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ANTI-AIDS AGENTS, 4.[1] TRIPTERIFORDIN, A NOVEL ANTI-HIV PRINCIPLE FROM *TRIPTERYGIUM WILFORDII*: ISOLATION AND STRUCTURAL ELUCIDATION

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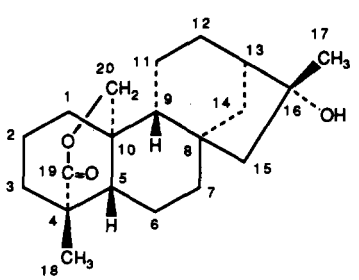
ABSTRACT.—A new kaurane-type diterpene lactone, tripterifordin [**1**], has been isolated from the roots of *Tripterygium wilfordii*. The structure of **1** was elucidated by spectroscopic methods, including the concerted application of a number of 2D nmr techniques that involved the ^{1}H-^{1}H COSY, heteronucleus-detected variants of the heteronuclear chemical shift correlation (HETCOR), phase-sensitive NOESY, and long-range HETCOR. Compound **1** shows anti-HIV replication activity in H9 lymphocyte cells with an EC_{50} of 1 μg/ml.

The roots of *Tripterygium wilfordii* Hook. (Celastraceae), a poison liana plant occurring in areas of southern China, are known as "Lei Gong Teng" in Chinese folklore. Because of its toxicity, "Lei Gong Teng" was only used as a pesticide. Since the 1960's, it has been found to possess antitumor, anti-inflammatory, and immunosuppressive activities. Many tricyclic diterpenes were previously isolated from this plant. These include triptolide and tripdiolide as major antitumor principles (2). Recently, "Lei Gong Teng" and its preparation "Leigongteng Duodai" have been used for the treatment of various diseases, including dermatitis, rheumatoid arthritis, systemic acne rosacea, and nephritis. In the course of our continuing search for novel potent anti-AIDS agents, the EtOH extract of the roots of this plant was found to show significant anti-HIV activity. Bioassay-directed fractionation of the active extract has led to the isolation and charac-



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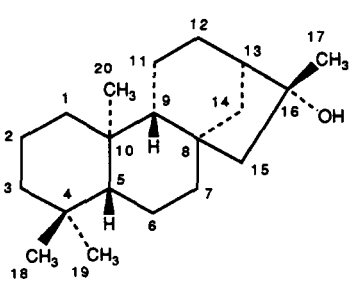

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[1]For Part 3, see Tatematsu *et al.* (1).

terization of a new anti-HIV principle, tripterifordin [**1**]. We report herein on the isolation and structural elucidation of **1**.

RESULTS AND DISCUSSION

The roots of *T. wilfordii* were extracted with 95% EtOH. Si gel chromatography of the active fractions followed by flash chromatography afforded tripterifordin [**1**].

Compound **1**, isolated as needles (mp 255–256°), has the molecular formula $C_{20}H_{30}O_3$ as deduced from its hrms in combination with its ^{1}H- and ^{13}C-nmr spectra. The ir spectrum indicated the presence of hydroxyl (3315 cm^{-1}) and lactone (1735 cm^{-1}) groups. The ^{1}H-nmr spectrum (Table 1) showed signals for two tertiary methyls at δ 1.20 and δ 1.40 and one oxymethylene at δ 4.15 and δ 5.21. The ^{13}C-nmr spectrum (Table 1), the distortionless enhancement by a polarization transfer (DEPT) experiment, and the ^{1}H-^{13}C heteronucleus-detected variants of the heteronuclear chemical shift correlation (HETCOR) experiment of **1** indicated a carbon count of 20 carbons and a hydrogen count of 29 carbon-bond hydrogens. The carbon types were exhibited

TABLE 1. Proton and Carbon Resonance Shift Assignments for Tripterifordin [**1**] and Compound **2**.

