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Structures of Xuxuarines, Stereoisomeric Triterpene Dimers from *Maytenus chuchuhuasca*

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Abstract: Nine novel stereoisomeric triterpene dimers, xuxuarines Aα (**1**), Aβ (**2**), Bα (**3**), Bβ (**4**), Cα (**5**), Cβ (**6**), Dα (**7**), Dβ (**8**) and 7',8'-dihydroxuxuarine Aβ (**9**), were isolated from a South American medicinal plant "xuxuá" (*Maytenus chuchuhuasca* Raymond-Hamet et Colas). Spectroscopic and chemical evidences showed that their structures were composed of one quinoid and one aromatic triterpenes joined together by two ether linkages formed between the two A rings, and MD calculations confirmed thier conformations.

INTRODUCTION

As a part of our studies on biologically active compounds in South American medicinal plants,[1] we studied on active principles of the genus *Maytenus* plants of the Celastraceae family, which have been widely used as a folk medicine in South America.[2-5] "Xuxuá" (*Maytenus chuchuhuasca* Raymond-Hamet et Colas) and related medicinal plants have been used for the treatment of rheumatism, and as an antitumoral agent for skin cancer, by the inhabitants of the Amazonian basin.[5,6] By cytotoxicity-guided purification, a methanol extract of "xuxuá" gave pristimerin, tingenone and 22β-hydroxytingenone as its active principles.[7-9] Nine novel stereoisomeric triterpene dimers, named xuxuarines Aα (**1**), Aβ (**2**), Bα (**3**), Bβ (**4**), Cα (**5**), Cβ (**6**), Dα (**7**), Dβ (**8**) and 7',8'-dihydroxuxuarine Aβ (**9**) were also isolated. All of these compounds were found to be composed of one quinoid type triterpene and one aromatic triterpene, derived from tingenone and/or 22β-hydroxytingenone, linked together by two ether linkages formed between the two A rings. Some of them showed tumor cell growth inhibition activities.

In the present paper, we report the isolation, structural elucidation and conformational analysis of these nine triterpene dimers by spectroscopic (NMR, MS and CD spectral analyses), chemical evidences, and MD calculations.

RESULTS AND DISCUSSION

Isolation of xuxuarines

From a cytotoxic methylene chloride soluble portion of a methanolic extract of *Maytenus chuchuhuasca* Raymond-Hamet et Colas (bark; 5 kg), three active compounds, i.e. pristimerin, tingenone and 22β-hydroxytingenone and nine triterpene dimers, xuxuarines Aα (**1**: 0.0026%), Aβ (**2**: 0.0046%), Bα (**3**: 0.0012%), Bβ (**4**: 0.0026%), Cα (**5**: 0.0010%), Cβ (**6**: 0.0015%), Dα (**7**: 0.0015%), Dβ (**8**: 0.0015%) and 7',8'-dihydroxuxuarine Aβ (**9**: 0.0002%) were obtained.

Structures of xuxuarines Aα and Aβ

Xuxuarine Aα (**1**) was obtained as yellow amorphous powder. The molecular formula was shown to be $C_{56}H_{70}O_7$ by FAB-MS and ^{13}C-NMR spectra. ^{1}H-NMR signals at δH 2.44 (m; H-20), 2.85 (d, J = 14.3 Hz; H-22), 0.92 (d, J = 6.5 Hz; H-30), 2.42 (m; H-20'), 2.80 (d, J = 14.4 Hz; H-22'), 0.94 (d, J = 6.5 Hz; H-30'), and ^{13}C-NMR signals at δC 115.53 (d; C-1), 190.09 (s; C-2), 91.96 (s; C-3), 79.25 (s; C-4), 130.20 (s; C-5), 126.35 (d; C-6), 116.09 (d; C-7), 160.23 (s; C-8), 173.35 (s; C-10), 213.34 (s; C-21), 52.41 (t; C-22), 111.21 (d; C-1'), 144.68 (s; C-2'), 137.58 (s; C-3'), 127.65 (s; C-4'), 124.17 (s; C-5'), 187.41 (s; C-6'), 125.96 (d; C-7'), 170.46 (s; C-8'), 150.34 (s; C-10'), 213.34 (s; C-21'), 52.31 (t; C-22') were suggested that **1** was a triterpene dimer composed of two tingenone type triterpenes, one in quinoid form, and the other in aromatic form.

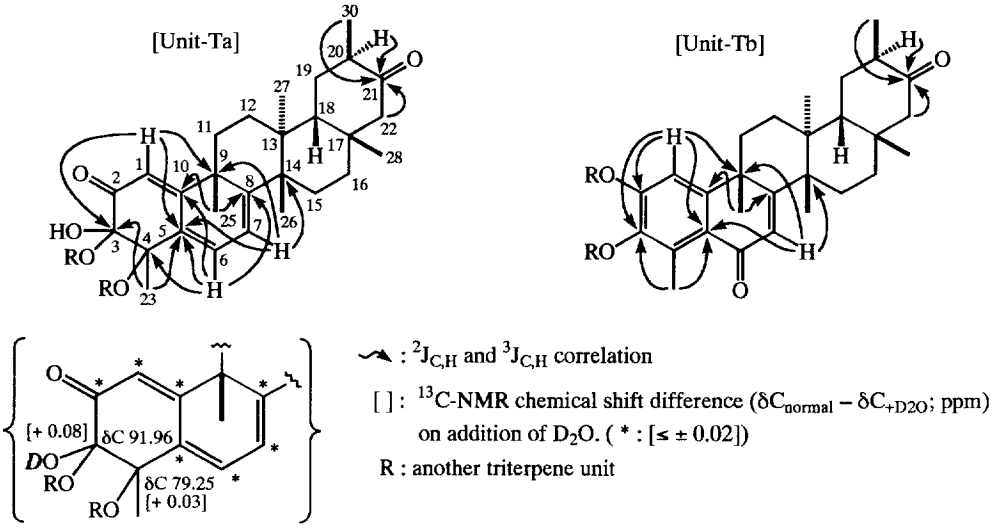


Figure 1. Partial structures of xuxuarine Aα (**1**).

The analyses of the HMQC and HMBC spectra showed that the quinoid triterpene unit (unit Ta) contained a conjugated ketone system with three conjugated double bonds in the A and B rings as the signals at δH 6.06 (d, J = 1.2 Hz; H-1), 6.23 (dd, J = 1.2, 6.6 Hz; H-6), 5.94 (d, J = 6.6 Hz; H-7), δC 115.53 (d; C-1), 173.35 (s; C-10), 130.20 (s; C-5), 126.35 (d; C-6), 116.09 (d; C-7), 160.23 (s; C-8), and two oxygenated adjacent quaternary carbons at C-3 and C-4 of the A ring as δC 91.96 (s; C-3) and 79.25 (s; C-4). As regards, the aromatic triterpene unit (unit Tb), the signals at δC 111.21 (d; C-1'), 144.68 (s; C-2'), 137.58 (s; C-3'), 127.65 (s; C-4'), 124.17 (s; C-5'), 187.41 (s; C-6'), 125.96 (d; C-7'), 170.46 (s; C-8'), 150.34 (s; C-10'), showed that it contained an aromatic ring system for A ring, one carbonyl group at C-6' and one conjugated double bond at C-7', 8' on B ring, and oxygenated carbons at C-2' and C-3' on A ring. There was no free phenolic hydroxyl group in **1**, since no bathochromic shift was observed in the UV spectrum on addition of alkali.[10] Furthermore, a high field shift (Δ 0.08 ppm) of C-3 observed in the ^{13}C-NMR spectrum by the addition of D_2O,[11] and the formation of one amide proton signal was also observed in the ^{1}H-NMR spectrum when treated with TAI reagent.[12] The above fact indicated that the only one hydroxyl group of the molecule suggested by the IR siganl at 3473 cm^{-1} was attached to C-3. In order to determine the linkages between the two units Ta and Tb, a NOESY spectrum of the methyl derivative of **1** was measured. NOE correlations between the introduced methoxy methyl group on the C-3 and the H-1' olefinic proton, and between the H-6 olefinic proton and the H-23 methyl group, revealed the configuration of the connection with two units. Besides, NOE correlation between the methoxy methyl group and the H-23 methyl group revealed that this 3,4-dioxy bond was *cis* configuration. Remained problem about stereochemistry of this *cis* 3,4-dioxy bond was cleared by the analysis of CD spectrum. That is to say, its spectrum showed positive first maxima value at 357 nm, which have resulted from α orientation about the *cis* 3,4-dioxy bond.[13]

