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Chemistry of endemic *Tanacetum mucroniferum* Hub.-Mor. & Grierson extracts and three new sesquiterpene lactones

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Abstract: *Tanacetum mucroniferum* Hub.-Mor. & Grierson was collected from Sakaltutan in Erzincan Province of Turkey. Comparative phytochemical investigation was carried out on the ethyl acetate and methanol extracts of the plant. The secondary metabolites were isolated and purified by chromatographic methods such as column chromatography, medium pressure liquid chromatography, high-performance thin-layer chromatography, and preparative thin-layer chromatography. The extracts of the aerial parts of *Tanacetum mucroniferum* yielded three new sesquiterpene lactones and some known compounds, namely two sesquiterpene lactones, six flavonoids, three coumarins, and one triterpene. Structures of isolated compounds were determined by IR, UV, ^{1}H NMR, APT, COSY, HSQC, HMBC, NOESY, and MS (EI, CI) spin decoupling, and some chemical reactions were carried out.

Key words: *Tanacetum mucroniferum*, sesquiterpene lactones, flavonoids, coumarin, triterpene

1. Introduction

The genus *Tanacetum* L. of the family Asteraceae is represented in Turkey by 60 taxa, of which 23 are endemic. *Chrysanthemum*, *Pyrethrum*, and *Leuchanthemum* are related to the genus *Tanacetum*. *Tanacetum* species show very small morphological differences. It makes the identification of the species very difficult. *Tanacetum mucroniferum* is an endemic species of Turkey found in Northeast and East Anatolia. This species is an intermediate species between *T. aucheranum* and *T. sipikorense*.[1]

The essential oil composition and DPPH scavenging activity of *Tanacetum mucroniferum* were reported. The essential oil showed low DPPH scavenging activity.[2] The stem extract of *Tanacetum mucroniferum* was investigated for inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The extract showed moderate AChE inhibition and low BChE inhibition.[3]

Phytochemical research revealed that sesquiterpene lactones are the principal secondary metabolites of this genus. The sesquiterpene lactones have chemosystematic significance in the family. The investigations of *Tanacetum* L. species are important to find new bioactive compounds, to find out the species of economic value, and to eliminate the systematic classification errors of the species.[4] According to our literature survey, there are no previous reports on the chemistry of *T. mucroniferum*. Herein, the phytochemistry of ethyl acetate and methanol extracts of *Tanacetum mucroniferum* is reported for the first time.

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2. Results and discussion

Ethyl acetate and methanol extracts of the aerial parts afforded three new compounds, ajanolide 1β,10α-epoxide (**2**), mucronolide (**10**), and 1α,3β,10α-trihydroxy-7α,11αH-germacra-4-en-12-6α-olide (**15**), and twelve known compounds: two sesquiterpene lactones, 9α-acetoxyartecanin (**11**) and arsanin (**12**); six flavonoids, cirsimaritin (**3**), jaceosidin (**4**), 5,3',4'-trihydroxy-3,6,7,5'-tetramethoxyflavone (**5**), salvigenin (**7**), 5-hydroxy-3$'$,4$'$,6,7-tetramethoxyflavone (**8**), and cirsilineol (**9**); three coumarins, scoparone (**6**), scopoletin (**13**), and umbelliferone (**14**); and one triterpene, α-amyrin (**1**).

Compounds **2** and **15** were detected on TLC as pale brown and compound **10** as a yellow spot when treated with $CeSO_4$. The ^{1}H NMR spectra of two compounds in $CDCl_3$ (compounds **2** and **15**) and one in pyridine (compound **10**) showed three oxygen functions' signals and an olefinic group in the molecules. The signals observed at δ 5.38 (H-14, brs) and δ 5.16 (H-15$'$, brs) 17 suggested an exocyclic methylene group in compound **10**. The structures were also verified with APT and HSQC experiments. The APT spectra gave four methyl, three methylene, six methine, two carbonyl, and two quaternary carbon signals for compound **2** (Table 1); three methyl, four methylene, six methine, two carbonyl, and two quaternary carbon signals for compound **10** (Table 2); and three methyl, three methylene, six methine, one carbonyl, and two quaternary carbon signals for compound **15** (Table 3). The structures of compounds **2**, **10**, and **15** are given in Figures 1, 2, and 3, respectively.

