A New Acetylenic Compound from the Rhizomes of *Atractylodes chinensis* and Its Absolute Configuration

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Received June 6, 2005; accepted September 28, 2005; published online September 30, 2005

A new acetylenic compound “atractyloyne”, (3*S*,4*E*,6*E*,12*E*)-1-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-3,14-diol (1) was isolated from the rhizomes of *Atractylodes chinensis* (Compositae) together with a known compound (4*E*,6*E*,12*E*)-3-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-1,14-diol (2). These structures were determined on the basis of the spectroscopic data and chemical evidence, and the absolute configuration of 1 was established by the modified 2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) method.

Key words *Atractylodes chinensis*; atractyloyne; absolute configuration; modified 2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) method; polyacetylene

The dried rhizomes of *Atractylodes chinensis* KOIDZUMI (*A. chinensis*) as well as *Atractylodes lancea* DE CANDOLLE (*A. lancea*) have traditionally been used as crude drugs in Japan. Both of them are defined as original plants of “Sojutsu” (*Atractylodes Lanceae* Rhizome) in Japanese Pharmacopeia, used as diuretic and stomachic.¹⁾ In the previous paper, we reported the isolation of several polyacetylenic compounds from the rhizomes of *A. lancea* act as improvement of the delay of gastric emptying.²⁾ So far several polyacetylenic compounds from the rhizomes of *A. lancea*,^{3–6)} and also the isolation of three polyacetylenic compounds from the rhizomes of *A. chinensis* were already reported.⁷⁾ In this paper, we reported the isolation and the structure determination of a new polyacetylenic compound, named “atractyloyne”.

Compound 1 was isolated as optically active pale yellow oil, $[\alpha]_D^{25} +45^\circ$ and has the molecular formula $C_{19}H_{24}O_4$ based on EI-MS and HR-ESI-MS. The UV spectrum showed typical absorptions at λ_{max} 336, 314 295, 266 and 250 nm for an ene-diyne-diene chromophore.⁸⁾ The IR spectrum of 1 showed the presence of a conjugated triple bond (2200 cm^{-1}), a conjugated double bond (1636 cm^{-1}), an ester carbonyl bond (1732 cm^{-1}) and a hydroxyl group (3402 cm^{-1}). The $^1\text{H-NMR}$ spectrum of 1 showed olefinic protons assignable to three trans double bonds at δ 5.86 (1H, d, $J=15\text{ Hz}$, $C_4\text{-H}$), 6.33 (1H, dd, $J=15, 11\text{ Hz}$, $C_5\text{-H}$), 6.70 (1H, dd, $J=15, 11\text{ Hz}$, $C_6\text{-H}$), 5.68 (1H, d, $J=15\text{ Hz}$, $C_7\text{-H}$), 5.87 (1H, d, $J=16\text{ Hz}$, $C_{12}\text{-H}$), 6.41 (1H, dt, $J=16, 5\text{ Hz}$, $C_{13}\text{-H}$), four methylene protons at δ 4.14, 4.26 (each 1H, m, $C_1\text{-H}$), 1.79–1.93 (2H, m, $C_2\text{-H}$), 4.26 (2H, m, $C_{14}\text{-H}$), 2.20 (2H, d, $J=7\text{ Hz}$, $C_3\text{-H}$), and two methyl protons at δ 0.96 (6H, d, $J=7\text{ Hz}$, $C_{3'}\text{-Me}$), respectively. The $^{13}\text{C-NMR}$ spectrum of 1 showed a carbonyl carbon at δ 173.5, two methyl carbons at δ 22.4 (two overlapping), four methylene carbons at δ 62.7, 60.8, 43.4, 36.1 and two methine carbons at δ 68.9 and 25.7, respectively. On hydrolysis by acid or boiling water, 1 afforded (4*E*,6*E*,12*E*)-tetradeca-4,6,12-triene-8,10-diyne-1,3,14-triol (3),^{9,10)} which was identified by ^1H - and ^{13}C -NMR spectra. In comparison of the ^{13}C -NMR spectrum of 1 with that of 2,⁶⁾ isovaleryloxy moiety of 1 was attached to C_1 hydroxy group. From these data, structure of 1 were determined to be (4*E*,6*E*,12*E*)-1-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-3,14-diol.

