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TRIPTININS A AND B, TWO LEUKOTRIENE D_4 ANTAGONISTIC 19(4 → 3)-*ABEO*-ABIETANES FROM *TRIPTERYGIUM WILFORDII*

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Key Word Index—*Tripterygium wilfordii*; Celastraceae; *abeo*-abietane; triptinin A; triptinin B; triptoquinone A; LTD_4 antagonist.

Abstract—Bioassay-directed separation of an ethanolic extract of the roots of *Tripterygium wilfordii* furnished two new 19(4 → 3)-*abeo*-abietanes, triptinin A and B, along with two known diterpenes, triptoquinone A and 11-hydroxy-14-methoxy-19(4 → 3)-*abeo*-abieta-3, 8, 11, 13-tetraen-19-oic acid, as leukotriene D_4 antagonist constituents. The structures of these diterpenes were elucidated by spectroscopic methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In the course of a screening programme for anti-allergic compounds from Chinese higher plants, we found that the ethanolic extract of the roots of *Tripterygium wilfordii* Hook [1] showed potent leukotriene D_4 (LTD_4) antagonistic activity in the bioassay using the smooth tracheal muscles of guinea-pig [2]. Bioassay guided separation of the extract furnished four active compounds (**1**–**4**). Among these, two compounds, triptinin A (**1**) and B (**2**), were found to be novel 19(4 → 3)-*abeo*-abietane diterpenes, while the other two compounds were identified as triptoquinone A (**3**) [3] and the related 19(4 → 3)-*abeo*-abietane (**4**) [4]. This paper describes the isolation and structure elucidation of these compounds, along with the results of their bioassay. Compounds **3** and **4**, previously isolated from *T. wilfordii* var. *regelii*, were reported to have inhibitory activity for interleukin-1α and -1β release [3–5]. The phytochemical background of *T. wilfordii* is described by Shishido *et al.* [5].

RESULTS AND DISCUSSION

The ethanolic extract of the roots of *T. wilfordii* was subjected to repeated silica gel and reversed phase HPLC to furnish compounds **1**–**4**.

Triptinin-A (**1**) was assigned the molecular formula $C_{21}H_{28}O_3$ (high resolution (HR) electron impact (EI) MS, m/z = 328.2049 [M^+ + 1.1 mu]$^+$) indicating eight double-bond equivalents in the molecule.

The ^{1}H-NMR spectrum showed two singlets δ 1.07 and 1.84 corresponding to the H-20 quaternary methyl group and H-18 olefinic methyl group, respectively. Two doublets at δ 1.19 and 1.20 (each with J = 7.3 Hz) were assigned to Me-16 and Me-17. A septet at δ 3.28 (J = 7.3 Hz) was ascribed to the C-15 methine proton. A three-proton singlet at δ 3.70 was assigned to the methoxy group attached to C-14. In the aromatic region, two *ortho* coupled protons at δ 7.04 (d, J = 8.3 Hz) and 7.09 (d, J = 8.3 Hz) were assigned to H-11 and H-12.

The H, H COSY spectrum showed cross-peaks between H-15 and H-16 and H-17, indicating the presence of an isopropyl group in the molecule. Cross-peaks were also observed between the C-11 and C-12 aromatic protons, indicating an isolated spin system and hence confirming the presence of the tetra-substituted benzene ring. The COSY interactions also revealed the presence of one CH_2–CH_2 and one CH–CH_2–CH_2 moiety in the molecule, assigned to H-1/H-2 and H-5/H-6/H-7, respectively. Nuclear Overhauser effect (NOE) experiments confirmed the location of the methoxy group at C-14. Irradiation of the proton at δ 3.70 enhanced the intensity of the C-7 methylene protons (δ 3.00 and 2.84) as well as the isopropyl methine proton (δ 3.28).

The carbon multiplicities, determined by insensitive

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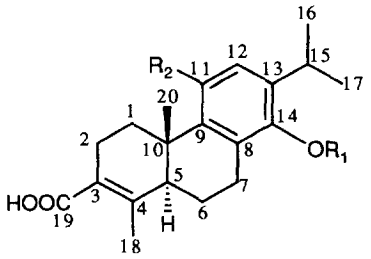
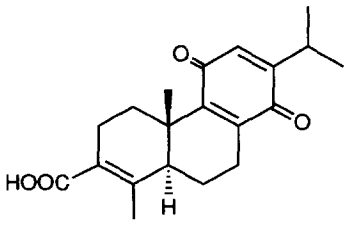


Triptinin-A (**1**): R_1=Me; R_2=H
Triptinin-B (**2**): R_1=H ; R_2=H
compound (**4**): R_1=Me; R_2=OH



