New Norditerpenoids with Trypanocidal Activity from *Vitex trifolia*

Fumiyuki KIUCHI,[a] Kenji MATSUO,[b] Michiho ITO,[b] Tran Kim QUI,[c] and Gisho HONDA*,[b]

[a] *Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences; 1 Hachiman-dai, Tsukuba 305–0843, Japan:* [b] *Graduate School of Pharmaceutical Sciences, Kyoto University; 46–29 Yoshida, Sakyo-ku, Kyoto 606–8501, Japan: and* [c] *Research Center for Applied Chemistry, Vietnam National University; Ho Chi Min City, Vietnam.*
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Trypanocidal constituents of the fruits of *Vitex trifolia* were investigated. Activity-guided isolation of the acetone extract resulted in the isolation of two new norditerpene aldehydes, 1 and 2, together with five known diterpenes: vitexifolin E (3), vitexifolin F (4), vitexilactone (5), 6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (6), and previtexilactone (7). *In vitro* minimum lethal concentrations of the isolated compounds against epimastigotes of *Trypanosoma cruzi* were 11 μM (1), 36 μM (2), 34 μM (3), 34 μM (4), 66 μM (5), 66 μM (6), and >265 μM (7).

Key words *Vitex trifolia*; Verbenaceae; norditerpene aldehyde; trypanocidal constituent; *Trypanosoma cruzi*

Vitex trifolia L. (Verbenaceae) is a deciduous shrub which has been used as an anti-inflammatory and sedative for headache, rheumatism and the common cold in Asian countries.[1)] In our search for anti-trypanosomal compounds from natural medicines used in Vietnam,[2)] an acetone extract of fruits of this plant showed potent trypanocidal activity against the epimastigotes of *Trypanosoma cruzi*, the etiologic agent of American trypanosomiasis (Chaga's disease).[3)] In this paper, we report the isolation and characterization of two new norditerpene aldehydes (**1**, **2**), together with five known constituents: vitexifolin E (**3**), vitexifolin F (**4**), vitexilactone (**5**), 6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**6**), and previtexilactone (**7**), as the trypanocidal constituents of the fruits of this plant.

Results and Discussion

Dried fruits of *V. trifolia* were extracted with acetone under reflux, and the extract, which showed strong *in vitro* trypanocidal activity,[2)] was separated by repeated column chromatography to give compounds **1**—**7**. Compounds **3**—**7** were known diterpenoids and their structures were identified with vitexifolin E (**3**),[4)] vitexifolin F (**4**),[4)] vitexilactone (**5**),[5)] 6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**6**),[6)] and previtexilactone (**7**)[5)] by comparison of their spectral data with those reported.

Compound **1** was obtained as a colorless oil. The molecular formula was determined to be $C_{19}H_{30}O_2$ by high-resolution fast atom bombardment mass spectrum (HR-FAB-MS *m/z*: 291.2316 $[M+H]^+$, Calcd for $C_{19}H_{31}O_2$: 291.2324). The IR and ^{1}H-NMR spectra showed the presence of an aldehyde (δ_H 9.66, d, J=6.7 Hz, δ_C 188.9) attached to a tri-substituted olefine (δ_H 5.77, d, J=6.7 Hz, δ_C 100.4). Compound **1** was easily air-oxidized to give an allylic acid, **1a**. Since the ^{13}C-NMR spectrum of **1** and **1a** (Table 1) showed similar chemical shifts with those of the decalin part of isoambreinolide (**8**),[7)] **1** and **1a** were concluded to have a 4,4,8,10-tetramethyl-*trans*-decalin skeleton. The whole structure of **1** was determined by the analysis of heteronuclear multiple bond connectivity (HMBC) and correlation spectroscopy (COSY) spectra, as shown in Fig. 1, and the stereochemistry of the double bond was concluded to be *E* from the nuclear Overhauser effect (NOE) observed between the H-12 protons and the aldehyde proton.

Compound **2** was obtained as a colorless oil. This compound showed a similar ^{13}C-NMR spectrum (Table 1) to that of **1**, except that it has an additional acetoxy group (δ_H 1.68, δ_C 21.3, 169.2). This acetoxy group was located at C-6, because the chemical shift of C-6 showed a very large downfield shift (47.9 ppm compared to the corresponding carbon in **1**). Irradiation of the H-6 proton resulted in NOEs on H-5 and H-17 (4α-methyl protons), which revealed the orientation of the acetoxy group to be β. NOEs were also observed between the aldehyde proton and H-12 protons, which indicated the stereochemistry of the double bond to be *E*. Thus, the structure of **2** was determined to be as shown in Fig. 1.

