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FLAVONES AND QUATERNARY ALKALOID FROM *TARCHONANTHUS CAMPHORATUS* L.

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من نبات التاركوننسس كامفوراتس تم فصل قلويد جديد وسمي تاركوناننسين أ بالإضافة إلى خمسة فلافون وهى ٥،٧،٤'-ثلاثى هيدروكسى-٦-ميثوكسى فلافون (هسبديولين) ، أبجنين ، ٥،٣،٤'-ثلاثى هيدروكسى-٧،٦-ثنائى ميثوكسى فلافون ، ٥،٧،٣،٤'-رباعى هيدروكسى-٦-ميثوكسى فلافون (نبيتن) ، ليتيولين ،. هذا وقد تم التعرف على هذه المركبات بواسطة الطرق المختلفة للتحليل الكيماوى والطيفى ودراسة الخواص الطبيعية وقد وجد أن جميع الفلافون قد تم فصلها لأول مرة من جنس التاركوننسس.

From Tarchonanthus camphoratus L. we isolated a new quaternary alkaloid Tarchonanthine (A) and five flavones viz. 5,7,4`-trihydroxy-6-methoxyflavone (hispedulin) (1), apegenin (2), 5,3`,4`-trihydroxy-6,7-dimethoxyflavone (3), 5,7,3`4`-tetrahydroxy-6-methoyflavone (Nepetin) (4) and Luteolin (5). The structure of these components were established through the spectral studies. All the isolated flavones were reported for the first time from the genus Tarchonanthus.

INTRODUCTION

Tarchonanthus camphoratus L. Family: Compositae is used in folk medicine[1] in the treatment of headache, toothache, venereal diseases and bronchitis. The infusion of the leaf has diaphoretic, narcosis and tonic effects, also used for the relief of inflammation, spasmodic abdominal pains and asthma. Bisabolene and dihydrocaffeic acid derivatives with 5,7-dihydroxyflavanone and tarconanthus lactone were isolated from *Tarchonanthus trilobus*.[2] Nothing was reported concerning the phytochemical investigation of *T. camphoratus*. As a part of our research programme we reported in this paper the isolation and structure elucidation of a new quaternary alkaloid and five flavones isolated for the first time from the genus *Tarchonanthus*.

EXPERIMENTAL

All mps are determined using Stuart scientific apparatus uncorrected. Mass spectra were carried out on Jeol, JMS, 600 H. NMR spectra were recorded at 400 and 100 MHz for ^1H and ^{13}C respectively in DMSO-d_6 and $CDCl_3$ (JNM-LA400), TLC carried out on silica gel (E-Merck) using the following solvent systems:

System I: Hexane-ethyl acetate (4:6).
System II: Chloroform-methanol (6:4).

Spots were visualized by their fluorescence at 254 nm in UV. or by spraying with Dragendorff's reagent and $AlCl_3$ (for flavonoids).

Plant material

The plant material was collected in October (1999) during the flowering stage, from Al-Shafa, near Taif city, Southwestern Saudi Arabia. Identity was confirmed by Prof. Dr. A. Fayed, Professor of Taxonomy, Faculty of Science, Assiut University. The aerial parts were dried and powdered.

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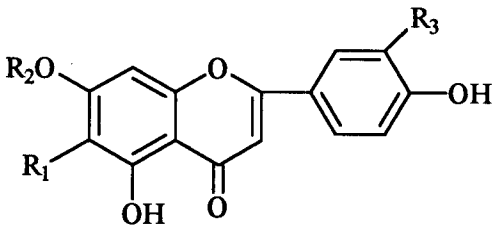
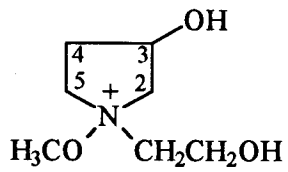
Extraction and isolation

The air dried aerial parts of the plant (2 kg) were percolated successively with hexane, chloroform and 70% ethanol. 20 g of the chloroformic extract (150 g) was subjected to column chromatography packed with silica gel (E-Merck, 800 g, 5x150 cm) using hexane-ethyl acetate gradient elution. Fractions, 250 ml each, were collected concentrated and monitored by TLC silica gel (System I). Elution with hexane-ethyl acetate (25:75) afforded compound **1**, hexane-ethyl acetate (22:78) afforded compound **2**, hexane-ethyl acetate (20:80) afforded compound **3**, hexane-ethyl acetate (15:85) afforded compound **4** and hexane-ethyl acetate (10:90) afforded compound **5**. Purification of the required compounds was accomplished by using silica gel column chromatography for each combined fraction using hexane-ethyl acetate gradient, where compounds (**1-5**) were isolated in pure form. 5 g of the ethanolic extract (40 g) was subjected to silica gel column chromatography (E-Merck, 200 g, 3x80 cm) using chloroform-methanol gradient. Similar fractions (100 ml each) were combined together.