Table 1. ^{1}H NMR (500 MHz, $CDCl_3$, δ), APT (125 MHz, $CDCl_3$, δ), HSQC, and HMBC of compound **2**.

^{1}H NMR		HSQC	HMBC	APT[a]	
H	**2**	**2**	**2**	**C**	**2**
1	2.76 dd (1H, J = 4.5, 10 Hz)	C-1	C-2, C-9, C-10	1	60.2
2	2.50 ddd (1H, J = 4.8, 4.8, 10.5 Hz)	C-2	C-1	2	30.9
2$'$	1.66 m (1H)	C-2	C-1, C-3, C-4	3	72.9
3	5.12 dd (1H, J = 2.2, 5.0 Hz)	C-3	C-1, C-2, C-4, C-5, C-15, OCO-	4	138.9
5	5.36 dq (1H, J = 1.4, 11.4 Hz)	C-5	C-3, C-6, C-7, C-15	5	124.8
6	5.46 brd (1H, J = 11.4 Hz)	C-6	C-4, C-5, C-7, C-12, C-11, C-15	6	80.0
7	2.04 (1H)	C-7		7	45.0
8	1.66 m (1H)	C-8		8	24.4
8$'$	1.40 m (1H)	C-8		9	40.4
9	2.33 ddd (1H, J = 2.4, 4.5, 11.8 Hz)	C-9	C-1, C-7, C-8, C-10, C-14	10	59.9
9$'$	0.98 ddd (1H, J = 3.0, 13.6, 12.5 Hz)	C-9	C-1, C-7, C-8, C-10, C-14	11	36.1
11	2.84 dq (1H, J = 7.0, 9.0 Hz)	C-11	C-7, C-8, C-12, C-13	12	178.8
13	1.11 d (3H, J = 7.0 Hz)	C-13	C-7, C-11, C-12	13	10.3
14	1.34 s (3H)	C-14	C-1, C-9, C-10	14	16.3
15	1.84 d (3H, J = 1.4 Hz)	C-15	C-3, C-4, C-5	15	23.1
-OAc	2.04 s (3H)	-OCO-		-OCO-	169.4
		-OAc		-OAc	21.0

Ajanolide 1β,10α-epoxide (**2**) and mucronolide (**10**) were heliangolide type and 1α,3β,10α-trihydroxy-7α,11αH-germacra-4-en-12-6α-olide (**15**) was a germacranolide type sesquiterpene lactone. The coupling constants of H-5/H-6 of compounds **2** and **10** were broad. The small value of $J_{5,6}$ is characteristic for heliangolide.[5]

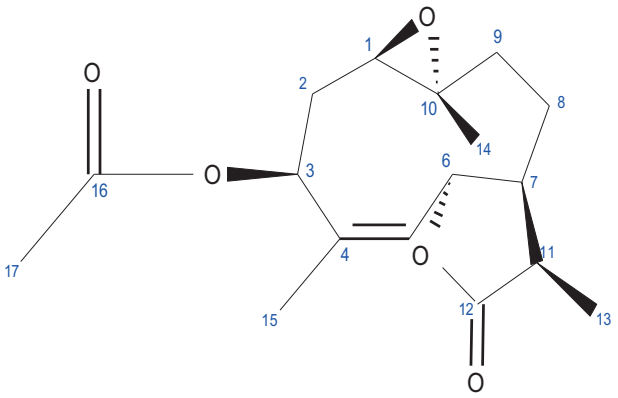

Figure 1. Molecular structure of compound **2**.

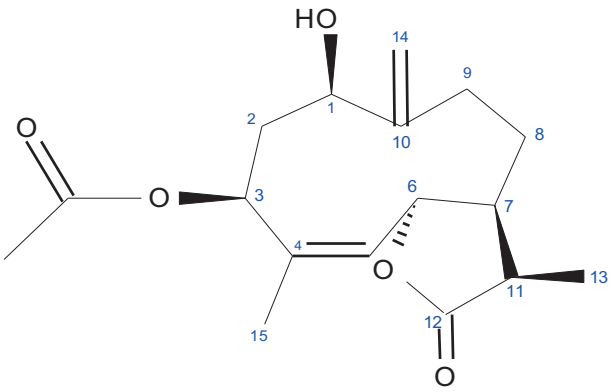

Figure 2. Molecular structure of compound **10**.