The minimum lethal concentrations (MLCs) of the isolated compounds **1**—**7** against epimastigotes of *Trypanosoma cruzi* were 11 μM (**1**), 36 μM (**2**), 34 μM (**3**), 34 μM (**4**), 66 μM (**5**), 66 μM (**6**), and >265 μM (**7**). The aldehyde **1** showed the strongest activity among the isolated compounds. The aldehyde function seems to play an important role in the activity, because the activity of the corresponding carboxylic acid **1a** was weaker (MLC=82 μM). The activities of compounds **2**—**6** were not high compared to the original acetone

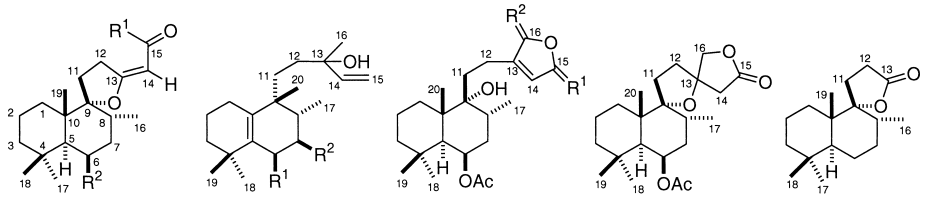

Chart 1

* To whom correspondence should be addressed. e-mail: ghonda@pharm.kyoto-u.ac.jp

Table 1. ^{13}C-NMR Data of the New Compounds

No.	**1**[a)]	**2**[a)]	**1a**[b)]	**8**[b,c)]
1	31.3[d)]	32.9	31.2	31.1
2	18.6	18.6	18.3	18.2
3	41.7	43.6	41.5	41.2
4	33.3	34.1	33.3	33.2
5	46.9	48.8	46.7	46.5
6	21.5	69.4	21.3	21.2
7	31.4[d)]	36.5	31.2	30.9
8	36.7	32.0	36.6	36.7
9	98.8	98.2	98.9	93.9
10	42.4	42.8	42.4	42.1
11	26.1	25.8	26.3	
12	30.0	30.1	32.2	
13	180.6	180.1	181.1	
14	100.4	100.6	86.6	
15	188.9	188.8	173.9	
16	15.7	15.3	15.7	15.6
17	33.3	33.1	33.3	33.0
18	21.9	23.7	21.8	21.8
19	16.6	19.3	16.7	15.5
MeCO		21.3		
MeCO		169.2		

a) in C_6D_6; *b*) in $CDCl_3$; *c*) data from ref. 7; *d*) interchangeable.

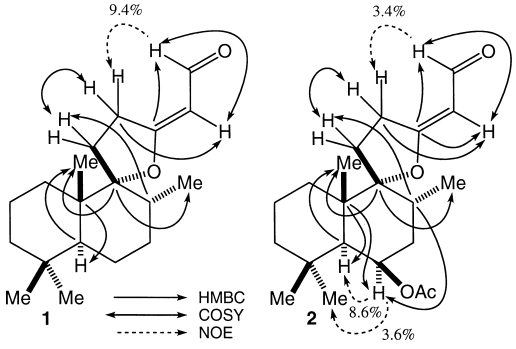

Fig. 1. Selected COSY, HMBC and NOE Data

extract. There may be compounds with stronger activity in the extract, or synergistic actions of the constituents may play an important role in the activity. Further study is necessary to clarify these points.

Experimental

General Experimental Procedures Optical rotations were determined on a JASCO DIP-370 polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-LA500 spectrometer. A solvent signal (residual proton signal or center signal of the deuterated carbon) was used as an internal standard, and chemical shifts are given in δ values. Mass spectra were measured on a JEOL JMS-HX/HX110A spectrometer. Gel permeation chromatography (GPC) was performed on a JAI-508 system with JAIGEL-H1 and H2 columns.