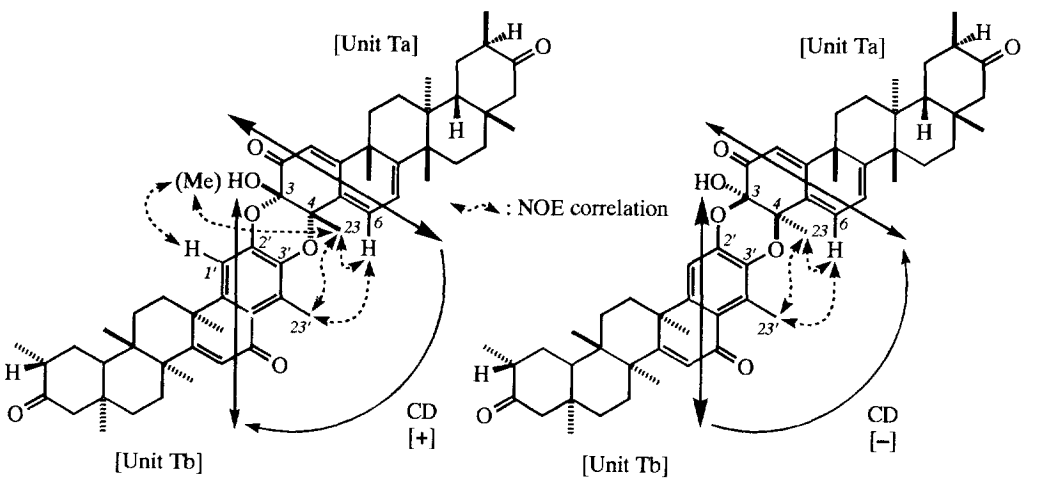


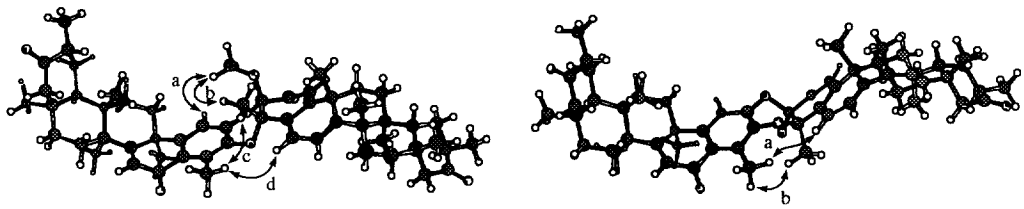
Figure 2. Structures of xuxuarines Aα (**1**) and Aβ (**2**).

Xuxuarine Aβ (**2**) was shown to have the same molecular formula, $C_{56}H_{70}O_7$, as **1** by FAB-MS and ^{13}C-NMR spectra. The HMQC and HMBC spectra of **2** revealed that its two triterpene units were identical with those of **1**. The difference between **1** and **2** was assumed to be in the stereochemistry of the two ether bonds linking the two units together. The NOE data taken from the NOESY spectrum of **2** were essentially identical with those of the methyl derivative of **1**. The CD spectrum of **2** showed a negative first Cotton effect at 397 nm, and a positive second Cotton effect at 331 nm, showing that the cis 3,4-dioxy group had β orientation in **2**.

These spectral analyses explained that the structures of xuxuarines Aα (**1**) and Aβ (**2**) were triterpene dimers stereoisomeric with the two ether linkages, as shown in Figure 2.

MD calculations of xuxuarines Aα and Aβ

For the purpose of confirming the orientation of the cis 3,4-dioxy bond of xuxuarines Aα (1) and Aβ (2), and analyzing complicated conformational features of them, it is necessary to use a computational method which can give us a result being independent on starting structure. We have already reported that the possibility of using molecular dynamics techniques as a tool for simulated annealing is tested.[14] The method, applied to a broad class of problems, has also shown its practical utility in the case of conformational problems.[15] High temperature MD calculations[16] for the simulation were performed with distance constraints derived from the NOE experiments of **1** and **2**, which were used to show that the solution structures are consistent with the experimental data. The each system was equilibrated for 5400 fs with a thermal bath at 900K and thereafter successively for 900 fs with a thermal bath 10K lower in temperature until a final temperature of 50K was obtained. Twenty cycles are performed, and each freezed conformation as sampled from the minimum temperature at 50K. Each low energy conformation was finally minimized by use of molecular mechanics calculation of TRIPOS force field.[17] Each snapshot with the lowest energy (**1**: 80.177 kcal/mol; **2**: 70.101 kcal/mol) was selected as a relevant conformation. It is obvious from the perspective view as shown in Figure 3 that each conformation is satisfied with the characteristic NOE relationship and is fulfilled for solution conformer.



Methyl derivative of xuxuarine Aα (**1**)

a = 2.900, b = 2.564, c = 2.850, d = 2.926 Å

Xuxuarine Aβ (**2**)

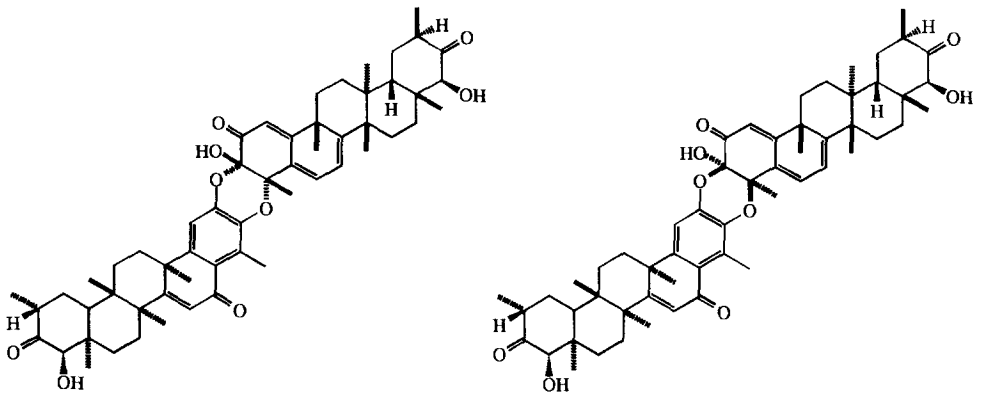
a = 2.970, b = 2.829 Å

Figure 3. Prespective views of the lowest energy conformers of methyl derivative of **1** (80.177 kcal/mol) and **2** (77.101 kcal/mol). The values of a - d represent the distances between two protons indicated by arrows.

