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Taxachitrienes A and B, Two New Bicyclic Taxane Diterpenoids from *Taxus chinensis*

Wei-Shuo Fang, Qi-Cheng Fang*, Xiao-Tian Liang, Yang Lu and Qi-Tai Zheng

(Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, People's Republic of China)

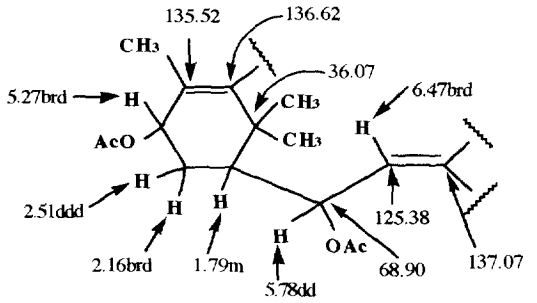
ABSTRACT: *Two new bicyclic taxane diterpenoids, taxachitrienes A(1) and B(2), were isolated from the needles of Taxus chinensis . Their structure were elucidated on the basis of spectroscopic data. The crystal structure of 2 was also presented. It is the first time for the isolation of bicyclic diterpenoid which is proposed as the biogenetic precursor for taxanes.*

The discovery of taxol,[1] an excellent antitumor agent, has promoted the relevant studies on taxol and taxane diterpenoids. In order to search for enough resources for mass production of taxol, we paid a lot of attention to the needles of *Taxus* sp. plants, as the regenerative resource. After successful semi-synthesis of taxol and its analogs from 10-deacetylbaccatin III,[2] which is abundant in the needles of *T. baccata* and *T. wallichiana*,[3] the needles of *Taxus* sp. plants have become the preferred source for producing taxol and other related compounds.

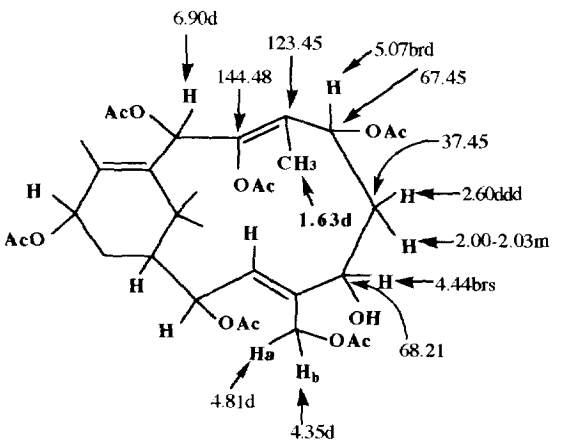
Taxus chinensis (Pilger) Rehd, indigenous to China, has been investigated as a source for producing taxol. In the mean time many taxoids were isolated.[4-10] We have found that the ethanolic extracts of needles of *T. chinensis* showed activity against several tumor cell lines *in vitro*. The further phytochemical studies on the ethanolic extracts led us to the discovery of taxachitrienes A(**1**) and B(**2**), two new diterpenoids with unique structures. It is the first time for the isolation of the taxane diterpenoids with a bicyclic skeleton, which was proposed by Pattenden *et al*[11,12] as the biogenetic precursor of taxanes.

Compound **1** occurs as white amorphous solid. Adduct ions at m/z 659($[M+Na]^+$) and 675($[M+K]^+$) revealed that its molecular weight was 636. By a combination analysis of FABMS and ^{13}C NMR data, its formula was proposed as $C_{32}H_{44}O_{13}$. HR-FABMS also gave the same formula. The unsaturation number for **1** was calculated as 11. Since the signals for six acetoxy groups in ^1H and ^{13}C NMR, and six carbon atoms in three double bonds were observed in ^{13}C NMR, only two unsaturation equivalents were left, and the skeleton of **1** was thus deduced to be bicyclic. Since a broad doublet at 6.47 ppm was correlated to a tertiary carbon signal at 125.38 ppm in ^{13}C-^1H COSYand a quaternary carbon atom at 137.07 ppm in Heteronuclear Multiple Bonds Correlation (HMBC) spectrum, we suggested the existence of a highly substituted double bond. A double-doublet at 5.78 ppm, coupled with the broad doublet at 6.47 ppm and a multiplet at 1.79 ppm in ^1H-^1H COSY, and a tertiary carbon atom at 68.90 ppm in ^{13}C-^1H COSY, was proposed to be attached to the carbon atom which was substituted by an acetoxy group. In ^1H-^1H COSY, the multiplet at 1.79 ppm was correlated with a double-double-doublet at 2.51 ppm and a broad doublet at