Triptoquinone-A (**3**)

nuclei enhanced by polarization transfer (INEPT) experiments, showed the presence of five methyl, four methylene, four methine and eight quaternary carbon atoms. The signals at δ 18.3, 21.3, 24.3 and 24.2 were assigned to methyl carbon atoms at C-19, C-20, C-16 and C-17, respectively. The signal at δ 60.9 was assigned to the MeO carbon, attached to C-14 of the aromatic ring. The ^{13}C NMR data further indicated the presence of a tetrasubstituted double bond (δ 133.0 and 131.4) and a carboxylic acid function (δ 173.7). No NOE interactions were observed between H-5 and H-20, which suggested a *trans* junction at C-5 and C-10.

On the basis of the data described above the structure of triptinin-A was formulated as 14-methoxy-19(4 → 3)-*abeo*-abieta-3, 8, 11, 13-tetraen-19-oic acid (**1**). The absolute stereochemistry (5α-hydrogen and C-10β-methyl) was assumed to be similar to triptoquinone A (**3**). The complete ^1H and ^{13}C assignments are listed in Table 1.

Triptinin-B (**2**) was found to have the molecular formula $C_{20}H_{26}O_3$ (HR-fast atom bombardment

Table 1. NMR data (^1H 270 MHz, ^{13}C 67.5 MHz, in MeOH-d_4) for compounds **1**, **2** and **4**

	1		**2**		**4**
C/H	δ_C	δ_H	δ_C	δ_H	δ_C
1	34.1	1.56 (*m*), 2.46 (*m*)	34.8	1.53 (*m*), 2.46 (*m*)	33.4
2	26.3	2.35 (*m*), 2.46 (*m*)	26.3	2.35 (*m*), 2.46 (*m*)	26.7
3	131.4[a]	—	132.2[b]	—	132.6[c]
4	133.0[a]	—	134.9[b]	—	133.6[c]
5	45.8	2.23 (*m*)	46.0	2.24 (*m*)	48.8
6	22.8	2.23 (*m*), 1.64 (*m*)	25.0	2.24 (*m*), 1.65 (*m*)	21.1
7	27.3	3.00 (*m*), 2.84 (*m*)	27.6	2.89 (*m*), 2.71 (*m*)	27.5
8	129.5	—	123.6	—	131.6
9	147.8	—	146.7	—	132.7
10	37.1	—	36.8	—	38.4
11	121.3	7.09 (*d*)	117.0	6.94 (*d*)	153.9
12	124.5	7.04 (*d*)	123.7	6.85 (*d*)	112.1
13	139.3	—	133.3	—	139.6
14	156.4	—	152.3	—	149.0
15	24.9	3.28 (*sep*)	23.4	3.26 (*sep*)	27.2
16	24.3	1.20 (*d*)	23.3	1.20 (*d*)	24.3
17	24.2	1.19 (*d*)	23.0	1.18 (*d*)	24.5
18	173.7†	—	—‡	—	173.8†
19	18.3	1.84 (*s*)	18.5	1.90 (*s*)	18.7
20	21.3	1.07 (*s*)	21.3	1.05 (*s*)	17.8
MeO	60.9	3.70 (*s*)	—	—	61.5

* The chemical shifts are referenced to TMS (δ 0.0) for ^1H and MeOH-d_4 (δ 49.0) for ^{13}C.
† Intensity of the signal was very weak.
‡ Obscure due to poor signal-to-noise ratio.
[a–c] Signals in the same column can be interchanged.

(FAB) MS, *m/z* 315.1980 $[MH+2.0\ mu]^+$). The M_r thus differs from **1** by one CH_2 unit. The NMR data of **2** were similar to those of **1**, except for the lack of a signal due to the methoxy group. Thus the structure of **2** was determined to be the *des*-methyl derivative of **1**, 14-hydroxy-19(4 → 3)-*abeo*-abieta-3, 8, 11, 13-tetraen-19-oic acid. The NMR assignments of **2** are given in Table 1, which were deduced on the basis of H, H COSY experiments and INEPT measurements.

The other two compounds were also found to have the 19(4 → 3)-*abeo*-abietane skeleton by spectral studies. Compound **3** was identified as triptoquinone A [3] by direct comparison (TLC and HPLC) with an authentic specimen provided by Prof. Takaishi. Compound **4** was characterized as 11-hydroxy-14-methoxy-19(4 → 3)-*abeo*-abieta-3, 8, 11, 13-tetraen-19-oic acid [4]. Table 1 also includes the ^{13}C NMR assignments of **4** which were obtained on the basis of H, H COSY, C, H COSY and C, H long-range COSY experiments.