Position	Compound		
	1[a]		**2**[b]
	δ ^{1}H	δ ^{13}C	δ ^{13}C
1	α 2.17, br d, J = 12.5 Hz β 1.05, m	40.6, t	42.0
2	1.71, m	20.9, t	18.6
3	α 1.87, br ddd, J = 1, 2, 12.5 Hz β 1.49, ddd, J = 3, 12.5, 12.5 Hz	40.6, t	42.0
4	—	43.0, s	33.2
5	1.29, m	51.2, d	56.2
6	α 1.21, m β 1.81, br ddd, J = 3, 3, 12.5 Hz	22.4, t	20.4
7	α 1.67, m β 1.47, ddd, J = 3, 12.5, 12.5 Hz	39.7, t	40.3
8	—	44.8, s	45.3
9	1.20, m	50.3, d	56.8
10	—	38.7, s	39.3
11	1.65, m	17.5, t	18.0
12	α 1.32, m β 1.64, m	26.0, t	26.9
13	1.89, br s	48.2, d	49.0
14	a 1.73, m b 1.92, br d, J = 12.5 Hz	37.9, t	37.7
15	α 1.64, d, J = 10.5 Hz β 1.57, d, J = 10.5 Hz	57.8, t	58.0
16	—	79.0, s	79.4
17	1.40, s	24.5, q	24.5
18	1.20, s	23.1, q	21.6
19	—	176.5, s	33.5
20	a 4.15, d, J = 12 Hz b 5.21, dd, J = 2, 12 Hz	73.9, t	18.0

[a]Measured in $CDCl_3$. Multiplicity of signals: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal. δ in ppm.

[b]Data for this compound are from Hanson *et al.* (3).

by DEPT as two methyls, nine simple methylenes, one oxymethylene, three methines, three quaternary carbons, one oxygenated quaternary carbon, and one carbonyl carbon. These assignments were consistent with the hrms empirical formula, supporting the presence of one hydroxyl group and six degrees of unsaturation/rings. The lack of any unsaturated carbon signals in the ^{13}C-nmr spectrum, coupled with the presence of the lactone moiety, suggested **1** to be a tetracyclic diterpene bearing one hydroxyl and one lactone ring.

A comparison of the carbon resonances with those of the related kauranoid diterpene *ent*-kauran-16-ol [**2**] (Table 1), suggested that **1** possesses the same kaurane-type skeleton, as it showed a ^{13}C-nmr spectrum similar to that of **2** (3) except for signals of C-4, C-5, C-9, C-18, C-19, and C-20. These differences were due to the fact that the signals of three methyl carbons which occur in **2** at δ 18.0 (Me-20), δ 21.6 (Me-18), δ 33.5 (Me-19) have been replaced by an oxymethylene carbon signal (δ 73.9), a methyl carbon signal (δ 23.1), and a carbonyl carbon signal (δ 176.5) respectively, in **1**. In addition, a downfield shift of C-4 (Δ 9.8 ppm) and upfield shifts of C-5 (Δ 5.0 ppm) and C-9 (Δ 6.5 ppm) were observed. These spectral differences suggested that the lactone ring in **1** could be located between C-19 and C-20. The position of the carbonyl group could be determined by using the long-range HETCOR (LR HETCOR) experiment which reveals the long-range coupling through two and three bonds. Long-range correlations observed in the LR HETCOR experiment are summarized in Figure 1. Strong and multiple correlation peaks were observed for all the protons two or three bonds away from carbon atoms. Importantly, the Me-18 protons (δ 1.20) showed two-bond correlation to the δ 43.0 (C-4) and three-bond coupling to the δ 40.6 (C-3), δ51.2 (C-5), and δ 176.5 (C-19), which in turn correlated with H-3β (δ 1.49), H_3-18, and H-5 (δ 1.29). The two C-20 protons, an oxymethylene, were long-range coupled to C-10 (δ 38.7, 2-bond), C-1 (δ 40.6, 3-bond), C-5 (3-bond), and C-19 carbonyl (3-bond) across the O ether linkage. Further, the H-1α (δ 2.17) proton was correlated to C-10 (2-bond), C-5 (3-bond), and C-3 (3-bond), while the two protons at C-3 were long-range coupled to C-2 (δ 20.9, 2-bond), C-1 (3-bond), C-4 (2-bond), C-5 (3-bond), C-18 (3-bond), and C-19 (3-bond). These correlations effectively established the structure of ring A and the lactone moiety, in which the carbonyl was unequivocally assigned to C-19.