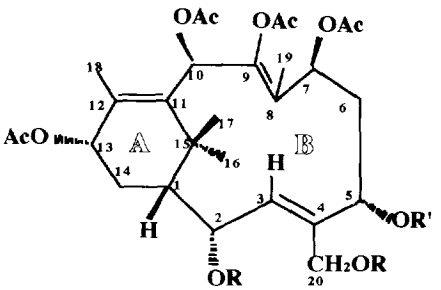
2.16 ppm, and the double-double-doublet at 2.51ppm was correlated to a broad doublet at 5.27 ppm. In HMBC, we observed that the broad doublet at 5.27 ppm was correlated to the carbon at 135.52 ppm, and the multiplet at 1.79 ppm was correlated to the carbon at 36.07 ppm and a quaternary carbon at 136.62 ppm. Since the signals of two quaternary carbon atoms at 135.52 and 136.62 ppm represented two carbon atoms at a fully-substituted double bond, we proposed that a cyclohexene ring existed.



The unusual doublet appeared at 6.90 ppm, which was coupled with the signal at 1.63 ppm with a small coupling constant(1.4 Hz). It may be ascribed to a proton in an unusual surrounding. Since the proton at 6.90 ppm was correlated to two pairs of olefinic carbon atoms, this proton must be flanked by two double bonds. A doublet representing three hydrogen atoms at 1.63 ppm, coupled with the doublet at 6.90 ppm in ^{1}H-^{1}H COSY, and correlated to two quaternary carbon atoms at 123.45 and 144.48 ppm and a tertiary carbon atom at 67.45 ppm in HMBC, was proposed to be attached to a fully-substituted double bond. The broad doublet at 5.07 ppm, correlated to the tertiary carbon atom at 67.45 ppm, was coupled with a double-double-doublet at 2.60 ppm and a multiplet at about 2.00-2.03 ppm (overlapping), and the latter was coupled with a broad singlet at 4.44 ppm. Both the double-double-doublet at 2.60 ppm and the multiplet at 2.00-2.03 ppm were correlated to a carbon atom at 37.45 ppm. A pair of doublets at 4.81 and 4.35 ppm were deduced to be the signal of -CH_2OAc. The signals of -CH_2OAc were correlated to the double bond carbons at 125.38 and 137.07 ppm, as well as a tertiary carbon atom at 68.21 ppm bearing a proton at 4.44 ppm (brs), therefore, a cyclododecadiene ring was suggested.



The assignment of the signals for the acetoxy groups in ^{1}H and ^{13}C NMR was all based on HMBC spectral data. The numbering of carbon atoms follows the taxoid convention.



1 R=Ac R'=H
2 R=H R'=Ac

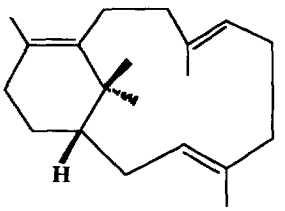


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Table 1 ^{1}H NMR data for compound **1** and **2**(500 MHz, $CDCl_3$)

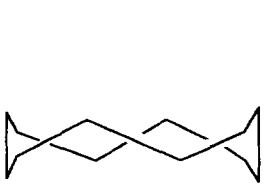
Proton	^{1}H NMR of **1**	difference NOE of **1**	^{1}H NMR of **2**
H-1β	1.79m	H-2, 14β, Me-16, 17	1.75dd(8.1; 4.1)
H-2β	5.78dd(11.5; 4.6)	H-1, 20a	4.68dd(10.4; 4.2)
H-3	6.47brd(11.5)		5.72d(10.4)
H-5β	4.44brs	H-6β	5.54brs
H-6α	2.00-2.03m		2.00-2.02m
H-6β	2.60ddd(16.0; 7.8; 3.0)		2.60ddd(16.5; 8.9; 2.0)
H-7α	5.07d(7.8)	H-10	5.39brd(8.9)
H-10α	6.90d(1.4)	H-7, Me-18	7.12s
H-13β	5.27brd(9.2)	H-14β	5.30(9.2)
H-14α	2.16brd(16.8)		2.00-2.02m
H-14β	2.51ddd(16.8; 9.2; 7.5)		2.52ddd(15.7; 9.2; 2.9)
Me-16	1.28s	H-2, 20, Me-17	1.23s
Me-17	1.11s	H-13, 14β, Me-16	1.08s
Me-18	1.92s	H-10	2.10s
Me-19	1.63d(1.4)	H-6β, 20b	1.58s
H-20a	4.81d(12.8)		4.36d(12.5)
H-20b	4.35d(12.8)		3.77d(12.5)
2-OAc	2.00s		
5-OAc			2.19s
7-OAc	2.17s		2.01s
9-OAc	2.21s		2.25s
10-OAc	1.95s		1.94s
13-OAc	2.09s		2.13s
20-OAc	2.01s		
5-OH	3.90brs		