Triptoquinone A (**3**) has a 5α-H and 10β-methyl absolute configuration [3]. Compounds **1** and **2** were isolated from a different variety of *T. wilfordii*. Therefore, it is reasonable to assume that they have the same absolute stereochemistry at C-5 and C-10 as **3**.

The anti-LTD_4 activity of compounds **1–4** is summarized in Table 2. After incubating with vehicle (DMSO), the tachea strip contracted dose dependently with LTD_4. The additions of **1–4** to the assay tissue bath shifted the dose–response curves to the right, and the curves were parallel to that of the control (vehicle) experiment. Therefore, these compounds appear to be competitive antagonists of LTD_4. These results explain one of the reasons why *T. wilfordii* is prescribed in Chinese medicines as an anti-asthmatic agent. These compounds (**1–4**) are the first diterpenoid compounds to be reported to have LTD_4 antagonistic activity [6].

Table 2. LTD_4 antagonistic activity of compounds **1–4**

Compound	$K_D^* \times 10^{-5}$
1	12.4
2	3.4
3	20.2
4	4.5

* Dissociation constant between receptor and antagonist. The value was determined at a concentration of 50 μg ml^{-1} of each compound.

EXPERIMENTAL

Isolation. The dried roots of the plant (1.0 kg), purchased in Tuong San, Hubei Province, China, in the summer of 1992, were extracted with EtOH (**21**) under reflux for 5 hr to give 30 g of crude extract. This extract was repeatedly chromatographed on silica gel columns using benzene–EtOAc, $CHCl_3$–Et_2O and finally $CHCl_3$–EtOAc–MeOH (14:10:1) systems. The active fractions were then chromatographed on a reversed phase (ODS) column. Final purification on a reversed phase (ODS) HPLC with MeOH–H_2O (10:1) as eluent gave compounds **1–4**. Typical R_t values for **1–4** were 4.4, 3.9, 4.2 and 3.8 min, respectively, when analysed by RP-HPLC (Inertsil ODS (Gaskuro Kogyo Co., Japan) 25 cm × 4.6 mm; MeOH–H_2O (10:1); flow rate, 1.0 ml min^{-1}).

Triptinin-A (**1**). 5 mg, amorphous powder, $[\alpha]_D$ +26.0 (MeOH, *c* 0.42)]. FAB-MS *m/z*: 367 $[M+K]^+$, 351 $[M+Na]^+$. EI-MS *m/z*: 328.2049 $[M]^-$ (calcd 328.2038), 313, 311, 284, 269. UV λ_{max}^{MeOH}: 222 (ε 7084), 258 (1288), 267 (1110). ^1H and ^{13}C NMR: Table 1.

Triptinin-B (**2**). 6 mg, amorphous powder, $[\alpha]_D$ +17.1 (MeOH, *c* 0.49). FAB-MS *m/z*: 337 $[M+Na]^+$, 315 $[M+H]^+$. HR-FAB-MS *m/z*: 315.1980 $[M+H]^+$ (calcd 315.1960). UV λ_{max}^{MeOH}: 219 (ε 7071), 266 (1633), 273 (1584).

Triptoquinone A (**3**). 15 mg, amorphous powder. HR-FAB-MS *m/z*: 329.1714 $[M+H]^+$ (calcd 329.1753). UV λ_{max}^{MeOH}: 229 (ε 9333), 258 (14000). ^1H and ^{13}C NMR: Table 1.

Compound **4**. 10 mg, mp 191–193°. FAB-MS *m/z*: 383 $[M+K]^+$, 367 $[M+Na]^+$. HR-EI-MS *m/z*: 344.1974 $[M]^+$ (calcd 344.1988). UV λ_{max}^{MeOH}: 220 (ε 8192), 282 (2851).

LTD_4 antagonistic activity assay. A trachea spiral strip, about 2–3 mm width × 3.5–4.5 cm long, from a 6–8 week-old Hartley guinea-pig was placed in a 10-ml tissue bath under a resting load of 1.0 g. The tissue tension changes were recorded by an isometric transducer. The tissue bath was filled with Kreb's soln containing indomethacin (1.4×10^{-6} M). It was maintained at 37° and constantly bubbled with 95% O_2 and 5% CO_2. To minimize variability between tissues, contractile responses were expressed as percentages of the maximal responses obtained to histamine (1×10^{-5} M). Pyrilamine (7×10^{-6} M) and a test compound was added to the tissue bath 30 min prior to the start of the LTD_4 response. A cumulative concentration response curve was obtained by successive increases in the bath concentration of the LTD_4 agonist according to the method of Van Rossum [2].

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