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Novel Sesquiterpene Esters with Alkaloid and Monoterpene and Related Compounds from *Tripterygium Hypoglaucum* : A New Class of Potent Anti-HIV Agents

Hongquan Duan[a], Yoshihisa Takaishi[a]* Masahiko Bando[b] Masaru Kido[b], Yasuo Imakura[c] and KuoHsiung Lee[d]

[a]Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770-8505 Japan, [b]Otsuka Pharmaceutical Co., Ltd., Kagasuno, Tokushima 771-01, Japan, [c]Faculty of Sciences, Naruto University of Education, Takashima, Naruto, Tokushima, 772-8502, Japan, [d]Natural Products Laboratory, Division of Medical Chemistry and Natural Products, School of Pharmacy, University of North Caqlolina, Chapel Hill, North Carolina 27599, U.S.A.

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Abstract: Two new sesquiterpene polyol esters (triptonine A and B) with alkaloid and monoterpene were isolated from *Tripterygium hypoglaucum* (Levl.) Hutch. Their structures were elucidated by spectroscopic means and X-ray analysis. Triptonine A (**1**) and hypoglaunine B[3] (**4**) demonstrated potent anti-HIV activity with EC_{50} values of 2.54 and 0.13μg/ml and therapeutic index values of 39.4 and >1000, respectively.
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The genus of *Tripterygium* has been used as traditional Chinese drugs for the treatment of cancer and as an insecticide for hundreds of years. Recently, *T. wilfordii* Hook has been used to treat rheumatoid arthritis and ankylosing spondylitis in some Chinese clinics[1]. In the course of our study on the sesquiterpene constituents of this genus, we have described the isolation of hyponine A, B, C[2] and hypoglaunine A, B, C, D[3] from the root barks of *T. hypoglaucum*. Our continuous study on the bioactive components in this plant led to the isolation of two novel sesquiterpene derivatives, named triptonine A (**1**) and B (**2**). In our anti-HIV active screening experiment for the above sesquiterpene alkaloid derivatives, we found a new class of potent anti-HIV agents; hypoglaunine B (**4**) showed extremely potent anti HIV activity with TI value of >1000.

By repeated column chromatography of the ethyl acetate soluble fraction from the methanol extract of the root bark (15.3kg) of *T. hypoglaucum*, a fraction containing a number of sesquiterpene alkaloids was obtained. Moreover, this fraction was separated on CC using HPLC (Inertsil PREP-ODS, GL Sciences and Si60, Hibar RT 250-25) to give **1** (98mg, 0.00064%) and **2**

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(13mg, 0.00008%).

Triptonine A (**1**), colorless needles, mp 284.0-285.5°C, exhibited a molecular formula, $C_{45}H_{55}O_{21}N$ (HR-EIMS)[4]. It showed hydroxy and ester carbonyl bands at 3438 and 1737 cm^{-1} in the IR spectrum, and the UV spectrum showed the presence of an aromatic moiety (224 and 264nm). Its ^1H and ^{13}C NMR spectal data[5] were very similar to those of hyponine A[2] except for the ester functions. Compound **1** was assumed to be an evonine type sesquiterpene alkaloid, having four acetyl groups and a monoterpene partial structure determined by ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC spectra (Fig. 1). The macrocycle structure accounted for 10 of the 19 degrees of unsaturation (deduced from the molecular formula), except for eight degrees of eight carbonyl carbons in four acetyl groups and this monoterpene partial structure; the remaining one degree indicated compound **1** has another ring in its structure. In its HMBC spectrum, the proton signals at δ_H4.69 (6"-H) and 5.41 (11-H) were correlated with the carbon signals at δ_C168.0 (C-9"), the signals at δ_H1.12 (10"-H_3) and 5.42 (7-H) with the signals at δ_C175.9 (C-1"). These facts indicated that another ring was formed by ester linkage between one sesquiterpene molecule and the partial structure of monoterpene at positions 7 and 11. In order to confirm the structure of **1**, X-ray analysis of **1** was undertaken[6]. The ORTEP drawing of the structure of **1** is shown in Figure 2.