The protons of the methyl group at C-16, which were shifted downfield to δ 1.40 due to its attachment to a carbon bearing a hydroxyl group, was correlated to C-16 (δ 79.0, 2-bond), C-13 (δ 48.2, 3-bond), and C-15 (δ 57.8, 3-bond). This evidence, coupled with the ^{1}H- and ^{13}C-nmr spectra, led to the assignment of the hydroxyl at C-16.

The LR HETCOR spectrum was also used for the assignment of all of the carbon resonances (Table 1). No correlation for the two protons at C-2 (δ 1.71) with 2-bond or 3-

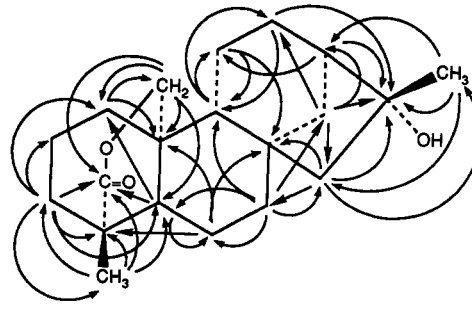

FIGURE 1. The long-range HETCOR responses of **1**.

bond carbons was observed in the LR HETCOR spectrum. The ^{1}H-^{1}H COSY, however, showed a strong coupling response between H_2-2 and H_2-1 as well as H_2-2 and H_2-3.

It was found that several proton resonances overlapped. However, a thorough examination of the 2D spectra, including the ^{1}H-^{1}H COSY and HETCOR, has led to an unambiguous assignment as shown in Table 1. Thus, the ^{1}H-^{1}H COSY spectrum identified the three different spin systems for protons associated with C-1, C-2, and C-3; C-5, C-6, and C-7; and C-9, C-11, C-12, C-13, and C-14; while the HETCOR study established the association of protons with relevant carbons. This evidence further confirmed the carbon skeleton connectivity of **1**.

The stereochemistry of **1**, particularly the β configuration of the methyl group at C-16, was ascertained by phase-sensitive 2D ^{1}H nOe (PHNOESY) and 1D ^{1}H nOe difference spectral evidence. A strong nOe response was observed in PHNOESY between the three protons at C-17 (δ 1.40) and the β-proton at C-11 (δ 1.65) together with the β-proton at C-12 (δ 1.64). This established that the Me-16, H-11β, and H-12β are in the same orientation. In addition, an nOe response was observed between H-15β and H-9. A strong nOe response was also observed between H_b-20 and H_b-14. Other nOe responses are shown in Figure 2.

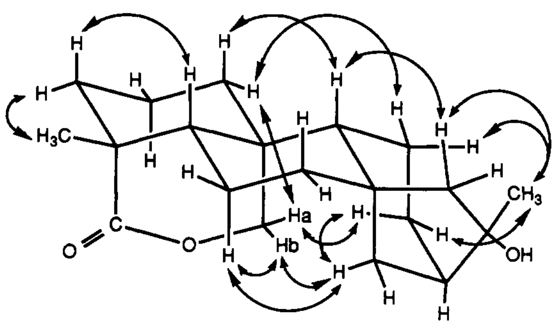

FIGURE 2. The phase-sensitive NOESY responses of **1**.