Table 2 ^{13}C NMR data for compound **1** and **2**(125 MHz, $CDCl_3$)

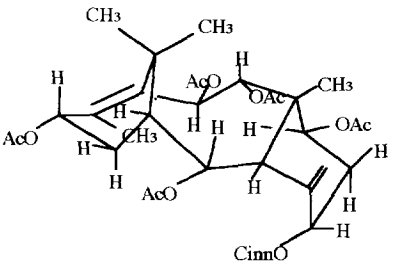
Carbon	DEPT	^{13}C NMR of **1**	HMBC of **1**	^{13}C NMR of **2**
C-1	CH	46.41	H-2, 3, 13, 14α, 14β, Me-16, 17	48.18
C-2	CH	68.90	H-1, 14α, 2-OAc	66.48
C-3	CH	125.38	H-1, 2, 6α, 20	127.49
C-4	C	137.07	H-2, 3, 6β, 20	135.44
C-5	CH	68.34	H-3, 7, 20	70.00
C-6	CH2	37.45	H-7	34.68
C-7	CH	67.45	H-6, Me-19	70.40
C-8	C	123.45	H-6, 7, 10, Me-19	124.32
C-9	C	144.48	H-7, 10, Me-19	143.32
C-10	CH	68.21		68.57
C-11	C	136.62	H-1, 10, Me-16, 17	135.03
C-12	C	135.52	H-10, 13, 14, Me-18	136.12
C-13	CH	69.01	H-1, 14α, 14β, Me-18	67.05
C-14	CH2	25.15	H-2, 13	25.12
C-15	C	36.07	H-1, 10, 14, Me-16, 17	36.21
C-16	CH3	24.57	Me-17, 18	25.56
C-17	CH3	33.98	H-2, Me-16	32.66
C-18	CH3	17.02	H-13	16.28
C-19	CH3	12.92	H-7	12.43
C-20	CH2	59.78	H-3	58.23
2-OCOCH3	C	169.84	H-2, 2-OAc	
OCOCH3	CH3	21.33		
5-OCOCH3	C			167.83
OCOCH3	CH3			20.44
7-OCOCH3	C	172.43	H-7, 7-OAc	169.47
OCOCH3	CH3	21.19		21.18
9-OCOCH3	C	167.99	9-OAc	170.29
OCOCH3	CH3	20.30		21.54
10-OCOCH3	C	167.99	H-10, 10-OAc	167.98
OCOCH3	CH3	20.72		20.77
13-OCOCH3	C	170.69	H-13, 13-OAc	170.29
OCOCH3	CH3	21.49		21.51
20-OCOCH3	C	170.65	H-20, 20-OAc	
OCOCH3	CH3	20.74		

The conformation of A ring was deduced as boat form, since NOE between Me-17 and H-13 was observed. Meanwhile, H-1, 13 and Me-17 were in *cis* -relationship, based on NOE results. H-1β and 13β were designated, in accordance with the configuration of taxane diterpenoids and verticillene (**3**), synthesized by Pattenden *et al*. and proposed as biogenetic precursor for taxane diterpenoids. The conformation of B ring was difficult to determine, as the twelve-membered ring was rather flexible. The dominant conformation for cyclododecane[13] was the starting point when we studied the configuration and conformation for B ring(see Fig.1). The NOEs among Me-18, H-10 and H-7, which were observed in the taxane diterpenoids with 6/8/6 ring system,[14] were also observed in **1**. It revealed that 9-OAc and Me-19 were in *cis* -relationship, while for the *trans* -relationship it was improbable for Me-18, H-10 and H-7 to be close to each other. Therefore, 10β-OAc and 7β-OAc substitution pattern was also proposed, in accordance with the NOE results and the configuration for most taxoids. Since the NOEs between H-1 and H-2, and H-2 and Me-16 were observed, we suggested that they were in *cis* -relationship and 2-OAc was substituted at 2α position. The fact that the coupling constant between H-2 and H-3 was 11.5 Hz and no NOE between them was observed indicated that they may be in *trans* -relationship, and H-20 was also *trans* -related to H-3. The NOEs between Me-19 and H-20b, H-2β and H-20a were the basis for assignments of H-20a and H-

