Bull. Pharm. Sci., Assiut University, Vol. 24, Part 2, December 2001, pp. 145-151.

METHOXYLATED FLAVONOIDS FROM *TANACETUM SANTOLINOIDES* (DC.)

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نبات التناسيتم سانتولينويد أو البيريثرم سانتولينويد هو نبات معمر مصرى ينمو فى سيناء. من الخلاصة الكلوروفورمية لهذا النبات تم فصل و التعرف على أربعة مركبات فلافونيدية تحتوى على مجموعات ميثوكسيلية. وقد أمكن التعرف على هذه المركبات باستخدام الطرق الطيفية المختلفة مثل الرنين النووى المغناطيسى البروتونى والكربونى والرنين النووى المغناطيسى ثنائى الإحداثيات (بروتون-بروتون) و (بروتون-كربون) وكذلك مطياف الكتلة ووجد أن هذه المركبات هى أرتيميتين ، جاسيوزيدين ، كريزيوريول وديوزميتين بالإضافة إلى استجماستيرول جلوكوزيد. وتجدر الإشارة الى أن هذه المركبات تفصل لأول مرة من النبات موضوع البحث.

From the chloroform extract of the aerial parts of Tanacetum santolinoides (DC.), four methoxylated flavonoids were isolated .Characterisation and structure elucidation were conducted through spectral data (^{1}H-NMR, ^{13}C-NMR, ^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-COSY, HMBC and MS experiments). The isolated flavonoids were identified as artemetin, jaceosidin, chrysoeriol and diosmetin. More over stigmasterol-3-O-ß-glucoside was also isolated and identified. This is the first report for the isolation of these compounds from the title plant.

INTRODUCTION

Tanacetum santolinoides (DC.) Feinbr.& Fertig (*Pyrethrum santolinoides* (DC.), Asteraceae is an Egyptian perennial herb growing wild in the rocky mountains of Sinai.[1]

The aerial parts of the plant are reputed among the Bedouins to be effective in cholera and the flowers are said to kill goats.

Previous studies on the chemical constituents of *Tanacetum* santolinoides (DC) led to the isolation of sesquiterpene lactones[2-7] and flavonoids[8] named 6-hydroxyluteolin 6,7,3`-trimethyl ether (cirsilineol), 6-hydroxyluteolin 6,7,3`,4`-tetramethyl ether and quercetagenin 3,6,7,4`-tetramethyl ether.

We present here the isolation of four methoxylated flavonoids from the aerial parts of the plant. The characterisation and structure elucidation of the isolated flavonoids were conducted through spectral data (^{1}H-NMR, ^{13}C-NMR, ^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-COSY, HMBC and MS experiments). The isolated flavonoids were identified as artemetin, jaceosidin, chrysoeriol and diosmetin. More over stigmasterol-3-O-ß-glucoside was also isolated and identified. This is the first report for the isolation of these compounds from the title plant.

EXPERIMENTAL

General experimental procedures

Mps were uncorrected. ^{1}H, and ^{13}C-NMR spectra were recorded in $CDCl_3$ unless otherwise mentioned at 300 MHz and 75 MHz, respectively, onVarian Unity 300 "Uni 300" spectrometer using TMS as internal standard. MS spectra on JEOL JMS 600, 70 eV. UV spectra were measured in MeOH and different ionizing and complexing reagents using Shimadzu, UV-1601PC (Shimadzu corporation, Japan). For CC, silica gel (E.Merck), and pre-packed columns [Lobar-Kieselgel columns (40-

Received in 18/1/2001 & Accepted in 28/2/2001

63 μm) (Merck) size B (310x25 mm)] were used. Precoated silica gel 60 F_{254} plates (E. Merck) were used for TLC.