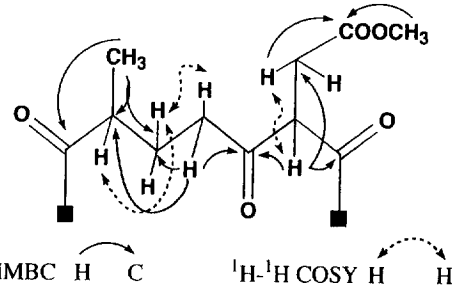


Fig.1 The monoterpene partial stucture of **1**

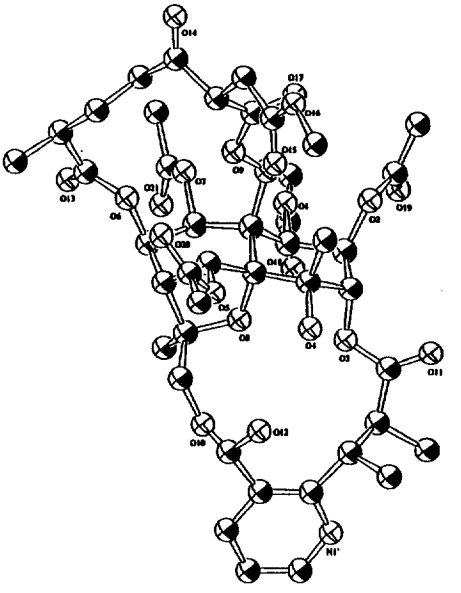
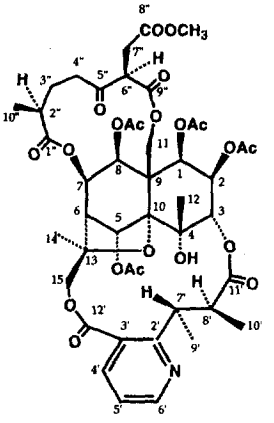
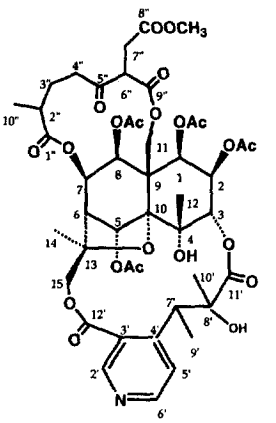


Fig.2 The ORTEP drawing of **1**

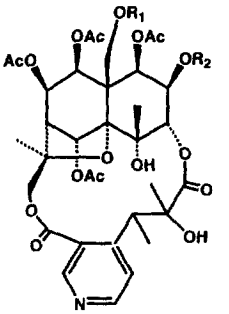
Triptonine B (**2**), $C_{45}H_{55}O_{22}N$, contained four acetyl groups and a monoterpene partial structure such as **1**. Its ^1H, ^{13}C NMR spectral data[7] were very similar to that of **1**, except for the pyridine unit, and also this pyridine unit was very similar to that of hypoglaunine A (**3**)[3]. Thus, compound **2** was assumed to be an isomeric evonine-type sesquiterpene alkaloid. By the elucidation of 2D NMR spectral data, the structure of compound **2** was determined as shown. (Fig.3)



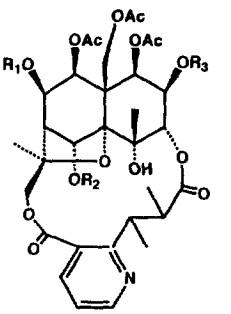
1



2



	R_1	R_2
3	Furanoyl	Ac
4	Ac	Furanoyl



	R_1	R_2	R_3
5	Nic	Furanoyl	Ac
6	Ac	Ac	Bz

Fig.3

More than fifty macrocycle sesquiterpene pyridine alkaloids have been isolated from Celastraceae plants, but the only example of a compound related to triptonine A (**1**) is cathedulin-K 20[8] which has a dimacrocycle structure. However, triptonine A (**1**) and B (**2**), which have a monoterpene structure bonded to the sesquiterpene molecule by ester linkage, are unique sesquiterpenoids first found in a natural source.

