This secondary metabolite was isolated as a new constituent, along with the known triterpenoids, betulinic acid and β-amyrin. All these compounds were purified by repeated column chromatography of the MeOH extract of *Nepeta floccosa*. The structure elucidation of the new compound was accomplished by the combined mass spectrometry (MS), infrared (IR) and ultraviolet (UV) absorption spectroscopy, one- (^{1}H- and ^{13}C-) and two-dimensional (H–C correlations; HMBC and HSQC) NMR techniques. The known compounds were identified by comparison of their physical and spectroscopic data with those reported in the literature.

Introduction. – The genus *Nepeta* is one of the largest genera of the family labiatae (Lamiaceae) with *ca.* 250 species distributed mainly in Southwest and Central Asia, Europe, North Africa, and North America [1]. About 67 *Nepeta* species are found in Iran and *ca.* 58 in Pakistan. Several *Nepeta* species possess diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue, sedative, insecticidal, acaricidal, antiviral, anti-inflammatory, and antioxidant properties [2]. Some of the Iranian *Nepeta* species are reported to have a great influence in Iranian folk and traditional medicine and are used for the treatment of nervous, respiratory, and gastrointestinal diseases [1]. During the course of our phytochemical investigations on medicinal plants from different areas of Pakistan [3][4], we investigated *Nepeta floccosa*, and the isolation and structure elucidation of a new compound **1** (*Fig. 1*), and two known metabolites, betulinic acid and β-amyrin, is reported in the present paper.

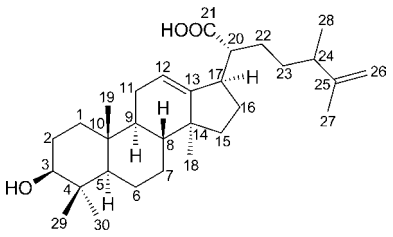

Fig. 1. *Structure of compound* **1**

Results and Discussion. – Chromatographic separation of the methanolic extract of *Nepeta floccosa* afforded three compounds (*Fig. 1*). Betulinic acid [5] and β-amyrin [6] were isolated as known constituents and their structures were confirmed by the comparison of their data with those reported in the corresponding literature.

Compound **1** was purified from the $CHCl_3$-soluble fraction of the MeOH extract, and it gave a pink color on TLC plates when sprayed with ceric sulfate. The molecular formula of compound **1** was determined as $C_{30}H_{48}O_3$ by HR-EI-MS (*m/z* 456.3596 ($C_{30}H_{48}O_3$; calc. 456.3603)), which was supported by ^{1}H-, ^{13}C-, and DEPT NMR data. The EI-MS showed fragment-ion peaks at *m/z* 248, 203, and 133 corresponding to a 12,13-didehydro-triterpenoid skeleton. The COOH group and the nature of the alkyl chain were indicated by the presence of fragment-ion peaks at *m/z* 412, 283, and 83 in the EI-MS of compound **1** (*Fig. 2*). The IR spectrum of **1** showed absorptions for a OH (3500 cm^{-1}) and a COOH group (1700 cm^{-1}), and a trisubstituted C=C bond (3045, 1650, and 815 cm^{-1}).

The analysis of the ^{1}H-, ^{13}C-, DEPT, and HMBC-NMR data of **1** (*Table* and *Fig. 3*) revealed the presence of six Me and ten CH_2 groups (one terminal CH_2), one sp^2 olefinic CH C-atom, seven CH groups, two sp^2 quaternary C-atoms, three sp^3 quaternary C-atoms, and one C=O C-atom of COOH group. The signal of the H-atom geminal to the OH group, H–C(3), was observed at δ(H) 3.44–3.46 as a *multiplet*. The olefinic H-atom, H–C(12), of the ring C gave rise to a *triplet* at δ(H) 5.49. The side chain of **1** exhibited signals of a tertiary and a secondary Me group (δ(C) 19.4 and 16.4, resp.) two CH_2 groups (δ(C) 37.6 and 26.1), a CH group C-atom (δ(C) 38.5 (C(24))), and one C=C bond (δ(C) 151.3 and 109.9). These ^{1}H-NMR signals, correlated through HSQC spectra, revealed the presence of one vinylic Me group (δ(H) 1.77 (s)), a secondary Me group (δ(H) 1.05 (*d*, *J* = 7.8)), a CH H-atom (δ(H) 1.54–1.55 (*m*)), and a methylidene group (δ(H) 4.76 and 4.94 (2 br. *s*)). These