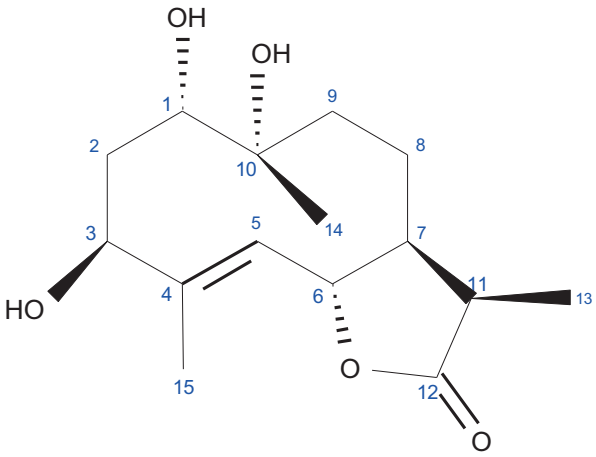

Figure 3. Molecular structure of compound **15**.

Ajanolide 1β,10α-epoxide (**2**) was obtained from chemical transformation of ajanolide A,[5] but this compound had not been isolated from a natural source before.

Spectral data of **10** were similar to those of 1β-hydroxy-3β-acetoxygermacra-4,10(14)-dien-6β,11β(H)-12,6-olide.[6] The coupling constants of H-6/H-5 and H-6/H-7 of 1β-hydroxy-3β-acetoxygermacra-4,10(14)-dien-6β,11β(H)-12,6-olide were J = 10.0, 9.8 Hz, but the coupling constant of H-6/H-5 of compound 10 was J = 6.6 Hz and H-6/H-7 was broad. In the spin decoupling experiment of compound **10**, irradiation of the H-6 signal collapsed the H-5 signal to a singlet and broad double doublet at 2.61 (H-7) to a double doublet.

Spectral data of **15** were very similar to 1α,3β,10α-trihydroxy-7α,11βH-germacra-4Z-en-12,6α-olide,[7] with the exception of a few differences in the coupling constants of H-6. The coupling constants of H-6/H-5 and H-6-H-7 of 1α,3β,10α-trihydroxy-7α,11βH-germacra-4Z-en-12,6α-olide were J = 11.0, 11.0 Hz, but the coupling constants of H-6/H-5 and H-6/H-7 of compound **15** were J = 6.3, 9.3 Hz.

Here, it was reported that three new sesquiterpene lactones, ajanolide 1β,10α-epoxide (**2**), mucronolide (**10**), and 1α,3β,10α-trihydroxy-7α,11αH-germacra-4-en-12-6α-olide (**15**), were isolated from a natural source for the first time. Additionally, the flavone 5,3′,4′-trihydroxy-3,6,7,5′-tetramethoxyflavone (**5**) was reported for the first time from the family Asteraceae and the sesquiterpene lactone 9α-acetoxyartecanin (**11**) for the first time from *Tanacetum* genus. These compounds had previously been isolated from different natural sources.

3. Experimental

3.1. Plant materials

Plant materials were collected during the flowering period from Sakaltutan in Erzincan Province in 2008. The voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy, İstanbul University (Voucher No. ISTE 85425), Turkey. Plant material was identified by Professor Neriman Özhatay.

3.2. Extraction of plant material

Air-dried and powdered plant material (1200 g) was macerated with hexane, ethyl acetate, and methanol, respectively. Each extract was concentrated under reduced pressure to give a solvent-free residue, and 4 g of crude hexane extract, 17.6 g of crude ethyl acetate extract, and 63 g of methanol crude extract were obtained from the aerial parts of *T. mucroniferum*. All of the extracts were kept in a deep freezer (–15 °C) until the day they were used for isolation.

3.3. Isolation of compounds

Ethyl acetate extract was fractioned on a silica gel column using hexane, ethyl acetate, and methanol as eluents of increasing polarity. A total of 31 fractions were obtained. Fractions were compared by TLC using appropriate mobile phases and $CeSO_4$ was sprayed to visualize the spots on TLC plates. Fractions containing similar bands on the chromatograms were combined to obtain the final seven fractions. The isolated compounds were obtained by further separation of the fractions.