Structures of xuxuarines Bα and Bβ

Xuxuarines Bα (**3**) and Bβ (**4**) were both yellow amorphous powder, with the same molecular formula $C_{56}H_{70}O_9$, i.e. having two more oxygen than **1** and **2**. Their NMR spectra, signals at δH 6.08 (d, J = 1.4 Hz; H-1), 6.26 (dd, J = 1.4, 6.6 Hz; H-6), 5.98 (d, J = 6.6 Hz; H-7), 6.77 (s; H-1'), 6.26 (s; H-7') for **3**, and δH 6.08 (d, J = 1.5 Hz; H-1), 6.56 (dd, J = 1.5, 6.9 Hz; H-6), 6.13 (d, J = 6.9 Hz; H-7), 6.74 (s; H-1'), 6.24 (s; H-7') for **4**, were generally similar to those of Aα and Aβ, but the signals at δH 2.61 (m; H-20), 4.45 (s; H-22α), 3.6 (br-s; HO-22β), 2.61 (m; H-20'), 4.51 (s; H-22'α), 3.6 (br-s; HO-22'β), δC 213.48 (s; C-21), 76.44 (d; C-22), 213.42 (s; C-21'), 76.40 (d; C-22') for **3**, and δH 2.60 (m; H-20), 4.49 (br-s; H-22α), 3.62 (br-s; HO-22β), 2.60 (m; H-20'), 4.51 (br-s; H-22'α), 3.62 (br-s; HO-22'β), δC 213.43 (s; C-21), 76.50 (d; C-22), 213.35 (s; C-21'), 76.27 (d; C-22') for **4**, showed that it consisted of two 22β-hydroxytingenone type triterpenes, one in quinoid form and the other in aromatic form. Analyses of their HMQC, HMBC and NOESY spectra analogously revealed that in them, the two units were linked together by two ether bondings as in **1** and **2**. Xuxuarine Bα (**3**) showed a Cotton effect curve similar to that of **1**, and Bβ (**4**) showed a curve similar to that of **2** in the CD spectra. Therefore, the two ether linkages of Bα and Bβ were of **1** type and **2** type, respectively.

Consequently, the structures of xuxuarines Bα (**3**) and Bβ (**4**) were determined to be a set of stereoisomeric triterpene dimers, each consisted of two 22β-hydroxytingenone type triterpenes, as shown below.



Xuxuarine Bα (**3**) Xuxuarine Bβ (**4**)

Figure 4. Structures of xuxuarines Bα (**3**) and Bβ (**4**).

Structures of xuxuarines Cα, Cβ, Dα and Dβ

All of xuxuarines Cα (**5**), Cβ (**6**), Dα (**7**) and Dβ (**8**) had the molecular formula $C_{56}H_{70}O_8$. The NMR spectral data, which were similar to those of Aα and Aβ suggested that each consisted of two triterpenes, one in quinoid form and the other in aromatic form, and the signal at δC 76.47 (d) for **5**, 76.35 (d) for **6**, 76.41 (d) for **7**, and 76.56 (d) for **8**, showed that one of the two had a β-hydroxy group at C-22. The MS spectral data determined whether the 22β-hydroxyl group was on the quinoid form triterpene or on the aromatic form triterpene: the fragmentation ion peaks at m/z 436, showing that C-22 hydroxy was on the quinoid form triterpene was observed in **5** and **6**, and those at m/z 420 and 452 suggested that C-22 hydroxy was on the aromatic form triterpene was observed in **7** and **8**. (Figure 5) The CD spectra revealed that **5** and **7** were of xuxuarine α type, and **6** and **8** of xuxuarine β type. Thus, the structures of these four triterpene dimers were clearly defined as shown in Figure 5.

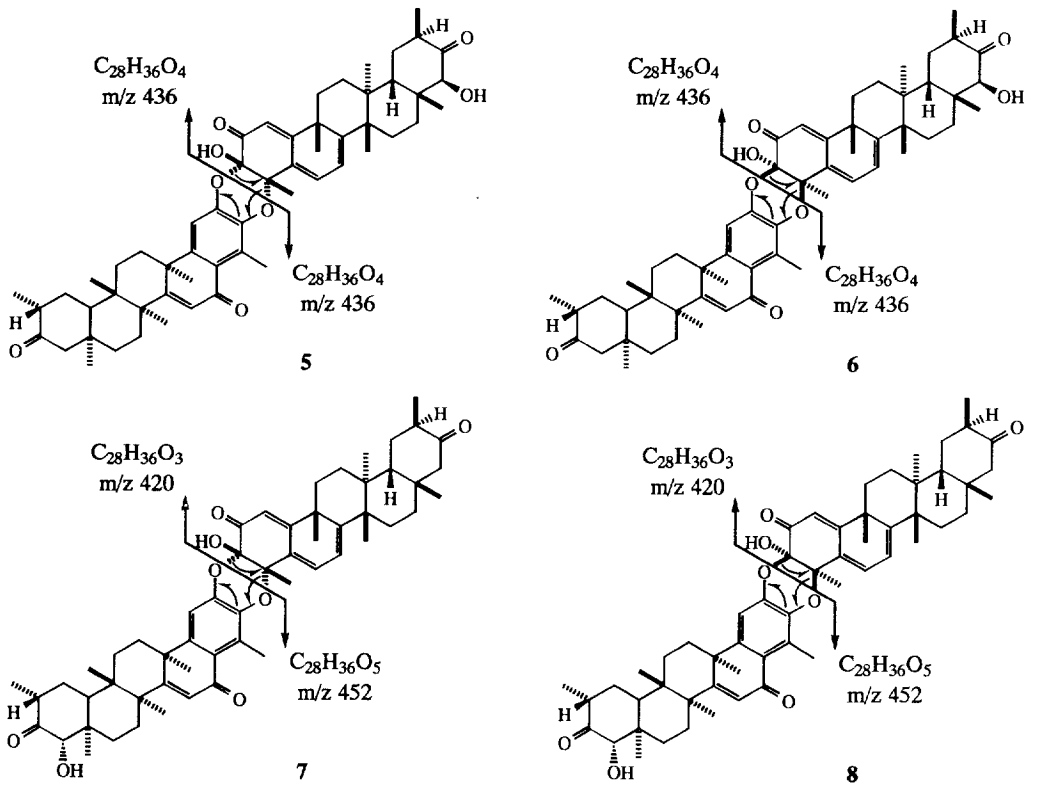


Figure 5. Structures and their MS spectra degradation patterns of **5** – **8**.

Structures of 7',8'-dihydroxuxuarine Aβ

Compound **9** was a triterpene dimer having the molecular formula $C_{56}H_{72}O_7$. In the NMR, δC 37.49 (t; C-7') and 41.90 (d; C-8), signals of aromatic triterpene unit suggested that C-7 to 8 double bond of the aromatic triterpene unit in **1** and **2** was saturated, in **9**. The fragmentation peaks at m/z 420 and 438 in the MS of **9** suggested this. The CD spectrum of **9** showed a Cotton effect curve similar to that of **2**. Therefore, the structure of **9** was decided to be 7',8'-dihydroxuxuarine Aβ. (Figure 6)

The complete assignments of the ^{1}H- and ^{13}C-NMR signals of all xuxuarines are shown in Tables 1 and 2, respectively. Assingments of all the ^{1}H- and ^{13}C-NMR signals were made by the HMBC and HMQC spectra.