In order to determine the absolute configuration at C_3 of 1, compound 1 was converted into the *S*- and *R*-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters.^{11,12)} The values of $\Delta\delta$ [δ (*S*-MTPA ester)– δ (*R*-MTPA ester)] were shown in Fig. 2. These results suggested that the absolute configuration at C_3 of 1 is *S*, and the structure of 1 was determined to be (3*S*,4*E*,6*E*,12*E*)-1-isovaleryloxy-tetradeca-4,6,12-triene-8,10-diyne-3,14-diol, named “atractyloyne” (Fig. 1).

In this study, a new acetylenic compound 1 and a known compound 2 were isolated from the rhizomes of *A. chinensis* for the first time to our knowledge. Because the compound 1 was partly converted to 3 by hydrolysis, as described above, it was suggested that both 1 and 2 in the rhizomes were con-

Fig. 1. Structures of Isolated Compounds 1, 2 and Hydrolyte 3

Fig. 2. Chemical Shifts Difference for the (*S*)-MTPA Ester (1_a) and (*R*)-MTPA Ester (1_b) in ppm

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verted to **3** through the boiling water extraction process. Therefore, improving effect of the rhizomes of *A. chinensis* on stomach disorder should consider the amount of **1** and **2** as well as the amount of bioactive compound **3**.²⁾

Experimental

General Experiment Procedures The UV spectrum was obtained with a HITACHI U-3000 spectrophotometer, whereas the IR spectrum was measured with a JASCO FT/IR-8300 spectrometer. Optical rotation was measured with a JASCO DIP-1000 digital polarimeter. The HR-ESI-MS and EI-MS were taken on a MICROMASS Q-Tof micro and a KRATOS CONCEPT 1S spectrometer, respectively. The ¹H- and ¹³C-NMR spectra were measured with a JEOL EX-400 (¹H-NMR: 400 MHz, ¹³C-NMR: 100 MHz) or BRUKER AM-500 (¹H-NMR: 500 MHz, ¹³C-NMR: 125 MHz) spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm).

Crude Drug Material The plant material was purchased in China, and identified using analysis of the internal transcribed spacer of nuclear ribosomal DNA by Ms. M. Shiba in our laboratory.⁸⁾ A voucher specimen (No. THS-77081) has been deposited in our institute.

Extraction and Isolation Dried, ground rhizomes of the material (1.0 kg) were extracted with MeOH (10 l) under reflux for 2 h. The extract was concentrated under reduced pressure and suspended in water (500 ml). The suspension was extracted with EtOAc (500 ml $\times$ 3), and the organic layer was concentrated to give the EtOAc fraction (93 g). The EtOAc fraction was dispersed into *n*-hexane (2 l) with vigorous stirring followed by filtration to afford the *n*-hexane-insoluble portion (25 g). Subsequently, the *n*-hexane-insoluble portion (20 g) was chromatographed on silica gel (6 cm i.d. $\times$ 34 cm, 500 g) eluted stepwise with a mixture of acetone and benzene (0:100 $\rightarrow$ 20:80). The 15% acetone-benzene eluent was repeatedly subjected to fractionation using a prep ODS-column (45% aqueous MeOH, flow rate 3.5 ml/min, detection at 334 nm) to give **1** as a pale yellow oil (1.27 g) and **2** as a pale yellow oil (0.57 g).

Compound **1**: Pale yellow oil; $[\alpha]_D^{25} +45^\circ$ ($c=0.139$, MeOH); UV λ_{max} nm (MeOH, log ϵ): 336 (4.41), 314 (4.55), 295 (4.41), 266 (4.41), 250 (4.45), 212 (4.20); IR ν_{max} (liquid membrane method) cm^{-1} : 3402, 2200, 1732, 1636, 986; EI-MS m/z : 316 [M]⁺; HR-ESI-MS m/z : 339.1570 [M+Na]⁺ (Calcd for [C₁₉H₂₄O₄+Na]⁺ 339.1572); ¹H- and ¹³C-NMR spectral data: see Table 1.

Preparation of MTPA Ester (S)-MTPA chloride (64.7 mg) and 3-[(dimethylamino) propyl] amine (12.2 mg) in anhydrous pyridine (0.40 ml) was added to a solution of compound **1** (20.3 mg) in CHCl₃ (0.50 ml). After stirring at room temperature for 10 h, the mixture was poured into water and extracted with EtOAc. The EtOAc extract was successively washed with water and saturated aqueous NaHCO₃, and then evaporated off. The residue was chromatographed on silica gel (1 cm i.d. $\times$ 5.5 cm, 2.0 g) eluted stepwise with a mixture of acetone and benzene (0:100 $\rightarrow$ 5:95) to give the (R)-MTPA diester (**1a**, 12.2 mg) of **1**.