	R_1	R_2	R_3
Compound 1	OCH_3	H	H
Compound 2	H	H	H
Compound 3	OCH_3	CH_3	OH
Compound 4	OCH_3	H	OH
Compound 5	H	H	OH



Compound A

Compound **A** was obtained from fractions (10-15) and purified by repeating the same technique on a smaller column.

Compound 1: Yellow needles (MeOH), (38 mg), R_F 0.51 using System I, mp. 265-267°, Mass spectrum (rel. int.%): 300 [M^+] (10), other characteristic fragments at 285 (68), 272 (10), 269 (7), 257 (57), 183 (11), 153 (11), 139 (19), 121 (13), 118 (33) and 69 (69), UV (Table 1), ^{1}H-NMR and ^{13}C-NMR (Tables 2 and 3) respectively.

Compound 2: Was obtained as yellow amorphous powder (MeOH),(18 mg), R_F 0.40 using System I, mp. 343-346°, MS (rel. int.%) 270 [M^+] (22), 269 (87), 241 (70), 240 (50), 153(78), 149 (45), 121 (74), 114 (34) and 69 (70), UV and ^{1}H-NMR (Tables 1 and 2) respectively.

Compound 3: Obtained as yellow prisms (MeOH), (20 mg), R_F 0.32 using System I, mp. 281-283°, MS (rel. int.%): 330 [M^+] (33), 329 (24), 315 (32), 300 (57), 299 (82), 270 (62), 257 (82), 197 (7), 182 (7), 167 (94), 153 (54), 121 (66) and 69 (100), UV, ^{1}H-NMR and ^{13}C-NMR (Tables 1, 2, 3) respectively.

Compound 4: Obtained as yellow needles (MeOH),(60 mg), R_F 0.28 using System I, mp. 259-261°, MS (rel. int.%): 316 [M^+] (56), 300 (41), 297 (28), 272 (49), 197 (12), 134 (60) and 69 (100), UV, ^{1}H-NMR and ^{13}C-NMR (Tables 1, 2 and 3) respectively.

Compound 5: Obtained as yellow needles (MeOH), (21 mg), R_f 0.23 using System I, mp. 326-328°, it showed physical, chemical, chromatographic characters and spectral data (UV, ^{1}H-NMR and MS) identical with those of luteolin previously isolated by the author.[3]

Compound A: White gum, (25 mg), R_F 0.11 using System II, IR (KBr) 3450 cm^{-1} (OH), MS (rel. int.%): 162 [M^+] (7), 149 (80), 95 (26), 85 (25), 83 (40), 69 (55), 57 (90) and 43 (100).

Table 1: UV spectral data of compounds **1,2,3** and **4**.

Comp. No.	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaoAc/H_3PO_3
1	274 334	274 326 (sh) 392	286 358	286 354	280 342	278 339
2	264 338	272 292 (sh) 390	270 298 (sh) 382	270 381	272 375	268 338
3	272 348	266 401	274 422	288 364	273 365	264 373
4	274 344	266 399	275 422	285 363	278 346	264 370

sh = shoulder

Table 2: ^{1}H-HNMR spectral data of compound **1** ($CDCl_3$), **2,3,4** (DMSO), 400 MHz.

Comp.	H-3	H-6	H-8	H-3`,5`	H-2`,6`	O-CH_3 (C-6)	O-CH_3 (C-7)	-OH
1	6.5 (s)	-	6.7 (s)	6.8 (d) J= 8.8 Hz	7.9 (d) J= 8.8 Hz	3.73 (s)	-	13.05 (s)
2	6.1 (s)	6.7 (s)	6.4 (s)	6.8 (d) J= 8.6 Hz	7.8 (d) J= 8.6 Hz	-	-	12.93 (s)
3	6.7 (s)	6.1 (br.s)	6.8 (s)	*6.8	7.4 (m)	3.71 (s)	3.91 (s)	12.94 (s)
4	6.5 (s)	6.4 (br.s)	6.6 (s)	6.8 (d) J= 8.7 Hz	7.3 (s) (broad)	3.73 (s)	-	13.05 (s)

*overlapped

Table 3: ^{13}C-NMR of flavones **1,3** and **4** (DMSO-d_6).