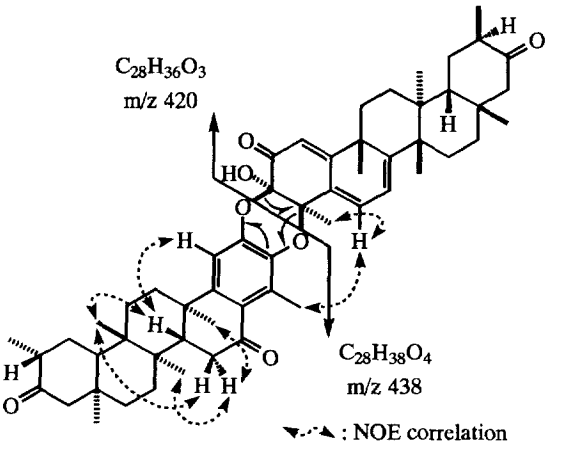
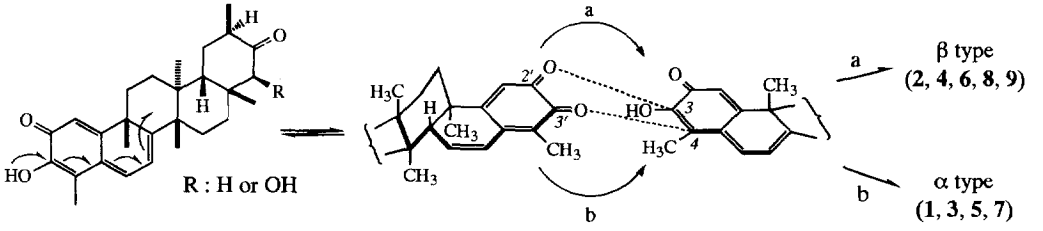


Figure 6. Structure of 7',8'-dihydroxyxuxuarine Aβ (**9**).

Biosynthetic mechanism for xuxuarines

A tentative route for the biosynthesis of xuxuarines is illustrated graphically in Scheme 1. Namely, a 2,3-diketon type triterpene is in an equilibrium state with its quinoid type one. One type molecule approaches to the other type molecule from above to form an adduct (by ortho-quinone Diels-Alder reaction) having a particular stereochemisty. Accordingly, these sets of stereoisomeric triterpene dimers are to be formed. It is interesting that some xuxuarines are prepared by combinations of tingenone and 22β-hydroxytingenone, that are of different oxidation stages.



Scheme 1. Proposed biosynthetic mechanism for xuxuarines α and β type.

Table 1. ^{1}H-NMR chemical shifts (ppm) for xuxuarines (**1 – 9**).

proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
H-1	6.06 (d, 1.2)	6.06 (d, 1.3)	6.08 (d, 1.4)	6.08 (d, 1.5)	6.11 (d, 1.4)	6.08 (d, 1.5)	6.08 (d, 1.4)	6.08 (d, 1.7)	6.10 (d, 1.6)
H-6	6.23 (dd, 1.2, 6.6)	6.52 (dd, 1.3, 6.9)	6.26 (dd, 1.4, 6.6)	6.56 (dd, 1.5, 6.9)	6.29 (dd, 1.4, 6.6)	6.56 (dd, 1.5, 6.9)	6.26 (dd, 1.4, 6.6)	6.55 (dd, 1.7, 6.9)	6.54 (dd, 1.6, 6.9)
H-7	5.94 (d, 6.6)	6.10 (d, 6.9)	5.98 (d, 6.6)	6.13 (d, 6.9)	6.00 (d, 6.6)	6.13 (d, 6.9)	5.97 (d, 6.6)	6.12 (d, 6.9)	6.13 (d, 6.9)
H-20	*2.44 (m)	*2.44 (m)	2.61 (m)	*2.60 (m)	2.63 (m)	2.61 (m)	2.44 (m)	2.46 (m)	2.49 (m)
H-22α	#2.85 (d, 14.3)	2.84 (d, 14.4)	4.45 (s)	4.49 (br-s)	4.48 (br-d,3.2)	4.49 (br-s)	2.82 (d, 14.4)	2.86 (d, 14.3)	2.88 (d, 14.2)
HO-22β	----	----	3.6 (br-s)	3.62 (br-s)	3.59 (d, 4.2)	3.63 (br-s)	----	----	----
Me-23	1.56 (s)	1.57 (s)	1.58 (s)	1.59 (s)	1.61 (s)	1.60 (s)	1.58 (s)	1.59 (s)	1.57 (s)
Mc-25	1.44 (s)	1.36 (s)	1.47 (s)	1.39 (s)	1.49 (s)	1.39 (s)	1.46 (s)	1.38 (s)	1.42 (s)
Me-26	1.22 (s)	1.21 (s)	1.25 (s)	1.24 (s)	1.27 (s)	1.25 (s)	1.24 (s)	1.23 (s)	1.27 (s)
Me-27	$0.96 (s)	0.96 (s)	0.98 (s)	0.96 (s)	1.00 (s)	0.96 (s)	0.96 (s)	0.95 (s)	0.99 (s)
Me-28	$0.96 (s)	0.96 (s)	0.81 (s)	#0.84 (s)	0.84 (s)	0.82 (s)	0.95 (s)	0.95 (s)	0.99 (s)
Me-30	¥0.92 (d, 6.5)	#0.92 (d, 6.6)	*1.02 (d, 6.2)	1.04 (d, 6.3)	1.05 (d, 6.2)	1.04 (d, 6.3)	0.95 (d, 6.2)	0.97 (d, 6.3)	0.97 (d, 6)
H-1'	6.76 (s)	6.73 (s)	6.77 (s)	6.74 (s)	6.80 (s)	6.75 (s)	6.77 (s)	6.74 (s)	6.60 (s)
H-7'	6.23 (s)	6.20 (s)	6.26 (s)	6.24 (s)	6.28 (s)	6.23 (s)	6.26 (s)	6.24 (s)	2.55, 2.60 (m)
H-20'	*2.42 (m)	*2.42 (m)	2.61 (m)	*2.60 (m)	2.48 (m)	2.44 (m)	2.62 (m)	2.59 (m)	2.48 (m)
H-22'α	#2.80 (d, 14.4)	2.84 (d, 14.4)	4.51 (s)	4.51 (br-s)	2.90 (d, 14.4)	2.87 (d, 14.5)	4.51 (br-s)	4.51 (br-s)	2.88 (d, 14.5)
HO-22'β	----	----	3.6 (br-s)	3.62 (br-s)	----	----	3.62 (br-s)	3.62 (br-s)	----
Me-23'	2.70 (s)	2.70 (s)	2.73 (s)	2.73 (s)	2.76 (s)	2.74 (s)	2.72 (s)	2.73 (s)	2.60 (s)
Me-25'	1.50 (s)	1.51 (s)	1.52 (s)	1.53 (s)	1.54 (s)	1.53 (s)	1.52 (s)	1.53 (s)	1.16 (s)
Me-26'	1.31 (s)	1.32 (s)	1.35 (s)	1.35 (s)	1.36 (s)	1.34 (s)	1.35 (s)	1.35 (s)	1.07 (s)
Me-27'	$0.95 (s)	0.93 (s)	0.96 (s)	0.99 (s)	0.98 (s)	0.99 (s)	0.97 (s)	0.99 (s)	1.24 (s)
Me-28'	$0.95 (s)	0.93 (s)	0.83 (s)	#0.81 (s)	1.00 (s)	0.99 (s)	0.83 (s)	0.84 (s)	0.99 (s)
Me-30'	¥0.94 (d, 6.5)	#0.94 (d, 6.6)	*1.01 (d, 6.2)	1.01 (d, 6.3)	0.98 (d, 6 2)	0.94 (d, 6.3)	1.01 (d, 6.2)	1.01 (d, 6.3)	0.99 (d, 6.6)

Measurements were performed in $CDCl_3$ at 400 MHz. Multiplicity and coupling constants (J/Hz) given in parenthesis.