Plant Materials Dried fruits of *V. trifolia* (mán kinh tu) were purchased from a market in Ho Chi Min City in June 2000. A voucher specimen (ESM-C00239) was deposited at the Experimental Station of Medicinal Plants, Faculty of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation Dried fruits of *V. trifolia* (2 kg) were extracted with acetone under reflux (3 h×3 times), and the extract was concentrated under reduced pressure to give 115.6 g of residue. A part of the residue (20 g) was applied to a silica gel column and eluted with hexane–acetone (9 : 1, 5 : 1, 2 : 1) to give seven fractions (fr. 1, 5.68 g; fr. 2, 1.87 g; fr. 3, 3.72 g; fr. 4, 2.04 g; fr. 5, 0.39 g; fr. 6, 1.01 g; fr. 7, 1.44 g). Fraction 3 was fractionated by silica gel column chromatography with hexane–EtOAc (2 : 1, 1 : 2) to give four fractions (fr. 3-1, 1.50 g; fr. 3-2, 1.56 g; fr. 3-3, 696 mg; fr. 3-4, 631 mg). Fraction 3-3 was separated with GPC ($CHCl_3$) and then Lobar® Si-60 (benzene–acetone=15 : 1) and Lobar® RP-18 (80% MeOH) column chromatography to give **1** (32 mg) and **4** (38 mg). Repeated separation of fr. 3-4 by GPC ($CHCl_3$) and Lobar® Si-60 (benzene–acetone=9 : 1, $CHCl_3$–acetone=19 : 1) gave **2** (10 mg), **3** (9 mg), **5** (17 mg) and **6** (12 mg). Repeated separation of fr. 2 (300 mg) with silica gel column chromatography ($CHCl_3$–EtOAc=19 : 1, hexane–EtOAc=3 : 1) gave **7** (10 mg).

Compound **1**: Colorless oil, $[\alpha]_D$ −3.4° (*c*=0.64, MeOH). IR (KBr) cm^{-1}: 2935, 1655, 1624, 1574, 1153. HR-FAB-MS *m/z*: 291.2316 (M+H$^+$, Calcd for $C_{19}H_{31}O_2$: 291.2324). +FAB-MS *m/z* (%): 291 (M+H$^+$, 100), 154 (16). UV λ_{max} (MeOH) nm (ε): 270 (19792). ^{1}H-NMR (C_6D_6, 500 MHz) δ: 9.66 (1H, d, *J*=6.7 Hz, H-15), 5.77 (1H, br d, *J*=6.7 Hz, H-14), 2.50 (2H, m, H-12), 1.50 (1H, m, H-11), 1.48 (1H, m, H-5), 1.46 (1H, m, H-6), 1.40 (1H, m, H-2), 1.36 (1H, m, H-2), 1.31 (1H, m, H-8), 1.30 (2H, m, H-7), 1.25 (1H, m, H-3), 1.13 (1H, m, H-11), 1.09 (1H, m, H-3), 1.07 (1H, m, H-1), 1.00 (1H, m, H-6), 0.94 (1H, m, H-1), 0.81 (3H, s, H-17), 0.74 (3H, s, H-18), 0.58 (3H, s, H-19), 0.50 (3H, d, *J*=5.8 Hz, H-16). ^{13}C-NMR: Table 1.

Air-Oxidation of 1 Compound **1** (8 mg) dissolved in $CHCl_3$ (3 ml) was left at room temperature for 3 d. The solution was concentrated and the residue was separated on silica gel ($CHCl_3$–acetone=19 : 1) to give **1a** (6 mg).

Compound **1a**: Colorless oil, $[\alpha]_D$ +2.0° (*c*=0.40, EtOH). IR (KBr) cm^{-1}: 2930, 1680, 1609, 1464, 1151. HR-FAB-MS *m/z*: 307.2271 (M+H$^+$, Calcd for $C_{19}H_{31}O_3$: 307.2273). +FAB-MS *m/z* (%): 307 (M+H$^+$, 76), 289 (100), 69 (57). UV λ_{max} (EtOH) nm (ε): 246 (5934). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.28 (1H, br s, H-14), 3.11 (1H, m, H-12), 3.03 (1H, m, H-12), 2.09 (1H, td, *J*=13.1, 7.3 Hz, H-11), 1.79 (1H, m, H-8), 1.77 (1H, m, H-11), 1.61 (1H, m, H-6), 1.57 (1H, m, H-1 or H-7), 1.47 (1H, m, H=5), 1.44 (2H, m, H-2), 1.35 (1H, m, H-3), 1.34 (1H, m, H-6), 1.31 (2H, m, H-7 or H-1), 1.14 (1H, td, *J*=13.4, 3.7 Hz, H-3), 1.13 (1H, td, *J*=12.2, 3.4 Hz, H-1 or H-7), 0.92 (3H, s, H-19), 0.89 (3H, s, H-17), 0.82 (3H, s, H-18), 0.77 (3H, d, *J*=6.4 Hz, H-16). ^{13}C-NMR: Table 1.

