Tanacetamide D: A New Ceramide from *Tanacetum artemisioides*

by **Javid Hussain***[a]), **Meamoona Munir**[a]), **Zahid Hassan**[b]), **Naseem Bano**[a]), **Saima Arshad**[b]), and **Viqar Uddin Ahmad**[b])

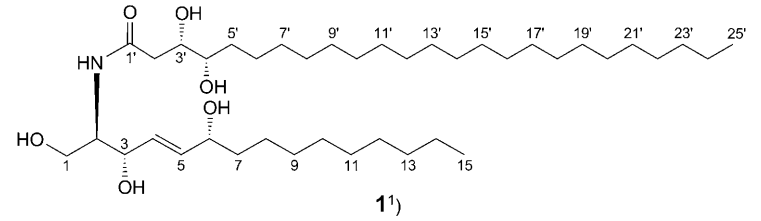
[a]) Department of Chemistry, Kohat University of Science and Technology, Kohat, Pakistan (phone: +92-922-554565; fax: +92-922-554556; e-mail: javidhej@yahoo.com)

[b]) H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

One new ceramide (= long-chain base linked to a fatty acid *via* an amide bond), tanacetamide D (**1**), was isolated from *Tanacetum artemisioides*. Besides this, the two known constituents 5-demethylnobiletin (**2**) and 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone (**3**) were isolated for the first time from this species. The structure elucidation of the isolated compounds were based primarily on 2D-NMR techniques including correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and nuclear *Overhauser* effect spectroscopy (NOESY) experiments.

Introduction. – The genus *Tanacetum* (tribe Anthemideae) with *ca.* 70 species is distributed over Europe and West Asia [1]. Several members of the genus *Tanacetum* have important medicinal properties, used for ages throughout the world [2]. The common *T. vulgare* has long been used in folk medicine as expectorants, vermifuges, antiseptics, and plasmolytics [3–5]. *T. parthenium*, commonly known as feverfew, is used as a herbal remedy for control of migraine and arthritis and *T. microphyllum* in Spanish folk medicine for ulcer and inflammatory conditions [6]. *T. artemisioides*, a pale yellow green annual shrub with several branches from the woody rootstock, grows in different parts of Pakistan [7]. The above medicinal properties prompted us to carry out a phytochemical investigation on *Tanacetum artemisioides*. Our current study has led to the isolation of one new compound, namely tanacetamide D (**1**). In addition, the two known constituents 5-demethylnobiletin (=2-(3,4-dimethoxyphenyl)-5-hydroxy-6,7,8-trimethoxy-4*H*-1-benzopyran-4-one; **2**) and 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone (=2-(3-hydroxy-4-methoxyphenyl)-5,6,7,8-tetramethoxy-4*H*-1-benzopyran-4-one; **3**) were isolated for the first time from this species.

Results and Discussion. – *Phytochemical Evaluation.* Compound **1** was isolated as a colorless oil and showed the molecular-ion peak at *m*/*z* 669.6234 corresponding to the molecular formula $C_{40}H_{79}NO_6$ by HR-FAB-MS. The IR spectrum revealed OH bands at 3630 cm^{-1} and bands at 1621 cm^{-1} due to a secondary-amide group. It also showed absorption bands at 2919, 2850, and 1465 cm^{-1} suggesting it to be a fatty acid amide. The ^{1}H-NMR spectrum (C_5D_5N, 400 MHz; *Table*) of compound **1** showed signals due to two terminal Me groups (Me(25′) and Me(15) at δ(H) 0.85 (*t*, *J* = 8.0 Hz, 6 H)), which appeared as a *t* of six H-atoms, an amide H-atom at δ(H) 8.61 (*d*, *J* = 8.7 Hz),



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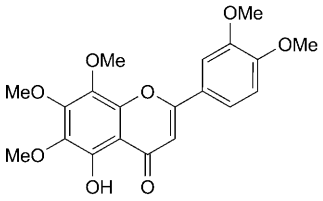

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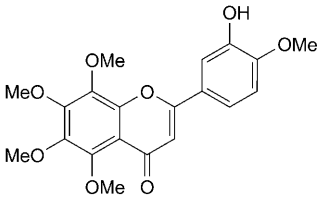

