ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Sesquiterpene Acid Derivatives from *Tarchonanthus camphoratus.*

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ABSTRACT

Fractionation of *n*-Hexane-CH_2Cl_2 (1:1) extract of dried leaves of *Tarchonanthus camphoratus* afforded seven Sesquiterpene acids derivatives (3-8, 10) and diazomethane treatment afforded four 1-2a and 9. The structures of these compounds were determined by extensive 1D and 2D NMR analyses, We report here the isolation and characterization of unreported sesquiterpene compounds isolated for the first time from *T. camphoratus.*

Keywords: *Tarchonanthus camphoratus*; Asteraceae, Sesquiterpenes acid derivatives.

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ISSN: 0975-8585

INTRODUCTION

The genus *Tarchonanthus,* of the family Asteraceae, tribe Tarchonantheae is a small genus with about four species and wide spread in Africa and Saudi Arabia and it is one of the most important species (in terms of frequency of Occurrence), it is closely related to genus *Brachylaena* [1]. The name of *Tarchonanthus* is derived from the Greek word meaning funeral flower, where the name *camphoratus* refers to the strong smell of camphor given off when the leaved are crushed [1]. *T. camphoratus* has a biological effect on nociception in mice and pyrexia on rats. *T. camphoratus* significally attenuated the fever produced by bacterial endotoxin. [2]. The camphor bursh is used for medicinal purposes. Problem such as blocked sinuses and headache can be healded by inhaling the smoke from the burning green leaves. Drinking boiled mixture of leaves and water can help to treat coughing, toothache, abdominal pain and bronchitis [1]. A previous investigation of *T. camphoratus* has revealed the presence of flavonoids [3, 4], alkaloids [3], sesquiterpenes [4, 5] and essential oil [6, 7]. We report here the isolation and characterization of unreported sesquiterpene compounds isolated for the first time from *T. camphoratus.*

MATERIALS AND METHODS

General

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (*l* = 5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; CI-MS, EIMS and HR-CI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-ECA500 (125 MHz) spectrometers with tetramethyl silane as an internal standard. HPLC detector, Shimadzu RID-10A refractive index detector; and HPLC column, YMC-Pack ODS-A (YMC, Inc., 250 x 4.6 mm i.d.) and (250 x 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase); and detection was achieved by spraying with 10% H_2SO_4 followed by heating.

Plant material

T. camphorates L. was collected during the flowering stage by Professor H. A. Albar, in October, 2008.

Extraction and separation

Dried and finely powdered leaves (**1** Kg) were extracted at room temperature with *n*-Hexan-CH_2Cl_2 (1:1). The extracts were concentrated in vacuo to obtain residues of **10** g from *T. camphoratus*. The extract of *T. camphoratus* was pre-fractionated by column chromatography (6x120 cm) on silica gel eluting with n-hexane (3 L) followed by a gradient of n-hexane-CH_2Cl_2 up to 100% CH_2Cl_2 and wash the column with CH_2Cl_2-MeOH (1:1) (2 L each of solvent mixture). The 50% *n-Hexan*-CH_2Cl_2 fraction was subjected to silica gel column (2 x 60) eluted with *n*-hexane-CH_2Cl_2–MeOH to give two fractions. Fraction 1 (*n*-hexane-CH_2Cl_2-MeOH, 7:4:0.25); Fraction 2 (*n*-hexane-CH_2Cl_2-MeOH, 7:4:0.5). Fraction 1 was further purified by TLC to obtain (**3, 4** and **10**); Fraction 2 was purified by HPLC (MeOH-H_2O, 70:30) followed by TLC to give **5**-**7** and **8**. A solution of 400 mg of fraction 2 in 100 ether was mixed was calculated amount of diazomethane in ether, the solution allowed to stand and evaporated at room temperature, and the oil crude product was chromatographed over Sephadex LH-20 followed by TLC to give **1**, **2** and **2a**. Treatment a large amount of Fr. 1 (250 mg) with excess Diazomethane to produce a large amount of products written before, but we find a difference, **9** was produced.