Compound **2**: Colorless oil, $[\alpha]_D$ −1.4° (*c*=0.50, MeOH). IR (KBr) cm^{-1}: 2936, 1734, 1655, 1624, 1248, 1213, 1153. HR-FAB-MS *m/z*: 349.2383 (M+H$^+$, Calcd for $C_{21}H_{33}O_4$: 349.2379). +FAB-MS *m/z* (%): 349 (M+H$^+$, 100), 289 (16). UV λ_{max} (MeOH) nm (ε): 268 (16531). ^{1}H-NMR (C_6D_6, 500 MHz) δ: 9.65 (1H, d, *J*=6.4 Hz, H-15), 5.71 (1H, br d, *J*=6.4 Hz, H-14), 5.58 (1H, d, *J*=2.5 Hz, H-6), 2.58 (1H, m, H-12), 2.48 (1H, m, H-12), 1.80 (1H, m, H-8), 1.68 (3H, s, H-21), 1.55 (1H, m, H-7), 1.50 (1H, m, H-11), 1.50 (1H, m, H-7), 1.49 (1H, m, H-2), 1.49 (1H, m, H-5), 1.30 (1H, m, H-2), 1.18 (1H, m, H-3), 1.17 (1H, m, H-11), 1.15 (2H, m, H-1), 1.01 (3H, s, H-19), 0.96 (1H, m, H-3), 0.95 (3H, s, H-18), 0.89 (3H, s, H-17), 0.42 (3H, d, *J*=6.7 Hz, H-16). ^{13}C-NMR: Table 1.

Vitexifolin E (**3**): Colorless prisms from hexane–acetone, mp 140—141 °C (lit.[4] mp 143—144 °C), $[\alpha]_D$ +115.7° (*c*=0.9, acetone) (lit.[4] +126.6°, *c*=1.4, acetone).

Vitexifolin F (**4**): Colorless oil, $[\alpha]_D$ +69.1° (*c*=0.35, acetone) (lit.[4] +94.6°, *c*=1.1, acetone).

Vitexilactone (**5**): Colorless needles from hexane–acetone, mp 148—149 °C (lit.[5] mp 144—146 °C), $[\alpha]_D$ −11.2° (*c*=0.85, $CHCl_3$) (lit.[5] −12.4° *c*=1.11, $CHCl_3$). Fully assigned ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.3 (C-15), 171.3 (C-13), 170.7 (C-21), 115.2 (C-14), 76.7 (C-9), 73.4 (C-16), 70.0 (C-6), 47.9 (C-5), 44.0 (C-10), 43.8 (C-3), 36.2 (C-7), 34.2 (C-4), 33.8 (×2, C-1, 18), 32.3 (C-8), 31.8 (C-11), 25.6 (C-12), 23.9 (C-19), 22.1 (C-22), 19.2 (C-20), 18.8 (C-2), 16.3 (C-17).

6-Acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**6**): Colorless oil, $[\alpha]_D$ −7.3° (*c*=0.8, acetone) (lit.[6] −10.0°, *c*=3.3, acetone).

Previtexilactone (**7**): Colorless prisms from hexane–$CHCl_3$, mp 224—225 °C (lit.[5] mp 214—215 °C), $[\alpha]_D$ −20.2° (*c*=0.85, $CHCl_3$). Fully assigned ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 174.8 (C-16), 170.4 (C-21), 93.9 (C-13), 85.8 (C-9), 78.4 (C-15), 70.3 (C-6), 48.8 (C-5), 43.9 (C-14), 42.89 (C-7), 42.86 (C-10), 37.8 (C-12), 36.5 (C-11), 34.2 (C-3), 34.1 (C-4), 33.1 (C-18), 31.2 (C-8), 29.3 (C-2), 23.7 (C-22), 21.9 (C-19), 19.8 (C-20), 18.7 (C-1), 17.2 (C-17).

Trypanocidal Assay[8] Epimastigotes of *Trypanosoma cruzi* (Tulahuen strain) were kept in GIT medium (Wako) supplemented with hemin (12.4 μM, Wako). The epimastigotes in GIT medium (10 μl) were incubated with a test sample dissolved in EtOH (5 μl) and autoclaved saline (185 μl). After 24 h of incubation, the movement of epimastigotes was observed under a microscope (×100). Each assay was performed in duplicate. Gentian violet, which is used to disinfect trypanosomes from transfusion blood in Latin America, is used as a positive control. The MLC of gentian violet under this assay condition was 6.3 μM.

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