*, #, $, ¥ : These sets of values may be interchangeable in each column.

Table 2. ^{13}C-NMR chemical shifts of xuxuarines (**1 – 9**).

C	1	2	3	4	5	6	7	8	9
1	115.53 (d)	114.96 (d)	115.57 (d)	115.02 (d)	115.60 (d)	115.05 (d)	115.51 (d)	115.02 (d)	115.14 (d)
2	190.09 (s)	189.34 (s)	190.18 (s)	189.43 (s)	190.26 (s)	189.45 (s)	190.16 (s)	189.42 (s)	189.45 (s)
3	91.96 (s)	91.13 (s)	92.05 (s)	91.15 (s)	92.26 (s)	91.17 (s)	92.01 (s)	91.16 (s)	91.19 (s)
4	79.25 (s)	76.82 (s)	79.36 (s)	76.83 (s)	79.43 (s)	76.90 (s)	79.32 (s)	76.89 (s)	76.85 (s)
5	130.20 (s)	132.01 (s)	130.29 (s)	132.00 (s)	130.38 (s)	132.15 (s)	130.21 (s)	132.12 (s)	132.31 (s)
6	126.35 (d)	128.44 (d)	126.52 (d)	128.56 (d)	126.64 (d)	128.61 (d)	126.48 (d)	128.55 (d)	128.39 (d)
7	116.09 (d)	117.13 (d)	116.24 (d)	117.21 (d)	116.34 (d)	117.28 (d)	116.16 (d)	117.24 (d)	117.19 (d)
8	160.23 (s)	163.06 (s)	160.12 (s)	162.85 (s)	160.18 (s)	162.95 (s)	160.33 (s)	163.12 (s)	163.23 (s)
9	41.52 (s)	43.51 (s)	41.54 (s)	43.59 (s)	41.60 (s)	43.52 (s)	41.61 (s)	43.49 (d)	43.65 (s)
10	173.35 (s)	172.63 (s)	173.56 (s)	172.65 (s)	173.72 (s)	172.78 (s)	173.55 (s)	172.75 (s)	172.71 (s)
11	33.14 (t)	32.99 (t)	34.48 (t)	33.23 (t)	33.48 (t)	33.32 (t)	33.17 (t)	33.08 (t)	33.16 (t)
12	29.67 (t)	*29.99 (t)	29.74 (t)	27.76 (t)	29.80 (t)	29.85 (t)	29.71 (t)	29.83 (t)	29.88 (t)
13	39.30 (s)	39.61 (s)	39.36 (s)	39.59 (s)	39.40 (s)	39.75 (s)	39.34 (s)	39.67 (s)	39.84 (s)
14	44.12 (s)	43.89 (s)	*43.96 (s)	43.42 (s)	43.98 (s)	43.67 (s)	44.19 (s)	43.97 (s)	44.00 (s)
15	28.18 (t)	#28.37 (t)	28.03 (t)	28.09 (t)	28.09 (t)	28.22 (t)	28.23 (t)	28.45 (t)	27.92 (t)
16	*35.45 (t)	$35.43 (t)	29.43 (t)	*29.50 (t)	29.48 (t)	29.47 (t)	35.35 (t)	35.45 (t)	*35.52 (t)
17	#38.04 (s)	38.02 (s)	44.78 (s)	#44.75 (s)	44.84 (s)	44.78 (s)	38.08 (s)	38.10 (s)	#38.22 (s)
18	$43.39 (d)	43.38 (d)	44.89 (d)	44.91 (d)	44.95 (d)	45.00 (d)	43.28 (d)	43.49 (d)	43.54 (d)
19	¥31.94 (t)	31.83 (t)	#32.03 (t)	31.84 (t)	32.08 (t)	31.91 (t)	32.00 (t)	31.91 (t)	31.96 (t)
20	41.69 (d)	41.75 (d)	40.76 (d)	40.76 (d)	40.80 (d)	40.83 (d)	41.76 (d)	41.83 (d)	$42.31 (d)
21	213.34 (s)	¥213.33 (s)	$213.48 (s)	$213.43 (s)	213.48 (s)	*213.44 (s)	213.50 (s)	*213.44 (s)	¥213.91 (s)
22	@52.41 (t)	@52.51 (t)	¥76.44 (d)	¥76.50 (d)	76.47 (d)	76.35 (d)	52.40 (t)	52.36 (t)	52.41 (t)
23	22.14 (q)	24.43 (q)	22.17 (q)	24.42 (q)	22.22 (q)	24.55 (q)	22.19 (q)	24.53 (q)	24.61 (q)
25	35.45 (q)	39.68 (q)	35.59 (q)	39.67 (q)	35.63 (q)	39.78 (q)	35.52 (q)	39.67 (q)	26.39 (q)
26	22.14 (q)	22.19 (q)	22.32 (q)	22.28 (q)	22.37 (q)	22.36 (q)	22.19 (q)	22.26 (q)	22.39 (q)
27	§19.79 (q)	§19.66 (q)	20.76 (q)	20.33 (q)	20.83 (q)	20.39 (q)	19.90 (q)	19.57 (q)	19.64 (q)
28	¶32.42 (q)	¶32.49 (q)	24.92 (q)	@24.95 (q)	24.97 (q)	24.94 (q)	32.46 (q)	32.46 (q)	@32.77 (q)
30	14.94 (q)	14.99 (q)	14.66 (q)	14.65 (q)	14.70 (q)	14.70 (q)	15.02 (q)	15.06 (q)	§15.17 (q)
1'	111.21 (d)	110.41 (d)	111.25 (d)	110.39 (d)	111.34 (d)	110.49 (d)	111.28 (d)	110.44 (d)	108.82 (d)
2'	144.68 (s)	145.14 (s)	144.69 (s)	145.12 (s)	144.74 (s)	145.18 (s)	144.68 (s)	145.18 (s)	145.38 (s)
3'	137.58 (s)	137.57 (s)	137.68 (s)	137.57 (s)	137.74 (s)	137.65 (s)	137.63 (s)	137.64 (s)	137.53 (s)
4'	127.65 (s)	128.38 (s)	127.82 (s)	128.47 (s)	127.91 (s)	128.56 (s)	127.74 (s)	128.55 (s)	129.69 (s)
5'	124.17 (s)	123.73 (s)	124.36 (s)	123.80 (s)	124.48 (s)	123.88 (s)	124.33 (s)	123.86 (s)	125.27 (s)
6'	187.41 (s)	186.99 (s)	187.44 (s)	187.04 (s)	187.56 (s)	187.09 (s)	187.50 (s)	187.07 (s)	200.06 (s)
7'	125.96 (d)	126.02 (d)	126.11 (d)	126.05 (d)	126.20 (d)	126.15 (d)	126.06 (s)	126.14 (d)	37.49 (t)
8'	170.46 (s)	170.06 (s)	170.21 (s)	169.88 (s)	170.50 (s)	170.09 (s)	170.30 (s)	169.88 (s)	41.90 (d)
9'	39.64 (s)	39.61 (s)	39.62 (s)	39.67 (s)	39.77 (s)	39.75 (s)	39.59 (s)	39.77 (s)	37.15 (s)
10'	150.34 (s)	151.04 (s)	150.40 (s)	151.04 (s)	150.51 (s)	151.14 (s)	150.36 (s)	151.11 (s)	152.32 (s)
11'	34.17 (t)	34.04 (t)	33.43 (t)	34.25 (t)	34.31 (t)	34.13 (t)	34.43 (t)	34.31 (t)	33.05 (t)
12'	30.03 (t)	*29.74 (t)	30.13 (t)	29.98 (t)	30.19 (t)	30.09 (t)	30.08 (t)	30.05 (t)	29.71 (t)
13'	40.07 (s)	40.07 (s)	40.11 (s)	40.04 (s)	40.21 (s)	40.16 (s)	40.07 (s)	40.10 (s)	39.53 (s)
14'	44.18 (s)	44.19 (s)	*43.93 (s)	43.91 (s)	44.32 (s)	44.29 (s)	43.94 (s)	43.97 (s)	40.04 (s)
15'	28.29 (t)	#28.30 (t)	28.15 (t)	28.09 (t)	28.43 (t)	28.39 (t)	28.11 (t)	28.15 (t)	28.56 (t)
16'	*35.31 (t)	$35.39 (t)	29.55 (t)	*29.39 (t)	35.56 (t)	35.52 (t)	29.51 (t)	29.56 (t)	*35.38 (t)
17'	#38.01 (s)	38.02 (s)	44.78 (s)	#44.71 (s)	38.18 (s)	38.12 (s)	44.75 (s)	44.81 (s)	#38.16 (s)
18'	$43.25 (d)	43.38 (d)	45.03 (d)	44.91 (d)	43.56 (d)	43.48 (d)	44.96 (d)	45.00 (d)	44.00 (d)
19'	¥31.85 (t)	31.83 (t)	#31.89 (t)	31.84 (t)	31.99 (t)	31.91 (t)	31.86 (t)	31.91 (t)	31.77 (t)
20'	41.69 (d)	41.75 (d)	40.76 (d)	40.76 (d)	41.86 (d)	41.85 (d)	40.75 (d)	40.83 (d)	$41.95 (d)
21'	213.34 (s)	¥213.22 (s)	$213.42 (s)	$213.35 (s)	213.48 (s)	*213.37 (s)	213.50 (s)	*213.29 (s)	¥213.32 (s)
22'	@52.31 (t)	@52.29 (t)	¥76.40 (d)	¥76.27 (d)	52.57 (t)	52.61 (t)	76.41 (d)	76.56 (d)	53.61 (t)
23'	12.82 (q)	13.06 (q)	12.89 (q)	13.09 (q)	12.94 (q)	13.14 (q)	12.89 (q)	13.12 (q)	13.21 (q)
25'	38.37 (q)	38.58 (q)	38.61 (q)	38.77 (q)	38.52 (q)	38.68 (q)	38.57 (q)	38.83 (q)	39.71 (q)
26'	20.65 (q)	20.72 (q)	20.84 (q)	20.85 (q)	20.78 (q)	20.81 (q)	20.81 (q)	20.90 (q)	14.97 (q)
27'	§19.52 (q)	§19.50 (q)	20.45 (q)	20.54 (q)	19.67 (q)	19.77 (q)	20.43 (q)	20.58 (q)	18.14 (q)
28'	¶32.40 (q)	¶32.38 (q)	24.98 (q)	@24.87 (q)	32.56 (q)	32.58 (q)	24.95 (q)	25.00 (q)	@32.52 (q)
30'	14.94 (q)	14.99 (q)	14.66 (q)	14.65 (q)	15.06 (q)	15.06 (q)	14.66 (q)	14.67 (q)	§15.11 (q)