Compound **2a**: ($CDCl_3$, 500 MHz): 21.9 (C-1), 21.9 (C-2), 43.7 (C-3), 72.0 (C-4), 54.4 (C-5), 43.4 (C-6), 43.8 (C-7), 40.7 (C-8), 20.0 (C-9), 34.6 (C-10), 103.42 (C-11), 170.98 (C-12), 21.7 (C-13), 18.5 (C-14), 22.5 (C-15), 52.7 (OCH_3), 77.7 (C-16).

Compound **5**: ($CDCl_3$, 500 MHz): 35.6 (C-1), 29.6 (C-2), 73.5 (C-3), 151.4 (C-4), 43.7 (C-5), 29.6 (C-6), 39.3 (C-7), 27.1 (C-8), 40.6 (C-9), 35.7 (C-10), 145.3 (C-11), 172.1 (C-12), 124.6 (C-13), 15.5 (C-14), 109.2 (C-15).

ISSN: 0975-8585

Compound **7**: ($CDCl_3$, 500 MHz): 160.4 (C-1), 126.4 (C-2), 202.2 (C-3), 42.7 (C-4), 48.4 (C-5), 29.9 (C-6), 39.1 (C-7), 26.7 (C-8), 37.8 (C-9), 36.1 (C-10), 144.7 (C-11), 171.8 (C-12), 124.9 (C-13), 17.2 (C-14), 11.7 (C-15).

RESULTS AND DISCUSSION

Fractionation of the *n*-Hexan-CH_2Cl_2 (1:1) extract of the leaves of *T. camphoratus* by column chromatography on Silica gel and Sephadex LH-20, in addition to, TLC and HPLC afforded acids (3-8, 10). The ^1H and ^{13}C-NMR spectrum of the remaining fraction indicated that we deal with acids, which cannot separate easily. We therefore make a diazomethane treatment, which purification of the products by TLC afforded 1-2a and 9.

Compound-9 is the methyl ester of 2a [8, 9], which we now establish the ^{13}C-NMR data for the first time (Table–1). The physical and spectral (IR, ^1H, ^{13}C, and MS) data of compounds, 1 [9], 2 [8, 9], 3 [10], 4 [10], 5 [11], 6 [12], 7 [13], 8 [14, 15], 9 [14, 16] and 10 [17] were found to be identical to those earlier. Compound 1 was purified by treatment with diazomethane (CH_2N_2) to afford 2 and 2a [8, 9]. The previously unreported ^{13}C NMR data of compounds 2a, 5 and 7 were given in the experimental section. The stereochemistry of 9 was proposed on the bases of chemical and IR spectrometer evidence. We now report an investigation of this stereochemistry based on ^1H-NMR coupling constant and the result of a series of different NOESY experiments (Fig. 1) which showed a clear effect between signals at (0.91 (H-14), 2.05 (H-15) and 4.43 (H-3). The structures of all compounds were confirmed by HMQC and HMBC experiments.

Chemotaxonomic significance

Tarchonanthus is apomorphic by its woolly florets and absence of pappus and placed in one group with Brachylance in the tribe Mutisieae [18]. However, Hansen did not accept *Tarchonanthus* and *Brachylance* within the Mutisieae. *Tarchonanthus* and *Brachylance* were earlier included as a subtribe in the tribe Inuleae. Additionally, Keeley and Jansen attempted to establish the DNA of the two genera and concluded that both genera constitute a branch distinct from all other tribes of the family Asteraceae and proceeded to describe a new tribe for the two genera. Our chemical investigation of *Tarchonanthus camphoratus* afforded almost typical structures as *Jasonia montana* and *Inula viscose* [10, 19]. These results showed a close relation of *T. camphorates to* the tribe Inula.