In searching for natural products as potential anti-AIDS agents, several compound series, such as coumarins[9], diterpenoids[10], triterpenoids[11], tannins[12, 13] were reported to have anti-HIV activity. In this paper, we reported a new class of potent anti-HIV agents, compound **1** and related sesquiterpene alkaloids isolated from *T. hypoglaucum*; their anti-HIV activity data are shown in Table 1. Triptonine A (**1**) inhibited HIV replication in H9 lymphocytes with an EC_{50} value of 2.54μg/ml and it inhibited uninfected H9 cell growth with an IC_{50} value of >100μg/ml, calculated therapeutic index value of 39.4. In general, TI>5.0 is considered to be significant activity; hypoglaunine B (**4**) showed extremely potent anti-HIV activity with a TI value of >1000, that is uncommon in a bioactive compound from a natural source.

Table 1. Anti-HIV activity of triptonine A (**1**) and related compounds

Compound	IC_{50}(μg/ml)	EC_{50}(μg/ml)	TI
Triptonine A (**1**)	>100	2.54	39.4
Hypoglaunine A (**3**)	>100	0.130	769
Hypoglaunine B (**4**)	>100	0.10	>1000
Hyponine E[14] (**5**)	1.95	0.172	11.3
Forrestine[14] (**6**)	>100	0.480	208
Cangoronine E-1[2]	56.8	0.9	63.4
AZT	500	0.032	15625

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4. *Triptonine A* (**1**). EI-MS *m/z* (rel. int.): 945 $[M]^+$(93), 914(10), 886(19), 857(60), 262(10), 220(21), 206(100), 178(40), 161(27), 150(14), 134(18), 107(69), 105(20), 95(22), 43(46). HR-EIMS: *m/z* 945.3254, $C_{45}H_{55}O_{21}N$, required 945.3267.

5. *Triptonine A* (**1**). ^{1}H NMR ($CDCl_3$): δ5.46 (*d*, 4.4, 1-H), 5.15 (*dd*, 2.0, 4.4, 2-H), 4.65 (*d*, 2.0, 3-H), 7.02 (*s*, 5-H), 2.43 (*d*, 3.9, 6-H), 5.42 (*dd*, 3.9, 5.9, 7-H), 5.25 (*d*, 5.9, 8-H), 5.41, 4.21 (each1H, *d*, 14.2, 11-H_2), 1.44 (*s*, 12-H_3), 1.75 (*s*, 14-H_3), 5.95, 3.65 (each1H, *d*, 11.7, 15-H_2), 8.01 (*dd*, 1.5, 7.8, 4'-H), 7.20 (*br t*, 6.5, 5'-H), 8.62 (*dd*, 1.5, 4.9, 6'-H), 4.60 (*q*, 6.8, 7'-H), 2.48 (*q*, 7.3, 8'-H), 1.32 (*d*, 6.8, 9'-H_3), 1.10 (*d*, 7.3, 10'-H_3), 2.54 (*m*, 2"-H), 1.90, 1.77 (each1H, *m*, 3"-H_2), 3.43, 2.91 (each1H, *m*, 4"-H_2), 4.69 (*dd*, 5.9, 9.3, 6"-H), 3.02 (*dd*, 9.3, 17.6, 7"-H_a), 2.90 (*dd*, 5.9, 17.6, 7"-H_b), 1.12 (*d*, 6.8, 10"-H_3), 1.75 (*s*, 1-OAc), 2.10 (*s*, 2-OAc), 2.18 (*s*, 5-OAc), 1.90 (*s*, 8-OAc), 3.61 (*s*, 9"-OMe). ^{13}C NMR ($CDCl_3$): δ73.7 (C-1), 68.8 (C-2), 75.9 (C-3), 70.7 (C-4), 73.9 (C-5), 50.3 (C-6), 69.8 (C-7), 71.1(C-8), 51.9 (C-9), 94.2 (C-10), 61.6 (C-11), 22.6 (C-12), 84.7 (C-13), 18.7 (C-14), 70.0 (C-15), 165.5 (C-2'), 125.1 (C-3'), 138.0 (C-4'), 121.3 (C-5'), 151.7 (C-6'), 36.5 (C-7'), 45.2 (C-8'), 11.9 (C-9'), 9.8 (C-10'), 174.1 (C-11'), 168.7 (C-12'), 175.9 (C-1"), 37.4 (C-2"), 28.2 (C-3"), 42.3 (C-4"), 204.5 (C-5"), 52.1 (C-6"), 32.8 (C-7"), 171.9 (C-8"), 168.0 (C-9"), 18.2 (C-10"), 169.1, 20.5 (1-OAc), 168.6, 21.1 (2-OAc), 170.3, 21.9 (5-OAc), 168.9, 20.6 (8-OAc), 52.1 (8"-OMe).