Carbon number	1	3	4
2	163.8	164.2	163.9
3	102.3	102.6	102.4
4	182.0	182.1	182.0
5	152.7	152.6	152.8
6	131.3	131.8	131.4
7	157.2	158.5	157.2
8	94.2	91.4	94.2
9	152.3	152.0	152.3
10	104.0	104.9	104.1
1`	121.1	121.3	121.6
2`	128.4	113.5	113.4
3`	115.9	145.8	145.8
4`	161.1	149.9	149.7
5`	115.9	115.9	116.0
6`	128.4	119.0	119.0
6-OMe	59.9	60.0	60.0
7-OMe	--	56.4	--

^{1}H-NMR: δ 2.0 (1H, m, H-4), 2.49 (1H, m, H-4), 3.18 (4H, overlaped, H_2-2,5), 3.25 (2H, m, $C\underline{H}_2CH_2OH$), 3.50 (2H, m, $CH_2C\underline{H}_2OH$), 3.82 (3H, s, OCH_3), 4.5 (1H, br.s, H-3) and 5.90 (1H, s, -OH at C-3), ^{13}C-NMR data are recorded in Table 4.

Table 4: ^{13}C-NMR of compound A (DMSO-d_6).

Carbon number	PPM	DEPT
2	72.50	CH_2
3	68.40	CH
4	32.80	CH_2
5	67.08	CH_2
$\underline{C}H_2$-CH_2-OH	55.30	CH_2
CH_2-$\underline{C}H_2$-OH	64.80	CH_2
-OCH_3	53.40	CH_3

RESULTS AND DISCUSSION

The aerial parts of *Tarchonanthus camphoratus* L. afforded five flavones and one new quaternary alkaloid.

Compound 1: Its UV absorption data (Table 1) showed that the compound **1** contains free-OH group at C-7 as indicated from the NaOAc shift, and a free 4`-hydroxy group (bathochromic shift in NaOMe of 58 nm for band I with an increase in intensity) and no ortho-dihydroxy group in A-ring ($AlCl_3$, $AlCl_3$/HCl). The ^{1}H-NMR spectrum showed the presence of signals for A-ring at δ 6.7 (1H, H-8) and 6.5 (1H, H-3) for the proton γ-pyrone ring, 6.8 (2H, H-3`,5`),7.9 (2H, H-2`,6`)for the protons of B ring and the singlet at δ 3.73 (3H, OCH_3) was attributed to the O-methyl group at C-6. Furthermore, a sharp singlet (1H) at δ 13.05 showed the presence of hydroxyl at the C-5 position.[4] The mass spectrum of **1** showed a peak at m/z 300 corresponding to the molecular ion $C_{16}H_{12}O_6$.

The ^{13}C-NMR (Table 2) showed that the methoxy group (downfield at δ 59.9) was flanked by ortho-oxygenated carbons (i.e 5,7-dihydroxy-6-methoxy flavone).[5] The structure was moreover supported by the close resemblance of the spectral data (UV, ^{1}H-NMR and ^{13}C-NMR) with those reported for 5,7,4`-trihydroxy-6-methoxy flavone (hispedulin).[4]

Compound 2: It is a flavone apigenin as indicated from UV, ^{1}H-NMR (Tables 1, 2) and MS which compared with those reported for apigenin.[6]