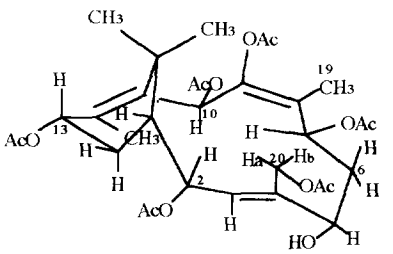
20b. The assignments of H-6α, 6β, 14α and 14β were also based on NOE results. The similar NOE results between taxachitriene A and taxinine J led us to conformational comparison between them, and conformation of taxachitriene A was deduced as that in Figure 1.



1a



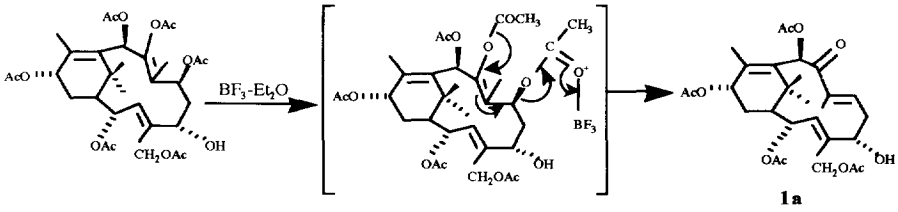
1b



1c

Fig. 1 Conformation of cyclododecane, taxinine J and taxachitriene A
1a. dominant conformation of cyclododecane
1b. conformation of taxinine J
1c. proposed conformation of taxachitriene A

It is noteworthy that an enol acetate substructure, usually not stable, was found to exist at C-9 position in **1**. For the purpose of searching for more evidence for the existence of such a substruture and studying the relations between **1** and other taxane diterpenoids through cyclisation, we treated **1** with BF_3-etherate. Unfortunately, we can not observe the cyclisation of **1** to taxanes, but did find some evidence for the existence of enol acetate substructure. After treatment of **1** with BF_3-etherate, a major product (**1a**) was isolated. The signal at 194.7 ppm in ^{13}C NMR spectrum revealed that it possesses an α,β-unsaturated ketone substructure, and the NOEs between Me-18 and H-10, as well as H-10 and H-7 could lead us to the conclusion that Me-19 and C-9 carbonylic oxygen were in eclipsed conformation, like **1**(see Fig. 1), thus Me-19 and H-7 were in *trans*-relation. The mechanism of its formation could be deduced as follows:



Compound **2** crystallized from acetone as colourless prism. Comparing the ^{1}H NMR spectra of **2** with those of **1**, we found that the number of peaks and the splitting of all the peaks were nearly the same except of loss of an acetoxy signal. The signal of H-5β in **2** was markedly shifted to a lower field, while the signals of H-2β and a pair of doublets representing H-20 were evidently shifted upfield(see Table 1). These observations revealed that an acetoxy group was attached to C-5 in **2** in place of a hydroxy group in **1**, and the other way round for C-2 and C-20. The chemical shifts of H-10α and H-7α varied a little since the molecular conformation was slightly modified. The assignments of all the ^{1}H and ^{13}C signals were based on HMBC spectra. Because it is the first time for the actual isolation of the previously proposed taxoid precursors, an X-ray diffraction experiment was performed on **2**, and the X-ray results also supported our structural deduction. Crystal structure of **2** was shown in Fig.2.