Compound **1a**: EI-MS m/z : 748; ¹H-NMR (CDCl₃): δ 3.96, 3.87 (2H, m, C₁-H), 1.94 (2H, m, C₂-H), 5.56 (1H, m, C₃-H), 5.73 (1H, dd, $J=15$, 6 Hz, C₄-H), 6.29 (1H, dd, $J=15$, 11 Hz, C₅-H), 6.60 (1H, dd, $J=15$, 11 Hz, C₆-H), 5.65 (1H, d, $J=15$ Hz, C₇-H), 5.77 (1H, dd, $J=16$, 1 Hz, C₁₂-H), 6.23 (1H, dt, $J=16$, 6 Hz, C₁₃-H), 4.79 (2H, m, C₁₄-H), 2.10 (2H, d, $J=7$ Hz, C₂-H), 2.01 (1H, m, C₃-H), 0.88 (6H, dd, $J=7$, 1 Hz, C_{3'}-Me-H), 3.48 (6H, m, C_{MTPA-OMe}-H), 7.43 (10H, m, C_{MTPA-Ph}-H).

(R)-MTPA chloride (70.1 mg) and 3-[(dimethylamino) propyl] amine (12.5 mg) in anhydrous pyridine (0.36 ml) were added to a solution of compound **1** (18.0 mg) in CHCl₃ (0.50 ml). Work up as mentioned above gave the (R)-di-MTPA ester (**1b**, 25.4 mg) of **1**.

Compound **1b**: EI-MS m/z : 748 [M]⁺; ¹H-NMR (CDCl₃): δ 4.03 (2H, dd, $J=7$, 6 Hz, C₁-H), 1.99 (2H, m, C₂-H), 5.52 (1H, dd, $J=13$, 7 Hz, C₃-H), 5.62 (1H, dd, $J=15$, 7 Hz, C₄-H), 6.14 (1H, dd, $J=15$, 11 Hz, C₅-H), 6.55 (1H, dd, $J=15$, 11 Hz, C₆-H), 5.56 (1H, d, $J=15$ Hz, C₇-H), 5.77 (1H, dd, $J=16$, 1 Hz, C₁₂-H), 6.22 (1H, dt, $J=16$, 6 Hz, C₁₃-H), 4.79 (2H, m, C₁₄-H), 2.11 (2H, d, $J=7$ Hz, C₂-H), 2.02 (1H, m, C₃-H), 0.90 (6H, d, $J=7$ Hz,

Table 1. ¹H- and ¹³C-NMR Spectral Data of **1**

	1 (CDCl ₃)	
	δ_C	δ_H
1	60.8	4.14 m, 4.26 m
2	36.1	1.79—1.93 m
3	68.9	4.32 m
4	139.1	5.86 d (15)
5	129.2	6.33 dd (15, 11)
6	143.8	6.70 dd (15, 11)
7	110.4	5.68 d (15)
8	80.7	—
9	76.6	—
10	74.6	—
11	80.8	—
12	109.0	5.87 d (16)
13	145.3	6.41 dt (16, 5)
14	62.7	4.26 m
1'	173.5	—
2'	43.4	2.20 d (7)
3'	25.7	2.09 m
3'-CH ₃	22.4	0.96 d (7)

Coupling constants (J) in Hz are given in parentheses.

C_{3'-Me}-H), 3.48 (6H, m, C_{MTPA-OMe}-H), 7.43 (10H, m, C_{MTPA-Ph}-H).

Hydrolysis of 1 A solution of **1** (2 mg) in 2 ml of MeOH was added to 8 ml of distilled water or 0.05 mol/l H₂SO₄, and each mixture was refluxed for 1 h. The reactants were directly subjected to HPLC analysis equipped with an ODS column (TSK GEL ODS-80T_S 4.6 mm i.d. $\times$ 250 mm, Tosoh, Tokyo, Japan). The solvents were 20 mM phosphoric acid (X), and acetonitrile (Y), and a liner gradient of 90% X and 10% Y changing over 60 min to 0% X and 100% Y was applied. The flow rate and the column temperature were set at 1.0 ml/min and 40 °C, respectively. The UV data of effluent from the column ranging from 200 to 400 nm were collected. The retention times were 36.4 min for **1** and 20.1 min for hydrolysate **3**.

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