The following solvent systems were used:

System I : $CHCl_3$-MeOH (95:5)
System II: $CHCl_3$-Acetone (90:10)

Plant material

The plant material was collected during flowering stage in March, 1997 from the rocky slopes in Sinai. The plant material was air-dried in the shade. The identity of the plant was confirmed by Prof. A. Fayed, Professor of plant taxonomy, Faculty of Science, Assiut University. A voucher specimen is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Egypt.

Extraction and isolation

4 kg of the air-dried powdered aerial parts were exhaustively extracted with 70 % methanol by maceration (4x15 L). The combined extracts were evaporated and the concentrated viscous product was diluted with distilled water (500 ml) and partitioned between petroleum ether (5x750 ml), $CHCl_3$ (5x750 ml) and finally with n-butanol (4x500 ml). The chloroformic extract was concentrated under reduced pressure to give viscous residue (20 g). This fraction was fractionated by CC (silica gel, 150x7 cm) eluting with $CHCl_3$ followed by MeOH-$CHCl_3$ gradients. Fractions 250 ml each, were collected and monitored by TLC (silica gel, Systems I and II).

Elution with $CHCl_3$-MeOH (98:2) afforded compound (**1**). Fractions eluted with $CHCl_3$-MeOH (95:5) afforded a mixture of compounds (**2**) and (**3**). This mixture was subsequently separated by further chromatography on an efficient Lobar silica gel pre-packed column, eluted with $CHCl_3$-Acetone (9:1) resulted in the isolation of compounds (**2**) and (**3**). Fractions eluted with $CHCl_3$-MeOH (9:1) afforded impure compound (**4**) which was purified by repeated column chromatography using SiO_2 to obtain pure compound (**4**).

Fractions eluted with $CHCl_3$-MeOH (85:15) afforded compound (**5**).

Compound (1): pale yellow prisms (MeOH) (35 mg), m.p 162-164°, R_f value= 0.9 in system I. UV λ_{max} (MeOH) 254, 273, 345; +NaOMe 289, 325 sh, 348 sh; +$AlCl_3$ 266, 280 sh, 300 sh, 377; +$AlCl_3$/HCl 265, 283, 364; +NaOAc 274, 340. EIMS, m/z (rel. int.%) 388 (M^+, 68.8), 373 (base peak), 345 (M^+-$COCH_3$, 64.8), 330 (40.1), 165 (89.4).

Compound (2): yellow crystals (MeOH) (25 mg), m.p 226-228°, R_f value= 0.78 in system I. UV λ_{max} (MeOH) 250 sh, 271, 344; +NaOMe 260, 274 sh, 331, 404; +$AlCl_3$ 260, 280, 300, 371; +$AlCl_3$/HCl 258, 283 sh, 293, 364; +NaOAc 274, 343. EIMS, m/z (rel. int.%) 330 (M^+, base peak), 229 (1.7), 312 (59.9), 301 (38.8).

Compound (3): yellow needles (MeOH) (20 mg), m.p 334-336°, R_f value= 0.63 in system I. UV λ_{max} (MeOH) 240, 268, 345; +NaOMe 262, 326 sh, 402; +$AlCl_3$ 261, 273, 295, 363, 388; +$AlCl_3$/HCl 260, 274, 291, 350, 384; +NaOAc 274, 318, 393. EIMS, m/z (rel. int.%) 300 (M^+, base peak), 272 (15.8), 258 (10.7), 229 (44.2), 153 (69.8), 152 (15.9), 148 (40.1), 133 (39.9), 124 (24.4).

Compound (4): yellow needles (MeOH) (30 mg), m.p 257-259°, R_f value= 0.54 in system II. UV λ_{max} (MeOH) 253, 268, 345; +NaOMe 269, 301 sh, 386; +$AlCl_3$ 265 sh, 273, 294, 363, 391; +$AlCl_3$/HCl 261 sh, 254, 293, 350, 382; + NaOAc 274, 320, 370. EIMS, m/z (rel. int.%) 300 (M^+, base peak), 272 (10.6), 258 (2.3), 229 (5.4), 153 (9.8), 152 (4.9), 148 (40.0), 133 (38.0), 124 (24.0).

