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SESQUITERPENE LACTONES AND FLAVONOID GLUCOSIDE WITH THE POTENT BIOLOGICAL ACTIVITIES OF *TARCHONANTHUS CAMPHORATUS* L.

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تم فصل مركبين من نوع سيسكتربين لاكتون ، أحدهما جديد ويسمى تاركوننثينوليد ومركب معروف وهو بارثينوليد وكذلك تم فصل فلافونيد جلوكوزيد لبتيولين-٧-أ-جلاكوزيد وقد تم التعرف على هذه المركبات بإستخدام الطرق الطيفية المختلفة من نبات تاركونانتس كامفوراتس. وقد تم عمل دراسة بيولوجية على النبات تبين فيها أن الخلاصة الكحولية للأوراق لها تأثير قوى كمسكن ومضاد للإلتهابات ومضاد للصرع.

From the chloroformic fraction of the leaves of Tarchonanthus camphoratus L (Compositae), a new guaianolide sesquiterpene lactone which were named tarchonanthenolide and the known germacranolide parthenolide were isolated. In addition luteoline 7-O-glucoside was obtained from the ethanolic fraction of the leaves. Moreover, the biological screening showed that the ethanolic extract of the leaves possesses a significant analgesic, anti-inflammatory and anticonvulsant activities.

INTRODUCTION

Reviewing the current literature, nothing was reported about the active constituents of *Tarchonanthus camphoratus* L. In a previous publication,[1] we reported the isolation of new alkaloid and five flavones as well as the folk medicinal uses of the plant. In this paper, as a part of our study on the active constituents of the leaves, the new sesquiterpene lactone tarchonanthenolide (2), the known sesquiterpene lactone parthenolide (1) and the flavonoid glucoside luteolin-7-O-glucoside (3) were isolated. The two known compounds (1,3) were isolated for the first time from the genus Tarchonanthus.

The biological screening of the ethanolic extract of the leaves showed a significant analgesic, anti-inflammatory and anticonvulsant activities.

EXPERIMENTAL

General

Melting points were uncorrected and determined using Stuart Scientific apparatus. ^{1}H-NMR and ^{13}C-NMR were recorded in DMSO-d_6 and $CDCl_3$ at 400 MHz and 100 MHz, respectively, using JNM-LA400. Mass spectra were carried out on Jeol, JMS, 600H. UV measurements were done on Perkin-Elmer model 550 spectrophotometer. The column chromatography and TLC with silica gel (E. Merck) was used.

Plant material

The plant was collected in October (1999) during 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. Herbarium specimens were kept at the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.

Extraction and isolation

The air dried aerial parts (leaves) of the

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plant (2 kg) were percolated successively with hexane, chloroform and 70% ethanol. Ten g of the chloroformic extract (150 g) was subjected to a column packed with silica gel (E. Merck, 400 g, 4x120 cm) using hexane-ethyl acetate gradient elution. Fractions, 250 ml each, were collected, concentrated and monitored by TLC silica gel (hexan-ethyl acetate 8:2 as solvent system and 10% H_2SO_4 as spraying reagent). Elution with hexane-ethyl acetate (90:10) afforded compound 1 which was crystallized from petroleum-Et_2O to yield 320 mg of colourless prisms, m.p 115-116°. Elution with hexane-ethyl acetate (80:20) gave after further fractionation on a smaller column chromatography using silica gel (E. Merck) and hexane-ethyl acetate gradient. Fifteen mg of a white gum was obtained which failed to give any crystalline substance. Five g of the ethanolic fraction (40 g) was chromatographed on a silica gel (E. Merck) column using chloroform-methanol graident. Fractions, 150 ml each, were collected and the same fractions were combined together, using chloroform-methanol (9:1) solvent system and $AlCl_3$ as spraying reagent for detection of each fraction, to give 80 mg of yellow crystals from methanol.

Acid hydrolysis

An ethanolic solution (20 mg) was refluxed on a boiling water bath with 1 N HCl for 1 hr. The excess acid was precipitated with Ag_2O. The solvent was evaporated and the aglycone was extracted with EtOAc and recrystallized from methanol. The sugar in the aqueous solution was examined on Whatmann No. 1 filter paper using EtOAc-pyridine-H_2O (5:5:4) mixture as solvent system. The aglycone was subjected to UV and ^{1}H-NMR analyses.

Compound 1: Colourless prisms (petroleum-Et_2O), 320 mg, m.p 115-116°, R_f= 0.40 (hexane-ethyl acetate 8:2), IR (KBr) 1754 cm^{-1} (γ-lactone ring), and 1656 cm^{-1} (unsaturation), MS m/z 248 [M^+] calculated for $C_{15}H_{20}O_3$, ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$), Tables 1 and 2 respectively.