Measurements performed in $CDCl_3$ at 100 MHz. Multiplicity given in parenthesis.

*, #, $, ¥, @, §, ¶ : These sets of values may be interchangeable in each column.

Cytotoxic activities of xuxuarines

Of these nine xuxuarines (Table 3), α type xuxuarines, i.e. **1**, **3**, **5**, **7** showed moderate cytotoxicities on cultured tumor cell lines P388 and L1210, but β type xuxuarines did not show any appreciable activity.

Table 3. Cytotoxic activity of xuxuarines against cultured cell lines.

	IC_{50} (mmol/l)		
	L1210	P388	KB
xuxuarine Aα (**1**)	0.094	0.060	>0.12
xuxuarine Aβ (**2**)	>0.12	>0.12	>0.12
xuxuarine Bα (**3**)	0.020	0.019	>0.12
xuxuarine Bβ (**4**)	>0.12	>0.12	>0.12
xuxuarine Cα (**5**)	0.092	0.059	>0.12
xuxuarine Cβ (**6**)	>0.12	>0.12	>0.12
xuxuarine Dα (**7**)	0.044	0.036	>0.12
xuxuarine Dβ (**8**)	0.070	>0.12	>0.12
7',8'-dihydroxuxuarine Aβ (**9**)	0.048	>0.12	>0.12

EXPERIMENTAL

General experimental procedures

Mp's were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. MS, UV, IR and CD spectra were taken with a VG Autospec spectrometer, a Hitachi 557 spectrophotometer, a Perkin Elmer 1710 spectrophotometer and a JASCO J-700 spectropolarimeter, respectively. Medium-pressure liquid chromatography (MPLC) was performed with a CIG column system (22 mm i.d. × 300 mm, Kusano Scientific Co., Tokyo) packed with 10 μm Silica gel or 20 μm ODS. HPLC was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm, GL Science Inc., Tokyo) packed with 10 μm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) and the spots were detected by heating after spraying with 10% H_2SO_4. 1D and 2D ^1H and ^{13}C NMR spectra were recorded on Bruker spectrometers (AM400 and AM500) at 303 K and processed on a Bruker data station with an Aspect 3000 computer. NOESY experiments were made with a mixing time of 0.6 s. The value of the delay to optimize one-bond correlation in the HMQC spectrum and suppress them in the HMBC spectrum were 3.2 msec, and the evolution delay for long-range couplings in the HMBC spectrum was set to 50 msec. The NMR coupling constants (J) were given in Hz.

Plant material

Dark reddish brown stem barks of *Maytenus chuchuhuasca* Raymond-Hamet et Colas (5 kg), commonly known as "xuxuá", were purchased in São Paulo, Brazil in 1992. The botanical identification was made by Dr. William Antonio Rodrigues (Instituto Nacional de Pesquisas da Amazonia). A voucher specimen has been deposited in the herbarium of the Tokyo College of Pharmacy.

Extraction and isolation

Crushed barks (5 kg) of *M. chuchuhuasca* Raymond-Hamet et Colas were extracted with hot MeOH (54 l) to give a MeOH extract (1.5 kg), which was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble fraction (155 g) was subjected to Silica gel cc using a CH_2Cl_2-ethyl acetate gradient system (1:0 - 0:1) to give twelve fractions. Cytotoxic fractions V (IC_{50} = 7.3 μg/ml; against P388) and VI (7.2 μg/ml) were further subjected to ODS MPLC first with CH_3CN - H_2O gradient system (8:2 or 7:3 - 1:0) to give pristimerin, tingenone and 22β-hydroxytingenone as active principles. Further elution of the ODS column with CH_3CN and then MeOH gave crude triterpene dimers fructions. Purification of these fractions by ODS HPLC with MeOH - H_2O (8:2, 7:3, etc) or CH_3CN - H_2O (7:3, etc) gave **1** - **9** as amorphous powders.