The ^{1}H-NMR and ^{13}C-NMR data of the isolated flavonoids were listed in Tables 1 and 2, respectively. The HMBC experiments were shown in Fig. 1.

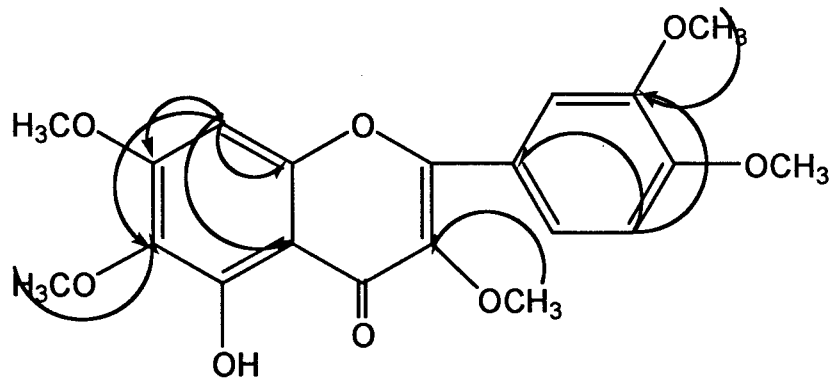
Compound (5): White amorphous powder ($CHCl_3$+MeOH) (50 mg). m.p 232-234°. It gives violet colour with vanillin-H_2SO_4.

Table 1: 300 MHz ^{1}H-NMR data of the isolated flavonoids.

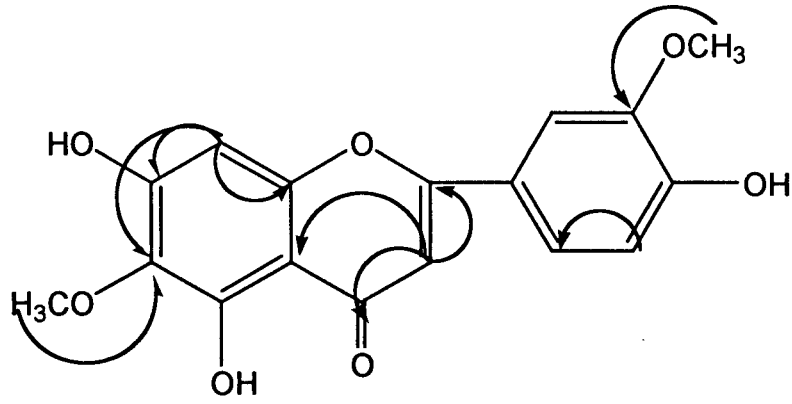
Proton No.	(1) ppm, multi (J Hz)	(2) ppm, multi (J Hz)	(3) ppm, multi (J Hz)	(4) ppm, multi (J Hz)
H-3	----------	6.82,s	6.82,s	6.74,s
H-6	----------	----------	6.19,d (2.1)	6.18,d (2)
H-8	6.48 ,s	6.60,s	6.49,d (2.1)	6.46,d (2)
H-2`	7.66,d (2)	7.55,d,(2)	7.54,d (2.3)	7.41,d (2.3)
H-5`	6.97,d (8.6)	7.0,d,(9)	6.93,d (8.9)	7.06,d (8.7)
H-6`	7.71,dd (8.6, 2)	7.5,dd, (9, 2)	7.54,dd (8.9, 2.3)	7.51,dd (8.7,2.3)
5-OH	12.60,s	13.0,s	12.9, s	12.85,s
OCH_3-3`	3.97,s	3.89,s	3.95,s	----------
OCH_3-3	3.85, s	----------	----------	----------
OCH_3-6	3.91,s	3.76,s	----------	----------
OCH_3-7	3.95,s	----------	----------	----------
OCH_3-4`	3.97,s	----------	----------	3.86,s

Table 2: ^{13}C-NMR data of the isolated flavonoids.