Fifth, sixth, and seventh fractions were refractionated via medium pressure liquid chromatography by using the gradient elution of the solvents: hexane, CH_2Cl_2, $CHCl_3$, ether, ethyl acetate, and methanol. The compounds were isolated by means of preparative TLC, using precoated silica gel 60 HF_{254} + 60 $HF_{254+366}$ +60 GF_{254}, with 0.2 mm layer and different solvent systems. The sample (15 mg) was applied to the layer for each plate. UV detection (254 and 366 nm) and $CeSO_4$ spray were used to locate the bands. Thus, compound **1** (7 mg)[8] was isolated from the fifth fraction; compounds **2** (8 mg), **3** (8 mg),[9] **4** (9 mg),[10,11] and **5** (5 mg)[12] were isolated from the sixth fraction; and compounds **6** (11 mg),[13–15] **7** (13 mg),[16] **8** (7 mg),[17] **9** (6 mg),[18,19] **10** (7 mg), **11** (9 mg),[20] and **12** (10 mg)[21] were isolated from the seventh fraction.

MeOH extract was fractionated with liquid–liquid chromatography using hexane, CH_2Cl_2, ethyl acetate, and BuOH:H_2O (70:30) as eluents and 4 fractions were obtained. The isolated compounds were obtained by further separations of the CH_2Cl_2 fraction.

CH_2Cl_2 fraction was refractionated on a silica gel column by using the gradient elution of $CHCl_3$, ether, ethyl acetate, and methanol. Fractions were compared by TLC using appropriate mobile phases and $CeSO_4$ was sprayed as a derivatization reagent. Fractions containing similar bands on the chromatogram were combined to obtain the final fractions. Thus, compounds **13** (5 mg),[22] **14** (4 mg),[23] and **15** (3 mg) were isolated from the CH_2Cl_2 fraction.

3.4. Identifications of compounds

Structures of isolated compounds were determined by means of spectral analyses including ^{1}H-NMR, APT, COSY, HSQC, HMQC, spin decoupling, NOESY, UV-Vis, FTIR, MS techniques, and some by chemical reactions. NMR techniques were run in $CDCl_3$ using TMS as internal standard on a 500 MHz Bruker Avance III. UV-Vis spectra were recorded using UV-1800. IR spectra were recorded using a Perkin Elmer FTIR instrument

with attenuated total reflectance attachment. Mass spectra were recorded using a TOF/Q-TOF ion trap mass spectrometer. Dual ESI was used as an ion source in MS.

3.4.1. 3β-Acetoxy-1β,10α-epoxy-6β(H)-7,11α(H)-germacra-4Z-en-6,12-olide (ajanolide 1β,10α-epoxide) (2)

Crystalline; the melting point was 127.3 °C and specific rotation [a]^{22}= –14.15. ^{1}H NMR (500 MHz, $CDCl_3$, δ) and APT (125 MHz, $CDCl_3$, δ) given in Table 1; ESIMS m/z: $[M+Na]^+$($C_{17}H_{24}O_5$) 331, [331- $CH_3COOH+H]^+$ 272, [272- (OCOCCH_3+H)-CH_3]$^+$ 185 and [185- CO+2]$^+$ 159.

Table 2. ^{1}H NMR (500 MHz, pyridine, δ), APT (125 MHz, pyridine, δ), HSQC, and HMBC of compound **10**.