Compound 2: White gum, 15 mg, R_f= 0.30 (hexane-ethyl acetate 8:2), IR (KBr) cm^{-1}: 3480 (OH) and 1771 (γ-lactone), MS m/z 266 [M^+] calculated for $C_{15}H_{22}O_4$; ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$), Tables 1 and 2 respectively.

Compound 3: Yellow crystals, 80 mg, R_f= 0.58 (chloroform-methanol 9:1), m.p 255-257°. UV λ_{max} nm: 350, 260, 270 (MeOH); 400, 300 sh, 270 (NaOMe); 430, 330 sh, 300 sh, 270 ($AlCl_3$); 388, 360, 294 sh, 270 ($AlCl_3$/HCl); 410, 360 sh, 260 (NaOAc); 370, 265 (NaOAc/H_3BO_3). ^{1}H-NMR (DMSO-d_6) at δ H: 7.43 (2H, m, H-6` and H-2`), 6.89 (1H, d, J= 7.08 Hz, H-5`), 6.76 (2H, d, J= 8.2 Hz, H-6 and H-8), 6.43 (1H, s, H-3) and 5.08 (1H, d, J= 7.1 Hz, H-1 glucose). ^{13}C-NMR data are cited in Table 3.

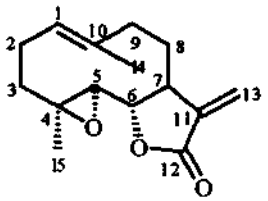
Biological screening

1- Analgesic effect

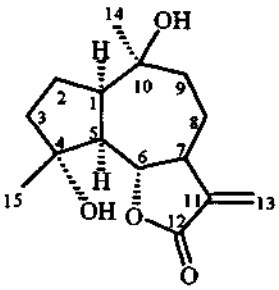
The analgesic effect of the ethanolic extract was studied using the hot plate method as described by Jacobs and Bosovski.[2] Fifteen mice of either sex each weighing 200-250 g were divided into 3 groups each of 5 animals. The first group was left as control. The second group was injected subcutaneously with indomethacin (8 mg/kg) and considered as standard. Animals of the last group were injected subcutaneously with the ethanolic extract. After treatment each mouse was placed on a hot plate thermostatically controlled at 55°. The time elapsed until the mouse jumped was considered as the reaction time for the analgesic activity. The parameter was recorded 15, 30, 60 and 120 min after administration. Results are listed in Table (4).

2- Anti-inflammatory effect

The anti-inflammatory effect was done according to the method described by Vinegar,[3] where a pedal inflammation in rat paws was induced by subcutaneous injection of kaolin (1 ml, 10%). Fifteen adult rats of both sexes each weighing from 200-250 g were equally divided into 3 groups and inflammation was induced in the right paw of all animals. At the beginning of test, the paw's thickness were measured in mm.



Compound 1



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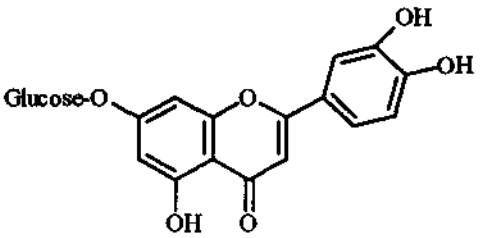

Compound 3

Table 1: ^{1}H-NMR spectral data of compounds **1** and **2** in $CDCl_3$.

H	1	2
1	5.06 (dr) (J= 11.0 Hz)	2.58 (dd) (J= 6, 9 Hz)
2α	2.01-2.21 (m)	1.90 (m)
2ß	2.44 (m)	1.62 (m)
3α	1.25 (m)	-
3ß	2.01-2.21 (m)	-
5	2.79 (d) (J= 9 Hz)	2.30 (dd) (J= 12, 12 Hz)
6	3.86 (dd) (J= 8, 9 Hz)	4.16 (dd) (J= 9.7, 9.8 Hz)
7	2.78 (m)	2.65 (m)
8α	2.01-2.21 (m)	2.07 (m)
8ß	2.88 (m)	1.40 (m)
9α	2.01-2.21 (m)	1.75 (m)
9ß	2.38 (m)	1.90 (m)
13α	6.10 (d) (J= 3.2 Hz)	6.15 (d) (J= 3.2 Hz)
13ß	5.49 (J= 3 Hz)	5.46 (d) (J= 2.9 Hz)
14	1.71 (s)	1.15 (s)
15	1.30 (s)	1.27 (s)

Table 2: ^{13}C-NMR spectral data of compounds **1** and **2** in $CDCl_3$.