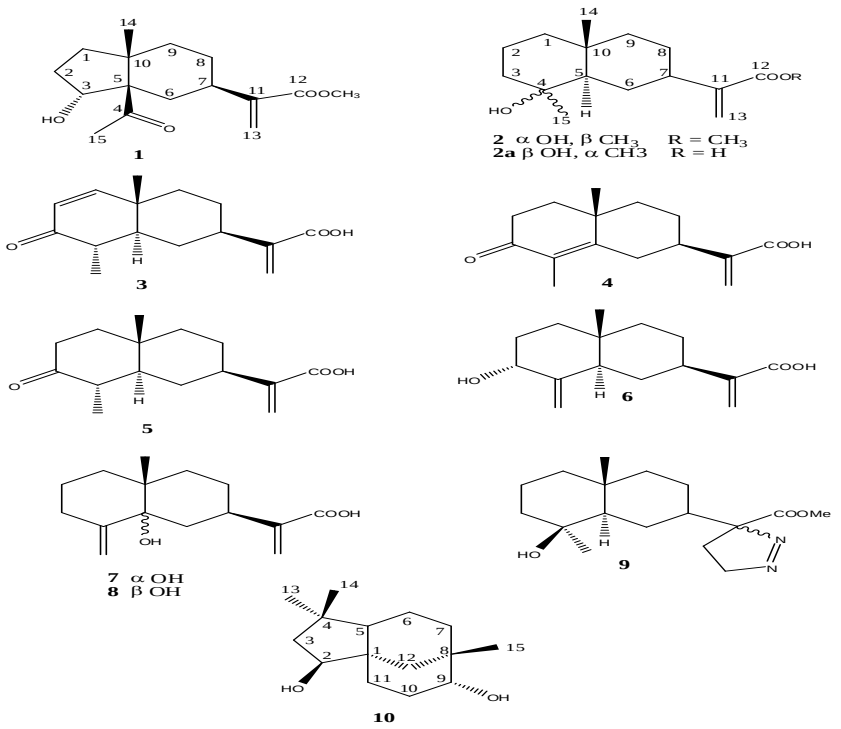

Figure 1: Structures of metabolites 1–10.

ISSN: 0975-8585

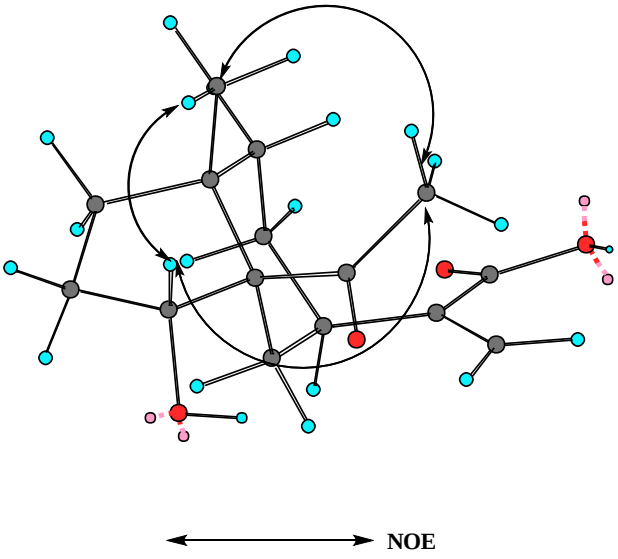

Figure 2: Selective NOESY correlation of 9.

Table 1: ^{13}C spectral data ($CDCl_3$ and C_5D_5N, 500MHz) of compound 9.

Position	$CDCl_3$	C_5D_5N
1	171.0 s	171.2 s
2	103.4 s	104.0 s
3	77.7 t	78.3 t
4	72.0 s	70.7 s
5	54.4 d	54.9 d
6	52.7 q	52.3 q
7	43.8 d	44.3 d
8	43.7 t	44.3 t
9	43.4 t	44.0 t
10	40.7 t	41.4 t
11	34.6 s	34.7 s
12	22.5 q	22.9 q
13	21.9 t	22.2 t
14	21.9 t	22.4 t
15	21.7 t	22.3 t
16	20.0 t	20.5 t
17	18.5 q	18.7 q

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