Carbon No.	Compound (1)	Compound (2)	Compound (3)	Compound (4)
C-2	155.86	163.70	164.18	163.59
C-3	138.87	102.72	103.76	103.26
C-4	178.89	182.12	181.87	181.77
C-5	152.33	152.70	161.47	161.47
C-6	132.36	131.32	98.89	98.89
C-7	158.79	157.24	163.73	164.26
C-8	90.34	94.29	94.13	93.99
C-9	152.82	152.38	157.39	157.38
C-10	106.64	104.04	103.26	103.56
C-1`	122.97	121.53	121.56	123.02
C-2`	110.91	110.19	110.17	112.19
C-3`	148.84	150.70	150.76	146.83
C-4`	151.44	148.00	148.07	151.20
C-5`	111.39	115.74	115.81	112.96
C-6`	122.16	120.32	120.42	118.80
OCH_3-3	60.88	----------	----------	----------
OCH_3-6	60.20	59.92	----------	----------
OCH_3	56.34	55.95 OCH_3-3`	56.01 OCH_3-3`	56.00 OCH_3-4`
OCH_3	56.12	----------	----------	----------
OCH_3	56.01	----------	----------	----------



Compound 1



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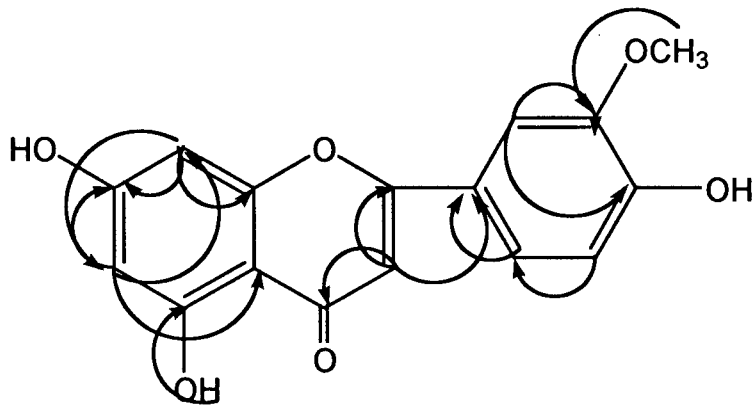
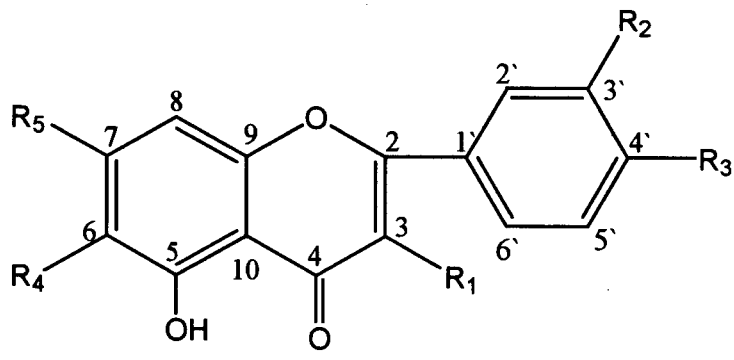
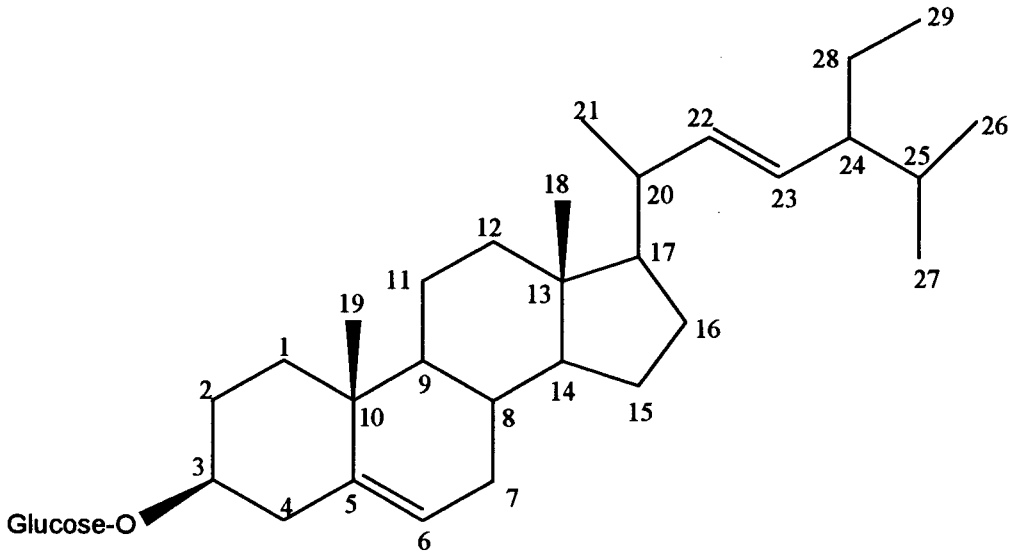