Bioassay

The extracts, fractions, and isolated compounds were routinely tested for their cytotoxicity by the MTT assay method[14] with slight modification.

Xuxuarine Aα (1)

Yellow amorphous powder; $[\alpha]_D$ +645.2° (c 0.61, $CHCl_3$); CD λ max (MeOH) nm (Δε), 357 (+19.4), 246 (-31.6); EI-MS / HR-MS m/z (%), 436 (M_{Tb}^+ + H, 67, calc. for $C_{28}H_{36}O_4$: 436.2614; found : 436.2610), 420 (M_{Tb}^+ + H, 63, calc. for $C_{28}H_{36}O_3$: 420.2664; found : 436.2638), 406 (16), 393 (5), 253 (20), 241 (37), 227 (25), 219 (30), 215 (28), 202 (100); FAB-MS m/z (%), 855 (M^+ + H, 14); IR ν max ($CHCl_3$) cm^{-1}, 3473, 1705, 1669, 1645, 1597, 1583, 1556; UV λ max (MeOH) nm (log ε), 206 (4.53), 222 (4.21), 252 (4.26), 296 (4.11), 379 (3.95); ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Reaction of Aα with TAI (trichloroacetyl isocyanate). : One drop of TAI was added to **1** (less than 1 mg) in an NMR sample tube, and mixed by shaking at once. By the reaction between the hydroxyl group in **1** and the isocyanate group of TAI, an amide proton signal (δ 8.45, s) in the ^{1}H-NMR gradually appeared, which was clearly confirmed after about 8 hours. There was no substantial change in the spectrum of this reaction mixture even after a week.

Preparation of methyl xuxuarine Aα. : 9.5 mg of **1** was dissolved in 1 ml of CH_3CN : MeOH (9 : 1) and treated with TMS-CHN_2[19] (2 eq.) and N,N-diisopropylethylamine for 18 hours at room temperature. The reaction mixture was partitioned between CH_2Cl_2 and H_2O, and the organic layer was concentrated. Then, the residue was chromatographed on Silica gel with hexane : AcOEt (62.5 : 27.5) to give 6.6 mg of Aα methyl ether.; IR ν max (CCl_4) cm^{-1}, 1713, 1686, 1654; ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 0.97 (3H, d, J = 6.3 Hz, Me-30)*, 0.98 (3H, s, Me-28)#, 0.98 (3H, s, Me-27)$, 0.99 (3H, d, J = 6.3 Hz, Me-30')*, 1.01 (3H, s, Me-28')#, 1.02 (3H, s, Me-27')$, 1.25 (3H, s, Me-26), 1.37 (3H, s, Me-26'), 1.46 (3H, s, Me-25), 1.57 (3H, s, Me-25'), 1.62 (3H, s, Me-23), 2.74 (3H, s, Me-23'), 2.84 (1H, d, J = 14.5 Hz, H-22α)¥, 2.91 (1H, d, J = 14.5 Hz, H-22'α)¥, 3.56 (3H, s, MeO-3), 5.94 (1H, d, J = 6.6 Hz, H-7), 5.97 (1H, d, J = 1.5 Hz, H-1), 6.18 (1H, dd, J =

1.5, 6.6 Hz, H-6), 6.29 (1H, s, H-7'), 6.91 (1H, s, H-1'), (*, #, $, ¥ : These sets of ascriptions may be interchangeable.); ^{13}C-NMR ($CDCl_3$, 100 MHz), δ 12.97 (q, C-23'), 15.06 (q, C-30)*, 15.10 (q, C-30')*, 19.74 (q, C-27)#, 19.94 (q, C-27')#, 20.76 (q, C-26'), 21.53 (q, C-23), 22.39 (q, C-26), 28.28 (t, C-15), 28.39 (t, C-15'), 29.78 (t, C-12), 30.07 (t, C-12'), 31.99 (t, C-21)$, 32.05 (t, C-21')$, 32.52 (q, C-28)¥, 32.58 (q, C-28')¥, 33.13 (t, C-11), 34.46 (t, C-11'), 35.43 (t, C-16)@, 35.51 (t, C-16')@, 35.56 (q, C-25), 38.19 (s × 2, C-17, 17'), 38.50 (q, C-25'), 39.29 (s, C-13), 39.78 (s, C-9'), 40.19 (s, C-13'), 40.97 (s, C-9), 41.89 (d × 2, C-20, 20'), 43.33 (d, C-18)§, 43.46 (d, C-18')§, 44.10 (s, C-14), 44.32 (s, C-14'), 52.48 (t, C-22)¶, 52.59 (t, C-22')¶, 53.21 (q, C-CH_3O), 79.82 (s, C-4), 95.03 (s, C-3), 110.89 (d, C-1'), 116.14 (d, C-7), 118.83 (d, C-1'), 124.60 (s, C-5'), 125.48 (d, C-6), 126.16 (d, C-7'), 127.97 (s, C-4'), 130.49 (s, C-5), 138.23 (s, C-3'), 144.30 (s, C-2'), 150.48 (s, C-10'), 159.44 (s, C-8), 169.67 (s, C-10), 170.58 (s, C-8'), 187.59 (s, C-6'), 192.47 (s, C-2), 213.58 (s, C-21)%, 213.70 (s, C-21')%, (*, #, $, ¥, @, §, ¶, % : These sets of ascriptions may be interchangeable.).

Xuxuarine Aβ (2)

Yellow amorphous powder; $[\alpha]_D$ -512.6° (c 0.40, $CHCl_3$); CD λ max (MeOH) nm (Δε), 397 (-12.2), 331 (+12.3), 261 (-60.0); EI-MS m/z (%), 436 (M_{Tb}^+ + H, 95), 421 (M_{Tb}^+ + 2H, 100), 420 (M_{Tb}^+ + H, 58), 406 (59), 391 (8), 253 (27), 241 (42), 227 (61), 215 (31), 201 (75); FAB-MS / HR-MS m/z (%), 855 (M^+ + H, 36, calc. for $C_{56}H_{71}O_7$: 855.5200; found : 855.5165); IR ν max ($CHCl_3$) cm^{-1}, 3467, 1704, 1667, 1647, 1597, 1583, 1564; UV λ max (MeOH) nm (log ε), 206 (4.55), 222 (4.23), 252 (4.25), 298 (4.12), 384 (4.06); ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Reaction of Aβ with TAI (trichloroacetyl isocyanate). : By the reaction between **2** and TAI, as in **1**, one TAI amide proton signal (δ 8.46, s) appeared in the ^{1}H-NMR spectrum.