C	1	2	DEPT
1	125.2	49.6	CH
2	24.1	24.8	CH_2
3	36.3	39.2	CH_2
4	61.5	79.7	C
5	66.3	55.2	CH
6	82.4	82.7	CH
7	47.6	47.0	CH
8	30.6	25.2	CH_2
9	41.1	43.3	CH_2
10	134.6	74.6	C
11	139.2	138.4	C
12	169.3	169.6	C
13	121.2	120.4	C
14	16.9	24.2	CH_3
15	17.2	23.4	CH_3

Table 3: ^{13}C-NMR spectrum of the flavonoid glucoside (compound 3) in DMSO-d_6.

C	Assignment	C	Assignment
2	164.4	1`	121.4
3	103.3	2`	113.5
4	181.9	3`	145.8
5	161.1	4`	149.9
6	99.8	5`	116.0
7	162.9	6`	119.2
8	94.7	Sugar glucose	
9	156.9	1"	99.5
10	105.3	2"	73.1
		3"	76.4
		4"	69.5
		5"	77.1
		6"	60.6

Table 4: The analgesic effect of the alcoholic extract.

Animal group	Tested compound	Reaction time after administration in min			
		15	30	60	120
Group I (control)	Untreated	1.282±0.203	1.146±0.357	1.172±0.026	1.142±0.09
Group II	Indomethacin (8 mg/kg)	1.425±0.058	2.520±0.413	1.98±0.112	1.367±0.114
Group III	Ethanolic ext. (100 mg/kg)	1.551±0.202	3.550±0.300	3.187±0.356	1.432±0.238

Therefore, the first group was kept as control non-treated. While indomethacin was orally administered to the second group at a dose of 8 mg/kg of body weight one hour after induction of inflammation. The ethanolic extract was orally administered to the third group at a dose of 100 mg/kg of body weight one hour after induction of inflammation and finally the paw thickness of all groups were measured at 1, 2, 3, 4 and 5 hours following administration. The percent of inhibition of paw oedema was estimated according to the formula: % inhibition = $(V_o - V_t) / V_o \times 100$, where V_o is the average paw thickness of control group and V_t is the average paw thickness of the treated group. Results were listed in Table (5).

3- Evaluation of the anti-convulsant activity of the ethanolic extract

The protective effect of a single intraperitoneal administration of the ethanolic extract (100 mg/kg) was studied in mice subjected to chemically-induced convulsions produced by intraperitoneal injection of pentylene-tetrazole (100 mg/kg) and compared with the protective effect produced by single

Table 5: The anti-inflammatory effect of the ethanolic extract.

Animal group	Tested comp.	% increase in paw volume (mean ±S-E after time				
		1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Group I (control)	Untreated	66.6±0.088	86.66±0.057	86.66±0.057	83.33±0.033	83.33±0.033
Group II	Indo-methacin (8 mg/kg)	60±0.091 9.9[a]	66.66±0.033 23.07[a]	56.66±0.033 34.61[a]	63.33±0.033 29.63[a]	63.33±0.033 29.63[a]
Group III	Ethanolic extract (100 mg/kg)	52.5±0.033 21.17[a]	51.25±0.044 40.86[a]	47.5±0.0216 42.3[a]	42.5±0.0478 52.77[a]	37.5±0.047 58.33[a]

[a] Percent of oedema inhibition.

Table 6: The anti-convulsant activity of ethanolic extract.

Treatment 8 dose	Onset of seizures	No. of dead mice	% Protection	Mean survivial time
Control (100 mg/kg)	2.043 ± 0.578	8/8	0	7.23 ± 0.632
Ethanolic extract (100 mg/kg)	10.333 ± 0.8819*	2/8	75	28.35 ± 0.791*
Sodium valproate (200 mg/kg)	6.04 ± 0.251*	4/8	50	26.5 ± 0.276*

*Highly significant difference from control group at $P < 0.01$

intraperitoneal administration of sodium valproate (200 mg/kg). Animals were observed over a period of 40 min. for the onset of convulsions, the mean survival time and the percentage of animals protected from death produced by the convulsive agent.

Groups of 24 albino mice weighing about 200-250 g were used. Mice were divided into 3 groups. Animals of the first group (control group) were intraperitoneally injected with the convulsive agent alone and the onset of seizures, the mean survival time of mice and the percent of surviving mice were determined. Animals of the second and third groups were intraperitoneally injected with the ethanolic extract and sodium valproate respectively 30 minutes before the injection of the convulsive agent. The same parameters i.e., mean survival time of mice, onset of seizures and percentage of surviving mice were computed. Results are listed in Table (6).

RESULTS AND DISCUSSION

Compound 1. The IR spectrum exhibited frequencies corresponding to an α, ß-unsaturated γ-lactone carbonyl at 1754 cm^{-1}, and unsaturation at 1656 cm^{-1}. The mass spectrum showed a molecular ion peak at m/z 248 which agreed with the molecular formula $C_{15}H_{20}O_3$.