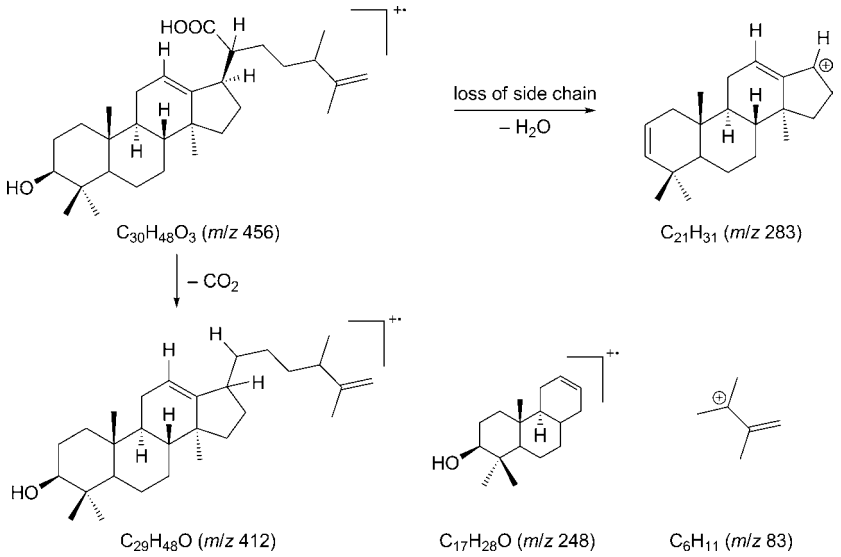

Fig. 2. *Key fragmentation pattern for floccosic acid* (**1**)

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD), *and HMBC Interactions for Compound* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC
1	0.94–0.96 (*m*, 1 H), 1.61–1.64 (*m*, 1 H)	39.2	–
2	1.84–1.85 (*m*, 2 H)	30.2	–
3	3.44–3.46 (*m*, 1 H)	78.1	C(1), C(2), C(29), C(30)
4	–	41.0	–
5	0.86–0.89 (*m*, 1 H)	55.8	C(1), C(6)
6	1.40–1.43 (*m*, 2 H)	18.7	–
7	1.30–1.33 (*m*, 2 H)	34.7	–
8	–	47.9	–
9	1.35–1.37 (*m*, 1 H)	50.9	C(8), C(14)
10	–	37.5	–
11	2.60–2.64 (*m*, 2 H)	32.8	–
12	5.49 (*t*, J = 3.9)	125.6	C(11), C(13), C(14)
13	–	139.2	–
14	–	42.8	–
15	1.82–1.83 (*m*, 2 H)	28.3	–
16	1.52–1.54 (*m*, 2 H)	31.1	–
17	3.53–3.56 (*m*, 1 H)	47.7	C(13), C(16), C(20)
18	1.02 (*s*, 3 H)	14.9	–
19	0.79 (*s*, 3 H)	16.3	–
20	1.72–1.75 (*m*, 1 H)	49.7	C(17), C(21), C(22)
21	–	178.9	–
22	1.26–1.28 (*m*, 1 H), 1.92–1.95 (*m*, 1 H)	26.1	–
23	2.22–2.25 (*m*, 2 H)	37.6	–
24	1.54–1.55 (*m*, 1 H)	38.5	C(23), C(25), C(26)
25	–	151.3	–
26	4.76 (br. *s*, 1 H), 4.94 (br. *s*, 1 H)	109.9	–
27	1.77 (*s*, 3 H)	19.4	C(25), C(26)
28	1.05 (*d*, J = 7.8)	23.9	–
29	0.99 (*s*, 3 H)	16.4	–
30	1.22 (*s*, 3 H)	28.6	C(3), C(5)