Compound 3

Fig. 1: HMBC experiments of the isolated flavonoids



	R_1	R_2	R_3	R_4	R_5
Artemetin (1)	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
Jaceosidin (2)	H	OCH_3	OH	OCH_3	OH
Chrysoeriol (3)	H	OCH_3	OH	H	OH
Diosmetin (4)	H	OH	OCH_3	H	OH



Compound **5**

300 MHz ^{1}H-NMR (DMSO-d_6) δ 5.30 (1H, m, H-6), 5.13 (1H, dd, J= 8.2, 15 Hz, H-22), 5.05 (1H, dd, J= 8.2, 15 Hz, H-23), 4.20 (1H, d, J= 7.8 Hz, H-1`), 3.63 (1H, dd, J= 4.3, 11 Hz, H-3), 3.2-3.6 (sugar protons), 0.79 (3H, d, J= 6.6 Hz, 27-CH_3), 0.64 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 0.98 (3H, d, J= 6.4 Hz, 21-CH_3), 0.89 (3H, d, J= 6.1 Hz, 26-CH_3), 0.80 (3H, t, J= 6.1 Hz, 29-CH_3). 75 MHz ^{13}C-NMR (DMSO-d_6) δ 140.41 (s,C-5), 137.97 (d, C-22), 128.79 (d, C-23), 121.13 (d, C-6), 100.78 (d, Glc. C-1`),76.91 (d, C-3), 76.74 (d, Glc. C-3`),76.70 (d, Glc. C-5`),73.43 (d, Glc. C-2`), 70.07 (d, Glc. C-4`), 61.06 (t, Glc. C-6`),56.14 (d, C-17), 55.41 (d, C-14), 50.55 (d, C-24), 49.58 (d, C-9), 45.12 (t, C-12), 41.82 (s, C-13), 41.70 (d, C-20), 38.29 (t, C-4), 36.80 (t, C-1), 36.18 (s, C-10), 33.45 (d, C-8), 33.33 (t, C-7), 31.39 (t, C-2), 29.24 (d, C-25), 28.58 (t, C-28), 27.74 (t, C-16), 25.14 (t, C-15), 23.83 (t, C-11), 22.58 (q, C-21-CH_3), 20.56 (q, C-27-CH_3), 19.66 (q, C-26-CH_3), 19.05 (q, C19-CH_3), 12.06 (q, C-29-CH_3), 11.74 (q, C-18-CH_3).

Acid hydrolysis

Ten mg of compound **5** were dissolved in 5 ml methanol and were refluxed with 10% methanolic HCl (2 ml), on a boiling water bath, for about 4 hr. The aglycone was extracted with Et_2O. The aqueous layer was neutralized by Amberlite MB-3 resin and dried. The sugar was identified as glucose by chromatographic study comparing with authentic sugars.