Compound 3: The MS displayed molecular ion peak at m/z 330 consistent with the molecular formula $C_{17}H_{14}O_7$ and in accordance with a flavone with three hydroxy and two methoxy groups. The UV absorption showed the presence of 3`,4`-ortho-dihydroxy groups ($AlCl_3$, $AlCl_3$/HCl and NaOAc/Boric acid spectra), blocked-OH at C-7 (soduim acetate shift), 4`-hydroxy group indicated by $NaOCH_3$ (increased intensity of band I) and free hydroxy at C-5 ($AlCl_3$/HCl shift). The ^{1}H-NMR (Table 2) revealed the presence of two methoxy groups, assigned to positions 6 and 7, as indicated from the singlet at δ 3.71 and 3.91 respectively. The signal at 6.7 (1H, s) is assigned to proton at C-3, signal at 6.8 (2H, overlaped) assigned to proton at C-8 and C-5, signal at 7.43 (2H, s) assigned to protons at C-2` and C-6` and signal at 12.94 (1H, s, C_5-OH). The ^{13}C-NMR (Table 3) showed that the compound **3** is a flavone with unsubstituted 2`,6`(C-3 at 102.0),[5] the presence of hydroxy at C-5 (the appearance of C-4 resonance at downfield shift 182.1). Two methoxy groups, one between two ortho-oxygenated carbons at δ 60.0 on C-6 and the other at 56.4 on C-7.[5] The already mentioned data as well as the close resemblance of these data with the data reported for related compounds,[7,8] supported the structure of compound **3** as 5,3`,4`-trihydroxy-6,7-dimethoxy flavone.

Compound 4: The mass spectrum showed a peak at m/z 316 corresponding to the molecular ion $C_{16}H_{12}O_7$. The UV spectra in different shift reagents indicated that **5** is almost similar to **3** but with free hydroxy at C-7 (sodium acetate shift reagent causes band II to move from 274 to 278 nm). The ^{1}H-NMR (Table 2) showed signals at δ 6.5 (1H, s, H-3), signal for A-ring proton at 6.6 (1H, s, H-8), signals for B-ring protons 6.8 (1H, d, H-5`), 7.3 (2H, br.s, H-2` and H-6`), signal at 3.73 for methoxy group at C-6 and signal at 13.05 (1H, s, C_5-OH). The ^{13}C-NMR (Table 3) showed a characteristic downfield shift signal at δ 60 for methoxy group at C-6 between two ortho-oxygenated carbons (i.e. 5,7-dihydroxy-6-methoxy flavone). The downfield shift of C-4 at 182.0 indicated the presence of hydroxy at C-5 in flavone.[5] The chemical shift of C-3 at δ 102.4 is characteristic for 2`,6` unsubstituted flavones.[5]

The above mentioned data suggest the flavonoid **4** is 5,7,3`,4`-tetrahydroxy-6-methoxy flavone. The structure of **5** was moreover supported by the close resemblance of the ^{13}C-NMR spectral data with those reported for 5,7,3`,4`-tetrahydroxy-6-methoxyflavone.[9]

Compound 5: Showed physical, chemical, chromatographic characters and spectral data (UV., ^{1}H-NMR and MS) which were identical with those of luteolin previously isolated by the author from Begnonia unguis-cati L.[3]

Compound A: White gum which respond positively to Dragendorff's test, IR (KBr) showed band at 3450 cm^{-1} indicated the presence of hydroxyl group. The mass spectrum showed molecular ion peak at m/z 162 calculated for $C_7H_{16}NO_3$. ^{1}H-NMR showed signals at δ 2.0, 2.49 (each one proton multiplet, 1H, m) assigned for two protons attached to C-4, signals at 3.18 (4H, overlapped) for protons attached to C-2 and C-5. A multiplet at δ 3.25, 3.50 assigned for two methylene groups one attached to the quaternary nitrogen and the other attached to hydroxy group. A singlet at δ 3.82 (3H, s) is due to methoxy group adjacent to the quaternary nitrogen. There is a broad signal at δ 4.5 (1H, br.s) attributed to one proton at C-3. A singlet at δ 5.90 (1H, s) assigned for the hydroxy group at C-3. The already mentioned data supported the proposed structure. Moreover ^{13}C-NMR and DEPT (Table 4) revealed the presence of two downfield signals at δ 72.5 and 67.08 assigned

for two methylene carbons adjacent to the quaternary nitrogen (C-2 and C-5 respectively), signal at δ 68.4 attributed to methine group (C-3). Signal at δ 64.8 is due to methylene carbon attached to hydroxy group, a downfield signal at 55.3 for methylene group attached to the quaternary nitrogen and signal at δ 53.4 for methoxy group adjacent to the quaternary nitrogen. There is a signal at δ 32.8 assigned to C-4 beta to the quaternary nitrogen. The above data compared with the data for 3-hydroxy-stachydrine,[3,10] provided further support for our conclusion for compound A and named tarchonanthine.

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