6. X-ray analysis of triptonine A (**1**): Crystal size 1.0 x 0.3 x 0.3mm. All data were obtained Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo *Ka* radiation. Crystal data: $C_{45}H_{55}O_{21}N$, Mr=945.92, orthorhombic, space froup $P2_12_12_1$, a=19.42(1) Å, b=25.26(1) Å, c=9.49(1)Å, V=4656(5) Å^3, Z=4.0, Dx=1.350g/cm^3, F(000)=2000, and μ(Mo*Ka*)=1.076cm^{-1}. Of the 6295 independent reflections which collected, 1661 reflections with $I > 3.0\sigma(I)$ were used for structure determination and refinemint. The final refinement converged with R=0.065 and Rw=0.084 for 604 parameters. The minimum and maximum peaks in the final difference Fourier map were -0.20 and 0.42e Å^{-3}.

7. *Triptonine B* (**2**). ^{1}H NMR ($CDCl_3$): 5.58 (*d*, 5.4, 1-H), 5.34 (*dd*, 2.4, 3.9, 2-H), 4.70 (*d*, 2.4, 3-H), 7.13 (*s*, 5-H), 2.52 (*d*, 3.4, 6-H), 5.53 (*dd*, 3.4, 3.9, 7-H), 5.32 (*d*, 3.9, 8-H), 4.29, 5.49 (each1H, *d*, 14.2, 11-H_2), 1.53 (*s*, 12-H_3), 1.63 (*s*, 14-H_3), 4.28, 5.49 (each1H, *d*, 11.2, 15-H_2), 8.99 (*s*, 2'-H), 7.81 (*d*, 5.4, 5'-H), 8.69 (*d*, 5.4, 6'-H), 4.24 (*q*, 6.8, 7'-H), 1.19 (*d*, 6.8, 9'-H_3), 1.34 (*s*, 10'-H_3), 2.62 (*m*, 2"-H), 1.91, 2.06 (each1H, *m*, 3"-H_2), 2.99, 3.27 (each1H, *m*, 4"-H), 4.74 (*t*, 7.3, 6"-H), 3.01 (2H, *d*, 7.3, 8"-H), 1.22 (*d*, 6.8, 10"-H_3), 1.98 (*s*, 1-OAc), 1.85 (*s*, 2-OAc), 2.24 (*s*, 5-OAc), 2.20 (*s*, 8-OAc), 3.68 (*s*, 9"-OMe). ^{13}C NMR ($CDCl_3$): δ73.3 (C-1), 70.8 (C-2), 77.8 (C-3), 70.6 (C-4), 74.6 (C-5), 50.6 (C-6), 69.7 (C-7), 68.1 (C-8), 52.3 (C-9), 93.3 (C-10), 61.7 (C-11), 22.0 (C-12), 83.6 (C-13), 18.8 (C-14), 69.8 (C-15), 151.5 (C-2'), 127.5 (C-3'), 151.8 (C-4'), 123. 6(C-5'), 152.7 (C-6'), 41.9 (C-7'), 76.8 (C-8'), 17.3 (C-9'), 24.1 (C-10'), 175.2 (C-11'), 167.7 (C-12'), 175.4 (C-1"), 38.1 (C-2"), 28.4 (C-3"), 42.1 (C-4"), 203.3 (C-5"), 52.1 (C-6"), 32.4 (C-7"), 171.9 (C-8"), 168.1 (C-9"), 18.3 (C-10"), 168.7, 20.5 (1-OAc), 169.1, 20.4 (2-OAc), 169.9, 21.8 (5-OAc), 168.5, 21.0 (8-OAc), 52.0 (8"-OMe).

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