^{1}H NMR		HSQC	HMBC	APT[a]	
H	**10**	**10**	**10**	**C**	**10**
1	4.40 brd (1H, J = 11.1 Hz)	C-1	C-9, C-14	1	73.0
2	2.51 ddd (1H, J = 2.4, 11.3, 15.5 Hz)	C-2	C-1, C-3, C-10	2	37.8
2′	2.41 ddd (1H, J = 3.9, 7.2, 15.3 Hz)	C-2	C-1, C-3, C-10	3	72.8
3	5.69 brd (1H, J = 6.6 Hz)	C-3	C-1, C-2, C-4, C-5, -OCO	4	139.1
5	5.43 ddq (1H, J = 1.2, 11.2, 6.8 Hz)	C-5	C-7, C-3, C-15	5	126.3
6	5.64 brd (1H, J = 6.6 Hz)	C-6	C-4, C-5, C-7, C-8, C-12	6	79.0
7	2.61 brdd (1H, J = 10.8, 10.5 Hz)	C-7	C-8, C-9, C-11, C-13	7	41.8
8	1.68 m (1H)	C-8		8	28.0
8′	1.31 m (1H)	C-8		9	28.0
9	2.68 ddd(1H, J = 4.1, 9.4, 14.9 Hz)	C-9	C-1, C-7, C-8, C-10	10	151.5
9′	2.32 ddd (1H, J = 5.1, 6.9, 14.2 Hz)	C-9	C-7, C-8, C-10, C-14	11	37.8
11	2.84 dq (1H, J = 7.5, 10.5 Hz)	C-11	C-7, C-8, C-12, C-13	12	181.0
13	1.07 d (3H, J = 7.4 Hz)	C-13	C-7, C-11, C-12	13	10.6
14	5.38 brs (1H)	C-14	C-1, C-9, C-10	14	114.7
14′	5.16 brs (1H)	C-14	C-1, C-9, C-10	15	22.7
15	1.71 brs (3H)	C-15	C-3, C-4, C-5, C-6, C-7,	-OCO-	170.2
-OAc	2.01 s (3H)	-OCO-		-OAc	21.2

3.4.2. 1β-Hydroxy-3β-acetoxy-4Z,10(14)-dien, 6β, 7α, 11α(H)-6,12-olide (Mucronolide) (10)

The melting point was 223.4 °C and specific rotation [a]^{22}= 17.19. ^{1}H NMR (500 MHz, pyridine, δ) and APT (125 MHz, pyridine, δ) given in Table 1; ESIMS m/z: $[M+Na]^+$($C_{17}H_{24}O_5$) 331, [331- $CH_3COOH]^+$ 271, [271- (OCOCCH_3)-CH_3]$^+$ 185 and [185- CO]$^+$ 157.

3.4.3. 1α,3β,10α-trihydroxy-7α,11αH-germacra-4-en-12-6α-olide (15)

^{1}H NMR (500 MHz, $CDCl_3$, δ) and APT (125 MHz, $CDCl_3$, δ) given in Table 1; ESIMS m/z: $[M+Na]^+$ ($C_{15}H_{24}O_5$) 307, [307- $H_2O]^+$ 289 and [289- $CO_2]^+$ 245.

Table 3. ^{1}H NMR (500 MHz, $CDCl_3$, δ), APT (125 MHz, $CDCl_3$, δ), HSQC, and HMBC of compound **15**.

^{1}H NMR		HSQC	HMBC	APT[a]	
H	**15**	**15**	**15**	**C**	**15**
1	3.82 brd (1H, J = 3.5 Hz)	C-1	C-2, C-9, C-14	1	77.5
2	2.13 m (1H)	C-2		2	41.3
2′	2.21 m (1H)	C-2		3	79.6
3	4.69 dd (1H, J = 8.3, 8.5 Hz)	C-3	C-2, C-5, C-15	4	138.2
5	5.11 dq (1H, J = 6.3, 1.5 Hz)	C-5	C-15	5	124.9
6	5.74 dd (1H, J = 9.3, 6.3 Hz)	C-6	C-7, C-8, C-11	6	82.7
7	2.13 m (1H)	C-7	C-8, C-9, C-13	7	46.0
8 and 8′	1.73 m (1H)	C-8		8	23.2
9	1.38 m (1H)	C-9		9	35.5
9′	1.49 m (1H)	C-9		10	87.6
11	2.61 dq (1H, J = 6.6, 15.6 Hz)	C-11	C-6, C-12, C-13	11	40.2
13	1.16 d (3H, J = 7.7 Hz)	C-13	C-7, C-8, C-11, C-12	12	179.9
14	1.26 s (3H)	C-14	C-1, C-9, C-10	13	12.4
15	1.54 brs (3H)	C-15	C-3, C-4, C-5, C-7	14	18.9
				15	20.3

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