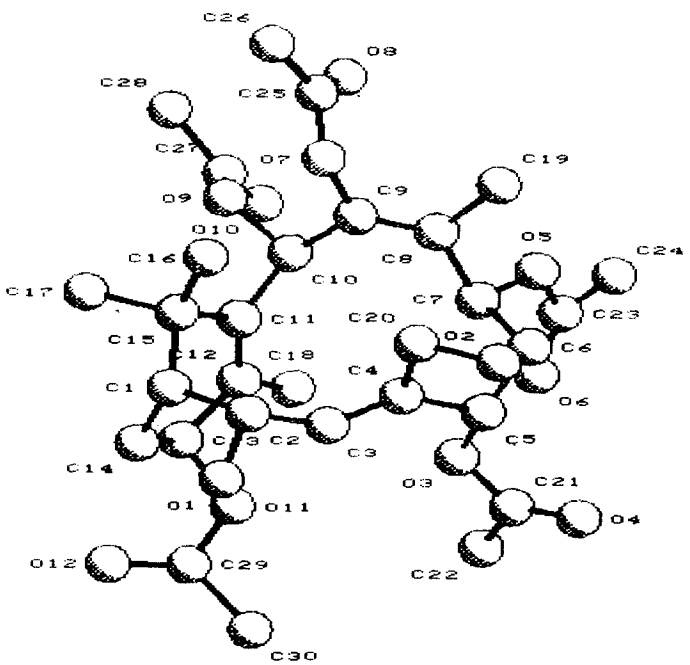


Fig. 2 Crystal structure of compound **2**

From the crystal structure of **2**, we could say that it is most probable that **2** and its analogs were indeed biogentic precursors for taxoids. Either C-8 attacking C-3 to give a 6/8/6 taxol-like skeleton or C-8 attacking C-20 to give a 6/10/6 skeleton will lead to the products with the same stereochemistry as that of natural taxoids. But the conformation of the bicyclic taxoids in an aprotic solvent such as chloroform or acetone discourages the transannular reaction, since C-8 and C-3, and C-8 and C-20 were not close to each other. It is probable that the conformation of taxachitriene in the protic solvent was rather different from that in the aprotic solvent, and the transannular reaction may proceed more easily.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.--Melting points were determined on an uncorrected Boetius melting point apparatus. Optical rotations were determined by Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Shimazu UV-240 spectrophotometer and IR spectra with a Perkin-Elmer 683 spectrometer. ^1H and ^{13}C NMR spectra were recorded with Bruker AM-500 appratus. FABMS spectra were recorded with a JMS-DX 300 MS spectrometer. Silica gel for chromatography is purchased from Qingdao Marine Chemical Factory, China.

ISOLATION.-- Needles of *T. chinensis* (14 kg) were extracted with 95% ethanol for 5 days, and then the extracts, added on a silica gel colunm, were eluted successively with P. ether (Fr. 1), P. ether/Me_2CO(6:1) (Fr. 2), P. ether/Me_2CO(2:1) (Fr. 3), Me_2CO (Fr. 4) and MeOH (Fr. 5). Fraction 3 was repeatedly chromatographed on the silica gel column and plates, and finally gave **1** (130 mg) and **2**(50 mg).

Compound **1** was obtained as white amorphous solid. mp 99-101 °C, $[\alpha]_D^{24}$ -9.9° (*c* =0.076, $CHCl_3$), UV(MeOH) λmax (log ε) 202(sh, 4.21), 204(4.23), 207(sh, 4.22); IR (dry film) νmax 3480, 2968, 1736, 1372, 1239, 1205, 1110, 1017, 960, 940 cm^{-1}; FABMS *m/z* 659([M+Na]$^+$)(19%), 475(17), 415(100), 373(100), 355(60), 313(100), 295(60); HR-FABMS $[C_{32}H_{44}O_{13}K]^+$ 675.2424 (calc. 674.4334); ^1H and ^{13}C NMR data see Tables 1 and 2.

Treatment of compound 1 with BF_3-etherate.-- Compound **1** (80mg) was dissolved in 2ml of dry ether, and 0.02ml of BF_3-etherate (distilled) was added and stirred at room temprature for 10h. The solution was diluted with water and partitioned with ether. The ether layer was washed with 5% $NaHCO_3$ and water successively, then dried with $MgSO_4$, and evaporated to dryness in vacuo. The residue (40mg) was chromatographed on a silica gel plate and developed with $CHCl_3$/MeOH(20:1). Seventeen mg of **1a** was obtained, and 11mg of **1** was recovered. **1a**: FABMS *m/z* 573([M+K]$^+$)(100%), 475(45), 373(90); ^1H NMR($CDCl_3$): 6.70(s, 1H, H-10α), 6.44(d, J=11.0 Hz, 1H, H-3), 6.42(d, J=13.1 Hz, 1H, H-7α), 5.72(dd, J=11.0, 4.8 Hz, 1H, H-2β), 5.36(d, J=8.7 Hz, 1H, H-13β), 4.78(d, J=12.9 Hz, 1H, H-20a), 4.68(brs, 1H, H-5β), 4.34(d, J=12.9 Hz, 1H, H-20b), 2.85(m, 1H, H-6β), 2.50(m, 1H, H-6α), 2.47(m, 1H, H-14β), 2.17(s, 3H, OAc), 2.13(s, 3H, OAc), 2.03(s, 3H, OAc), 2.01(s, 3H, OAc), 1.92(s, 3H, Me-18), 1.84(m, 1H, H-1), 1.78(s, 3H, Me-19), 1.25(s, 3H, Me-17), 0.90(s, 3H, Me-16). ^{13}C NMR($CDCl_3$): 194.7, 170.4, 170.3, 169.7, 169.6, 140.7, 139.5, 139.2, 135.7, 134.2, 124.0, 76.2, 69.4, 69.2, 68.4, 59.8, 46.3, 37.3, 34.2, 33.6, 29.7, 24.7, 24.0, 21.3, 20.9, 20.8, 17.3, 11.9.