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and signals of a *trans* CH=CH bond at δ(H) 5.45 (*dt*, $J = 5.4$, 15.4 Hz, H–C(4)) and δ(H) 5.57 (*dt*, $J = 5.4$, 15.4 Hz, H–C(5)). A signal at low field (δ(H) 5.11 (*m*)) was identified as arising from a CH group vicinal to N-atom of the amide group. Compound **1** showed the presence of four CH groups at δ(H) 4.36–4.39 (*m*, H–C(3′)), 4.31–4.34 (*m*, H–C(4′)), 4.25 (*t*, $J = 7.2$ Hz, H–C(3)), 4.60–4.68 (*m*, H–C(6)), and of a CH_2 group at δ(H) 4.55 (*dd*, $J = 4.7$, 10.7 Hz, H_a–C(1)) and 4.42 (*dd*, $J = 4.7$, 10.7 Hz, H_b–C(1)). The usual CH_2 groups associated with the chain appeared as a broad signal at δ(H) 1.25.

Table. *^{1}H- and ^{13}C-NMR Data of Compound* **1**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C) (DEPT)
CH_2(1)	4.55 (*dd*, $J = 4.7$, 10.7, H_a), 4.4 (*dd*, $J = 4.7$, 10.7, H_b)	62.0 (*t*)
H–C(2)	5.11–5.18 (*m*)	52.0 (*d*)
H–C(3)	4.25 (*t*, $J = 7.2$)	72.2 (*d*)
H–C(4)	5.45 (*dt*, $J = 5.4$, 15.4)	130.8 (*d*)
H–C(5)	5.57 (*dt*, $J = 5.4$, 15.4)	130.7 (*d*)
H–C(6)	4.60–4.68 (*m*)	72.4 (*d*)
CH_2(7)	2.20–2.26 (*m*)	32.2 (*t*)
Me(15,25′)	0.85 (*t*, $J = 8.0$)	14.2 (*q*)
C=O		175.5 (*s*)
CH_2(2′)	1.98–2.11 (*m*)	35.5 (*t*)
H–C(3′)	4.36–4.39 (*m*)	79.7 (*d*)
H–C(4′)	4.31–4.34 (*m*)	76.8 (*d*)
CH_2(5′)	1.72–1.78 (*m*)	30.1 (*t*)
$(CH_2)_n$ (chain)	1.25 (br. *s*)	29.9 (*t*)
OH	7.18 (br. *s*)	–
OH	7.55 (br. *s*)	–
OH	8.72 (*s*)	–
NH	8.61 (*d*, $J = 8.7$)	–

[1]) Arbitrary atom numbering; for the systematic name, see *Exper. Part.*

The ^{13}C-NMR spectrum (*Table*) of compound **1** showed signals due to two terminal Me groups of aliphatic hydrocarbon chains at δ(C) 14.2 and a characteristic signal at δ(C) 175.5 due to an amide C=O. Signals of a tertiary C-atom at δ(C) 52.0 and a downfield CH_2 signal appearing at δ(C) 62.0 supported the presence of a C-atom attached to an N-atom and the presence of an OH function, respectively. Two olefinic CH groups at δ(C) 130.8 and 130.7 suggested that this compound possesses a CH=CH moiety, and four other downfield signals at δ(C) 79.7, 76.8, 72.2, and 72.4 indicated the presence of 4 oxygenated CH groups. The remaining CH_2 groups of the chain showed their signals in the C-atom spectrum in the expected region [8][9].

The ^{1}H,^{1}H- and ^{1}H,^{13}C-connectivities were established by the ^{1}H,^{1}H-COSY and HMQC plots. This assembly of spectral data and the molecular formula suggested that compound **1** is a ceramide (= long-chain base linked to a fatty acid *via* an amide bond). The position of the C=C bond was confirmed by HMBCs (*Fig.*). Cross-peaks H–C(3)/C(4) and C(5), H–C(4)/C(3), C(5), and C(6), H–C(5)/C(3), C(4), and C(6), and H–C(6)/C(4) and C(5) in the HMBC plot (*Fig.*) confirmed the position of the C=C bond between C(4) and C(5) of the long-chain base part.

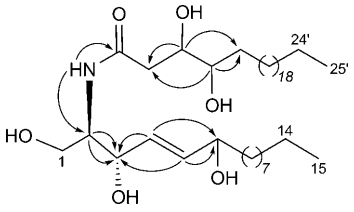

Fig. 1. *Important HMBCs of Compound* **1**[1])

The length of the fatty acid was determined by the characteristic peaks at *m*/*z* 57 ($C_4H_9^+$), 127 ($C_9H_{19}^+$), 281 ($C_{20}H_{41}^+$), 295 ($C_{21}H_{43}^+$), 355 ($C_{23}H_{47}O_2^+$), 397 ($C_{25}H_{49}O_3^+$), and 412 ($C_{25}H_{50}NO_3^+$) in the EI-MS. The characteristic peak at *m*/*z* 425 is due to the fragment ion $C_{26}H_{51}NO_3^+$.