A significant feature of the ^{1}H-nmr spectrum was the long-range W-coupling between H-1β (δ 1.05) and H_b-20 (δ 5.21), H-1α (δ 2.17) and H-3α (δ 1.87), and H-5 (δ 1.29) and H_a-20 (δ 4.15), indicating that they are in the same plane and the rings A and lactone are also in a chair conformation. A Dreiding model indicated the feasibility of this assignment.

The evidence described above led to the elucidation of tripterifordin [**1**] as shown. Because the amount of sample obtained was small, the absolute stereochemistry of **1** remains to be determined.

Tripterifordin [**1**] inhibits HIV replication in H9 lymphocyte cells with an EC_{50} of 1 μg/ml (6 μM). Tripterifordin did not inhibit uninfected H9 cell growth at 15 μM.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—The melting point was taken on a Fischer-Johns melting point apparatus and is uncorrected. The ir spectrum was recorded on a Perkin-Elmer 1320 spectrophotometer. The mass spectrum was determined on a VG 70-250 SEQ mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were measured on Varian XL-400 and Bruker AC 300 spectrometers with TMS as an internal standard. Aldrich Si gel 60 (5–25 μ) was used for cc, and pre-coated Si gel plates (Kieselgel 60 F_{254}, 0.25 mm thickness, Merck) were used for analytical tlc. Tripterifordin was detected by spraying with 50% H_2SO_4 solution containing 1% anisaldehyde in 95% EtOH, followed by heating.

PLANT MATERIAL.—The roots of *T. wilfordii* were collected in Fujian Province, China. The plant material was identified by Pharmacognosy Associate Professor Guan-Yun Gu, Vice-chairman of Scientific

and Technical Archives of Shanghai Medical University, Shanghai, China. A voucher specimen is available for inspection at Department of Chemistry of Natural Drugs, School of Pharmacy, Shanghai Medical University, Shanghai, China.

EXTRACTION AND ISOLATION OF TRIPTERIFORDIN.—The air-dried roots (25 kg) of *T. wilfordii* were powdered and extracted with 95% EtOH. The EtOH extract was extracted with $CHCl_3$. The $CHCl_3$-soluble fraction was chromatographed in hexane over Si gel with increasing polarity of $CHCl_3$. The active fraction [hexane-$CHCl_3$ (1:4)] was further purified by flash chromatography employing hexane followed by toluene as eluents. Further repeated flash chromatography after elution with hexane followed by Et_2O afforded 12 mg (0.000048% yield) of tripterifordin [**1**], which was purified by recrystallization.

TRIPTERIFORDIN [**1**].—White needles (Me_2CO); mp 255–256°; $[\alpha]_D$ −46.6° ($c = 0.93$, $CHCl_3$, R_f 0.37 (Et_2O); ir (KBr) 3315, 2975, 2920, 2917, 2850, 1735, 1722, 1455, 1435, 1395, 1380, 1345, 1130, 1095, 1030, 975, 920, 870 cm^{-1}; ^1H and ^{13}C nmr see Table 1; eims *m/z* $[M]^+$ 318.2178 ($C_{20}H_{30}O_3$, calcd 318.2195), 300, 285, 272, 260 (100%), 257, 245, 242, 232, 227, 215, 202, 187, 173, 161, 159, 147, 145, 131, 119, 105, 93.

HIV INHIBITION ASSAY.—HIV inhibition was measured as described previously (1, 4, 5). Briefly, the assay employed H9 lymphocytes (3.5×10^6 cells/ml) in the presence or absence of HIV-1 (IIIB strain, 0.01–0.1 $TCID_{50}$/cell) for 1 h at 37°. Cells were washed thoroughly and resuspended at a final concentration of 2×10^5 cells/ml in the presence or absence of compound. After incubation for 3 days at 37°, the cell density of uninfected cultures was determined by cell count to assess toxicity of the drug. A p24 antigen capture assay (1) was used to determine the level of virus released onto the medium of HIV-infected cultures.

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