functions were linked together on the basis of HMBC experiments (*Fig. 3*) to assemble the overall structure for **1**. The HMB long-range correlations displayed cross-peaks between the signal of Me H-atoms (H–C(27)) at δ(H) 1.77 (*s*, 3 H), and those of the methylidene C-atom (C(26) at δ(C) 109.9), the quaternary C-atom (C(25) at δ(C) 151.3), and the CH C-atom (C(24) at δ(C) 38.5). In addition, strong HMBCs between the signal of the C=O group C-atom (C(21) at δ(C) 178.9) and those of the CH H-atoms (H–C(20) at δ(H) 1.72–1.75) and (H–C(17) at δ(H) 3.53–3.56) were observed. These established connectivities further confirmed the substitution pattern and positions of different groups in the molecule as assigned by the mass fragmentation pattern (*cf. Fig. 2*).

The ^{1}H- and ^{13}C-NMR spectra of **1** resemble to those of known lanostene-type triterpenoids [7] with differences concerning the position of the olefinic groups and other substituents, which have already been confirmed on the basis of HMBC interactions (*Fig. 3*).

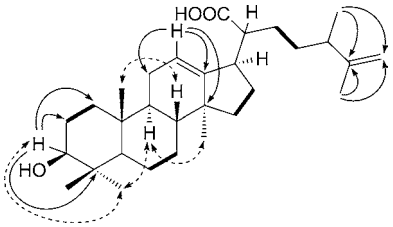

Fig. 3. *Key HMBCs* (H → C)*, and* ^{1}*H,*^{1}*H-COSY* (—)*, and NOESY* (H ⇠ ⇢ H) *correlations of compound* **1**

Comprehensive analyses of MS, and 1D- and 2D-NMR data led to the structure elucidation of compound **1**, named floccosic acid after the producing organism, *Nepeta floccosa*.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 0.040 – 0.062 mm; *Merck*). Anal. and prep. TLC: Precoated silica gel plates (*G60 F-254* or *G50 UV-254*, resp., *Merck*) IR spectra: *Nicolet-510P* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance 500* (500 and 125 MHz, resp.) spectrometer; δ in ppm rel. to CD_3OD (δ(H) 3.30, δ(C) 49.0) as internal standard, *J* in Hz. EI-MS and HR-EI-MS: *JEOL JMS HX 110* mass spectrometers; in *m*/*z*.

Plant Material. The entire plant of *N. floccosa* was collected at the Parachinar Kurram Agency, Khyber pakhtunkhwa, Pakistan, in 2009, and was identified by plant taxonomist at the Department of Botany, Government. Jehanzeb Post Graduate College, Saidu Shareef, Swat, Pakistan. A specimen of this plant was deposited with the Herbarium of the College.

Extraction and Isolation. Dried and powdered whole plants of *N. floccosa* (1.5 kg) were extracted with 80% MeOH at r.t. for 2 weeks. The crude extract (50 g) was then suspended in H_2O and extracted with the increasingly polar solvents hexane, $CHCl_3$, and finally AcOEt. The $CHCl_3$ fraction (25 g) was then subjected to CC (SiO_2; hexane, hexane/$CHCl_3$, and $CHCl_3$/MeOH) to yield 16 subfractions. Subfraction SF_{12} (1.3 g) was again subjected to repeated CC (hexane/$CHCl_3$ 2 : 8). *Floccosic acid* (**1**; 32 mg) was obtained in the form of an amorphous powder along with some other semi-pure compounds. These impure fractions were separated on prep. TLC plates to furnish the known compounds betulinic acid (12.4 mg) and β-amyrin (8.2 mg) by eluting with hexane/$CHCl_3$ 3 : 7 and hexane/$CHCl_3$ 4 : 6, resp.

Floccosic Acid (*=(3β,5α)-3-Hydroxy-4,4,14,24-tetramethyl-18-norcholesta-12,25-dien-21-oic Acid*; **1**). White amorphous powder. $[\alpha]_D^{27} = +4.8$ ($c = 0.003$, MeOH). IR (MeOH): 3500, 3045, 1700, 1650, 815. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-EI-MS: 456.3596 ($C_{30}H_{48}O_3^+$; calc. 456.3603).

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