The geometry of C=C bond at C(4) and C(5) was deduced to be (*E*) from the ^{1}H,^{1}H-coupling constant (^3J = 15.4 Hz). The structures of the known compounds **2** and **3** were established by comparing their spectral data and physical constants with reported data in [10][11].

Taxanomic Issues Concerning the Plant. The plant species has been used for blood pressure, diabetes, abdominal disorders, ringworms, flatulence, headache, and fever by the local communities of Kurrum and Gilgit [12]. It is locally known as Zoon in Gilgit and Zawail in Kurrum valley. The present survey of the plant at Kurrum valley in Pakistan showed that its population has considerably reduced, when it was collected from the same location for the research work. According to the local people and our ethnobotanical survey, the plant faces tremendous pressure of overgrazing and unsustainable collection. The external threats to the plant species advocate toward *in situ* conservation and sustainable collection for medicinal purposes.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 70–230 mesh). Flash chromatography (FC): SiO_2 (230–400 mesh). TLC: precoated SiO_2 *G-25-UV$_{254}$* plates; detection at 254 nm and by $Ce(SO_4)_2$ reagent. Optical rotations: *Jasco-DIP-360* digital polarimeter. IR Spectra: *Jasco-320-A* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR, COSY, HMQC, and HMBC Data: *Bruker* spectrometers operating at 400 MHz; δ in ppm rel. to Me_4Si as internal standard, J in Hz. EI- and CI-MS: *JMS-HX-110* with a data system; in m/z (rel. %).

Plant Material. The plant *Tanacetum artemisioides* was collected at Parachinar Kurram Agency, N.W.F.P. Pakistan, in July, 2005, and was identified by Dr. *Jahandar Shah* (plant taxonomist). A voucher specimen has been deposited with the Herbarium of our Botany department at Kohat University of Science and Technology (KUST), Kohat.

Extraction and Purification. The whole plant including roots (9.5 kg) of *T. artemisioides* was crushed and extracted 3 × with MeOH (20 l each) at r.t. The resulting MeOH extract (304 g) was suspended in H_2O and successively partitioned to provide hexane, $CHCl_3$, AcOEt, and BuOH fractions. The $CHCl_3$ fraction was subjected to CC (SiO_2 solvents of increasing polarity containing hexane, AcOEt, $CHCl_3$, and MeOH): *Fractions A–E*. The major *Fr. A* (eluted with hexane/AcOEt 8:2) was re-subjected to CC (SiO_2, various hexane/AcOEt mixtures). The fraction of the second CC which was eluted with hexane/AcOEt 6:3 was a mixture of two UV-active compounds; its repeated CC resulted in the isolation of **2** (13 mg) and **3** (15 mg). The fractions of the second CC which were eluted with hexane/AcOEt 5:5 showed one major spot on TLC; they were subjected to FC (SiO_2 hexane/AcOEt 4:6): **1** (10 mg).

Tanacetamide D (*=(−)-*N*-[(3*E*)-2,5-dihydroxy-1-(hydroxymethyl)tetradec-3-en-1-yl]-3,4-dihydroxypentacosanamide*; **1**): Colorless oil. $[\alpha]_D^{23} = -5.28$ ($c = 0.15$, $CHCl_3$). IR (KBr): 3630, 2919, 2850.2, 1621, 1465. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 57 (58.85, $C_4H_9^+$), 127 (1.05, $C_9H_{19}^+$), 281 (1.35, $C_{20}H_{41}^+$), 295 (1.29, $C_{21}H_{43}^+$), 355 (7.57, $C_{23}H_{47}O_2^+$), 397 (1.23, $C_{25}H_{47}O_3^+$), 412 (1.21, $C_{25}H_{50}NO_3^+$), 425 (1.18, $C_{26}H_{51}NO_3^+$), 60.1 (100), 82.9 (28.54), 83.0 (25.59), 95.1 (15.9), 97.1 (16.21), 109.1 (6.57), 111.1 (8.36), 123.0 (4.11), 140.2 (1.13), 218.2 (2.54), 225 (1.21), 245.1 (1.01), 262.2 (269).

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