The aglycone was identified as stigmasterol by comparison with authentic sample.

RESULTS AND DISCUSION

Compound (1): The UV spectroscopic absorptions were closely similar to those reported for polymethoxylated flavonoids.[9]

^{1}H-NMR data (Table 1) revealed four signals appeared at δ 3.85, 3.91, 3.95 and 3.97 integrated for 15 protons, attributable to five methoxy groups. The signal integrated for one proton which appeared as a singlet at δ 6.48 referred to the proton at 8 position, this could be established from HMBC experiments since H-8 showed two and three-bond couplings to C-9 at 152.82, C-7 at 158.79 and C-6 at 132.36, respectively. The positions of methoxy groups could be established at C-3`, C-4`, C-3, C-6 and C-7 positions from HMBC (Fig. 1).

Mass spectrum of compound **(1)** showed molecular ion peak M^+ at m/z 388 indicating a penta methoxyflavone.

The above mentioned data of compound **(1)** showed identity with those reported for artemetin.[10-12] This is the first report for isolation of artemetin from the title plant.

Compound (2): The UV spectra with standard reagents indicated that compound **(2)** is a flavone with free hydroxyl groups at 4` and 7 positions. The presence of hydrogen-bonded phenolic hydroxyl group at C-5 was deduced from ^{1}H-NMR spectrum which showed a sharp singlet at δ 13.0. The NMR experiments (^{1}H-NMR, ^{13}C-NMR, ^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-COSY and HMBC) indicated the presence of two methoxyl groups (each singlet at δ 3.76 and 3.89) assigned for methoxyls at C-6 and C-3`, respectively.

The above mentioned data (UV, ^{1}H-NMR, ^{13}C-NMR and MS) are in accordance with the data reported for jaceosidin.[10,13,14,15]

This is the first report of jaceosidin in *Tanacetum santolinoides*.

Compound (3): The ^{1}H-NMR and ^{13}C-NMR (Tables 1 and 2) data of compound **(3)** are similar to those of compound **(2)** except the disappearance of methoxyl signal at 6 position and this was confirmed from HMBC experiments (Fig. 1).

Its Ms exhibited a molecular ion peak at m/z 300 (base peak) consistant with the molecular formula of $C_{16}H_{12}O_6$ and in accordance with one methoxy and three hydroxyl groups in the flavonoidal rings. Fragments at m/z 152 and 153 indicated that ring A is substituted with two hydroxyl groups. Fragment at m/z 148 indicated that ring B is substituted by one hydroxy and one methoxy group.

The above mentioned data are in accordance with the data reported for chrysoeriol.[9,10,16]

This is the first report of chrysoeriol in *Tanacetum santolinoides*.

Compound (4): It has the same fragmentation pattern as compound (3) and has a methoxy group also in ring B. The only difference between (3) and (4) is the position of the methoxy group.

The ^{1}H-NMR spectrum (Table 1) in DMSO-d_6 confirmed that it is a flavone with one methoxy singlet at δ 3.86.

The H-6` signal appeared downfield from the one for H-2` is in accord with the presence of a C-4` methoxyl group rather than one at the C-3` position as in compound (3). Therefore the methoxyl group was positioned at C-4`.[17]

The above mentioned data are in accordance with the data reported for Diosmetin.[9,10,17]

This is the first report of Diosmetin in *Tanacetum santolinoides*.

Compound (5): was obtained as white amorphous powder melted at 232-234°. It gave positive tests for unsaturated sterols and/or triterpenes and carbohydrates and/or glycosides. It was identified as stigmasteryl 3ß-glucoside by comparison of its 300 MHz ^{1}H-NMR and 75 MHz ^{13}C-NMR data with those reported for stigmasteryl 3ß-glucoside and also through hydrolysis of this glycoside[18] (see Experimental).

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