The ^{1}H-NMR spectrum (Table 1) showed the exocyclic methylene protons at δ 6.10 (d, J= 3.2 Hz) and 5.49 (d, J= 3 Hz), the proton under the lactone oxygen (H-6) at 3.86 (dd, J= 8, 9 Hz) and two methyl singlets at 1.71 and 1.30. The ^{13}C-NMR spectrum (Table 2) and ^{1}H-NMR (Table 1) were identical with those reported for parthenolid.[4] The DEPT and COLOC experiments, which were reported for the first time here, confirmed the above results.

Compound 2. The IR spectrum displayed a γ-lactone group at 1771 cm^{-1} and a hydroxyl group at 3480 cm^{-1}. The mass spectrum of 2 showed a [M$^+$] ion peak at m/z 266 which agreed with the molecular formula $C_{15}H_{22}O_4$. The ^{1}H-NMR spectrum (Table 1) showed the exocyclic methylene protons at δ 6.15 (d, J= 3.2 Hz) and 5.46 (d, J= 2.9 Hz), the proton under the lactone oxygen (H-6) at δ 4.16 (dd, J= 9.7, 9.8 Hz). The downfield shifts of the two methyl singlets in ^{1}H-NMR at δ 1.15 and 1.27 indicated that these methyl groups should be adjacent to oxygen functions.[5] The ^{13}C-NMR spectrum (Table 2) which exhibited two signals at δ 79.7 and 74.6 also supported this finding.[5] The downfield shifts of the two methyl groups in ^{13}C-NMR at δ 23.4 and 24.2 indicated that these methyl groups adjacent to hydroxy groups.[5-7] From DEPT experiments (Table 2), it was determined that compound 2 possessed two methyl groups, five methylene, four methine and four quaternary carbons one of which, at δ 169.6 could be assigned to the lactone carbonyl. In ^{1}H-NMR spectrum the H-5 of compound 2 is at lower field than H-5 in the compounds previously isolated of the same basic structure with ß-orientation of the 4-OH function.[5] Furthermore, the shift of H-5 of compound 2 closed to that of the compounds with α-orientation of the 4-OH,[6,7] indicating the α-orientation of 4-OH function of compound 2. This supposition was reinforced by a significant downfield shift of C-4 at δ 79.7 in ^{13}C-NMR. The upfield signal of the methyl group at C-10 to δ 1.15 assumed to be due to the ß-orientation of the hydroxy group at C-10. This supposition was indicated by a change in the frequency of the C-10 signal downfield to δ 74.6 (^{13}C-NMR, Table 2) in comparison with similar guaianolides of α-oriented hydroxyl at C-10[8] and in the same time closed to that of compounds with ß-oriented hydroxyl at C-10,[5,7] indicating the ß-orentiation of the hydroxyl group at C-10 in compound 2. On the other hand, the downfield shift of H-1 at δ 2.58 (dd, J= 6, 9 Hz) in comparison with similar compound[5] indicated the α-orientation of H-1 with the ß-orientation of the hydroxyl group at C-10. In the ^{1}H-NMR spectrum (Table 1), the exocyclic methylene doublets which observed at δ 6.15 and 5.46 with coupling of 3.2 and 2.9 Hz indicating a transfused lacton.[9] Complete ^{1}H- and ^{13}C-NMR spectral assignments for compound 2 were determined from 2D-NMR studies (COLOC experiment). The spectroscopic data already mentioned support the suggested structure of compound 2 as 4α,10ß-dihydroxy-1α(H) guaianolide which was named tarchonanthenolide.

Compound 3. It showed UV, ^{1}H-NMR and ^{13}C-NMR data (Table 3) identical to those reported for luteolin-7-O-glucoside.[10,11] Acid hydrolysis afforded D-glucose and luteolin aglycone (co-chromatography with authentic sample using ethyl acetate-hexane 6:4 and comparative study of the UV and ^{1}H-NMR spectra with that of the authentic luteolin).[1]

Results of the biological screening

1- The analgesic effect of the ethanolic extract was done as prementioned in the experimental part. It was found from the data listed in Table (4) that the ethanolic extract showed a significant analgesic effect at P< 0.05 with respect to control.

2- The anti-inflammatory effect of the ethanolic extract was done as prementioned in the experimental part. From the data listed in Table (5), we found that the ethanolic extract of the plant *Tarchonanthus camphoratus* showed a significant anti-inflammatory effect at P< 0.01, where a marked reduction of the paw oedema was observed.

3- The anti-convulsant effect of the ethanolic extract was done as prementioned in the experimental part. From the data listed in Table (6), it can be concluded that:

a) The ethanolic extract showed a significant anticonvulsant effect at $P < 0.01$ where a marked relief of the convulsions was observed.

b) The anticonvulsant effect of the ethanolic extract was more potent than that of sodium valproate with a percent protection 25% more.

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