Compound **2** was obtained as colourless prism when crystallized from acetone. mp 225-227 °C, $[\alpha]_D^{14}$ +29° (*c* =0.10, MeOH), UV(MeOH) λmax (log ε) 206(4.22), 207(4.23); IR (dry film) νmax 3456, 2955, 1737, 1716, 1374, 1262, 1229, 1112, 1016, 734 cm^{-1}; FABMS *m/z* 617([M+Na]$^+$)(12%), 577(6), 475(7), 433(7), 415(31), 373(55), 355(44), 313(100), 295(60); Elemental analysis C 60.58%, H 7.17%, O 32.25% (calc. for $C_{30}H_{42}O_{12}$ C 60.60%, H 7.07%, O 32.33%); ^1H and ^{13}C NMR data see Tables 1 and 2.

X-ray crystallography of 2.--Taxachitriene B crystallized as monoclinic system, space group $P2_1$ with two molecules of composition $C_{30}H_{42}O_{12}$ in unit cell. Accurate cell constants were a=13.200(3)Å,

b=8.627(1)Å, c=15.884(5)Å, β=101.12(2)°, and V=1774.9(7)Å^3. All unique reflections with 2θ≤46° were collected on the Enraf-Nonius CAD-4 diffractometer using graphite monochromated MoKα radiation and 2θ/ω sacns. In the 2430 unique reflections 1993 observed reflections were judged(|Fo|>3σ|Fo|). The structure was solved by direct method(SHELXS-86). The residual factors were R=0.089 for 1993 observed reflections.

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REFERENCES

1. Wani, M.C.; Taylor, H.L.; Wall M.E.; Coggon, P.; McPhail, A.T.; *J. Am. Chem. Soc.* **1971**, 93, 2325-2327.

2. Gueritte-Voegelein, F.; Guenard, D.; Lavelle, F.; LeGoff, M.T.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, 34, 992-998.

3. Kingston, D.G.I. *Pharmac. Ther.* **1991**, 52, 1-34.

4. Zhang, Z.; Jia, Z.; Zhu, Z.; Cui, Y.; Cheng, J.; Wang, Q. *Planta Med.* **1990**, 56, 293-295.

5. Zhang, Z.; Jia, Z. *Phytochem.* **1991**, 30, 2345-2348.

6. Zhang, Z.; Jia, Z. *Kexue Tongbao (Chin. Sci. Bull.)* **1991**, 36, 593-595.

7. Zhang, S.; Chen, W.M.; Chen, Y.H. *Yaoxue Xuebao (Acta Pharm. Sin.)* **1992**, 27, 268-272.

8. Fuji, K.; Tanaka, K.; Li, B.; Shingu, T.; Sun, H., Taga, T ; *Tetrahedron Lett.* **1992**, 33, 7915-7916.

9. Li, B.; Tanaka, K.; Fuji, K.; Sun, H.; Taga T. *Chem. Pharm. Bull.* **1993**, 41, 1672-1673.

10. Fuji, K.; Tanaka, K.; Li, B.; Shingu, T.; Sun, H., Taga, T.; *J. Nat. Prod.* **1993**, 56, 1520-1531.

11. Jackson, C.B.; Pattenden, G. *Tetrahedron Lett.* **1985**, 26, 3393-3396.

12. Begley, M.J.; Jackson, C.B.; Pattenden, G. *Tetrahedron Lett.* **1985**, 26, 3397-3400.

13. Dale, J. "Stereochemistry and conformational analysis", Universitetsforlaget, Oslo, 1978, pp.1-230.

14. Woods, M.C.; Chiang, H.C.; Nakadaira, Y.; Nakanishi, K. *J. Am. Chem. Soc.* **1968**, 90, 522-523.

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