Preparation of methyl xuxuarine Aβ : By the method described for the preparation of methyl xuxuarine Aα, from 9.7 mg of **2**, 7.2 mg of Aβ methyl ether was obtained.; IR ν max (CCl_4) cm^{-1}, 1713, 1683, 1654 ; ^{1}H-NMR ($CDCl_3$, 400 MHz), δ 0.99 (3H, d, J = 6.7 Hz, Me-30), 1.00 (3H, s × 2, Me-28, 28')*, 1.0 (3H, d, J = 6.7 Hz, Me-30'), 1.01 (3H, s × 2, Me-27, 27')*, 1.28 (3H, s, Me-26), 1.37 (3H, s, Me-26'), 1.48 (3H, br-s, Me-25), 1.59 (3H, s × 2, Me-23, 25'), 2.53 (3H, br-s, Me-23'), 2.91 (2H, d, = 14.5 Hz, H-22α, 22'α), 3.54 (3H, br-s, MeO-3), 5.86 (1H, br-s, H-1), 6.09 (1H, d, J = 6.6 Hz, H-7), 6.25 (1H, s, H-7'), 6.55 (1H, br-d, J = 6.4 Hz, H-6), 7.03 (1H, br-s, H-1'), (* : These sets of ascriptions may be interchangeable.); ^{13}C-NMR ($CDCl_3$, 100 MHz), δ 13.10 (q, C-23'), 15.11 (q × 2, C-30, 30'), 19.74 (q, C-27)*, 19.98 (q, C-27')*, 20.82 (q, C-26'), 22.45 (q, C-26), 28.39 (t × 2, C-15, 15'), 29.80 (t, C-12)#, 30.18 (t, C-12')#, 31.98 (t, C-19)$, 32.07 (t, C-19')$, 32.52 (q, C-28)¥, 32.60 (q, C-28')¥, 33.07 (t, C-11), 34.35 (t, C-11'), 35.51 (t, C-16)@, 35.54 (t, C-16')@, 38.19 (s, × 2, C-17, 17'), 38.23 (q, C-25'), 38.23 (s, C-13)§, 39.31 (s, C-9')§, 39.88 (s, C-13')§, 40.19 (s, C-9)§, 41.89 (d × 2, C-20, 20'), 43.41 (d, C-18)¶, 43.52 (d, C-18')¶, 44.19 (s, C-14)%, 44.32 (s, C-14')%, 52.53 (t, C-22)!, 52.62 (t, C-22')!, 52.71 (q, CH_3O-3), 77 (s, overlapped with solvent peak, C-4), 91 (s, broadened, C-3), 110.36 (d, C-1'), 116.17 (d, broadened, C-7), 117.78 (d, C-1), 124.17 (s, C-5'), 126.14 (d, C-7'), 127.59 (s, C-6), 128.74 (d, C-4'), 131.92 (s, broadened, C-5), 138.16 (s, C-3'), 143.95 (s, broadened, C-2'), 151.01 (s, C-10'), 161.11 (s, broadened, C-8), 169.34 (s, broadened, C-10), 170.32 (s, C-8'), 187.31 (s, C-6'), 191.74 (s, C-2), 213.61 (s × 2, C-21, 21'), (*, #, $, ¥, @, §, ¶, %, ! : These sets of ascriptions may be interchangeable.).

Xuxuarine Bα (3)

Yellow amorphous powder; $[\alpha]_D$ +647.0° (c 0.63, $CHCl_3$); CD λ max (MeOH) nm (Δε), 359 (+21.2),

246 (-34.9); EI-MS m/z (%), 452 (M_{Tb}^+ + H, 19), 437 (M_{Ta}^+ + 2H, 41), 436 (M_{Ta}^+ +H, 79), 434 (18), 422 (17), 253 (24), 241 (53), 227 (25), 215 (22), 214 (21), 201 (100); FAB-MS / HR-MS m/z(%), 887 (M^+ + H, 53, calc. for $C_{56}H_{71}O_7$: 887.5098; found : 887.5146); IR ν max ($CHCl_3$) cm^{-1}, 3475, 1707, 1669, 1646, 1598, 1583, 1555; UV λ max (MeOH) nm (log ε), 206 (4.59), 221 (4.26), 252 (4.30), 294 (4.13), 378 (4.00), ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Xuxuarine Bβ (4)

Yellow amorphous powder; $[\alpha]_D$ -523.3° (c 0.41, $CHCl_3$); CD λ max (MeOH) nm (Δε), 397 (-10.0), 331 (+9.7), 262 (-48.2); EI-MS m/z (%), 452 (M_{Tb}^+ + H, 94), 437 (M_{Ta}^+ + 2H, 100), 436 (M_{Ta}^+ + H, 72), 422 (38), 253 (35), 241 (55), 227 (49), 217 (35), 215 (34), 201 (100); FAB-MS / HR-MS m/z(%), 887 (M^+ + H, 20, calc. for $C_{56}H_{71}O_7$: 887.5098; found : 887.5119); IR ν max ($CHCl_3$) cm^{-1}, 3473, 1728, 1708, 1647, 1597, 1583, 1564; UV λ max (MeOH) nm (log ε), 206 (4.48), 221 (4.15), 252 (4.16), 297 (4.03), 386 (3.97); ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Xuxuarine Cα (5)

Yellow amorphous powder; $[\alpha]_D$ +654.0° (c 0.43, $CHCl_3$); CD λ max (MeOH) nm (Δε), 358 (+19.7), 246 (-32.3); EI-MS / HR-MS m/z (%), 437 (M_{Ta}^+ + 2H and M_{Tb}^+ + 2H, 39), 436 (M_{Ta}^+ + H and M_{Tb}^+ + H, 100, calc. for $C_{28}H_{36}O_4$: 436.2614; found : 436.2574), 421 (59), 253 (25), 241 (48), 227 (45), 215 (32), 201 (86); FAB-MS m/z(%), 887 (M^+ + H, 29); IR ν max ($CHCl_3$) cm^{-1}, 3475, 1706, 1669, 1645, 1597, 1583, 1555; UV λ max (MeOH) nm (log ε), 206 (4.56), 221 (4.24), 252 (4.28), 294 (4.12), 379 (3.97), ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Xuxuarine Cβ (6)

Yellow amorphous powder; $[\alpha]_D$ -505.4° (c 0.71, $CHCl_3$); CD λ max (MeOH) nm (Δε), 397 (-14.2), 331 (+13.9), 262 (-67.0); EI-MS / HR-MS m/z (%), 437 (M_{Ta}^+ + 2H and M_{Tb}^+ + 2H, 34), 436 (M_{Ta}^+ + 2H and M_{Tb}^+ + 2H, 82, calc. for $C_{28}H_{36}O_4$: 436.2614; found : 436.2619), 421 (63), 406 (16), 253 (31), 241 (52), 227 (50), 215 (30), 201 (100); FAB-MS m/z(%), 871 (M^+ + H, 28); IR ν max ($CHCl_3$) cm^{-1}, 3472, 1706, 1647, 1598, 1583, 1564; UV λ max (MeOH) nm (log ε), 206 (4.61), 221 (4.28), 252 (4.29), 296 (4.16), 384 (4.10); ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Xuxuarine Dα (7)

Yellow amorphous powder; $[\alpha]_D$ +554.2° (c 0.38, $CHCl_3$); CD λ max (MeOH) nm (Δε), 358 (+21.7), 246 (-35.1); EI-MS / HR-MS m/z (%), 452 (M_{Tb}^+ + H, 60, calc. for $C_{28}H_{36}O_5$: 452.2563; found : 452.2736), 437 (51), 420 (M_{Ta}^+ + H, 57, calc. for $C_{28}H_{36}O_3$: 420.2664; found : 436.2547), 406 (48), 253 (30), 241 (48), 227 (72), 215 (39), 201 (100); FAB-MS m/z(%), 871 (M^+ + H, 13); IR ν max ($CHCl_3$) cm^{-1}, 3476, 1706, 1668, 1645, 1600, 1583, 1551; UV λ max (MeOH) nm (log ε), 205 (4.56), 222 (4.25), 251 (4.30), 295 (4.13), 378 (4.00), ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

Xuxuarine Dβ (8)

Yellow amorphous powder; $[\alpha]_D$ -517.0° (c 0.74, $CHCl_3$); CD λ max (MeOH) nm (Δε), 397 (-13.1), 331 (+12.7), 262 (-62.7); EI-MS / HR-MS m/z (%), 452 (M_{Tb}^+ + H, 14, calc. for $C_{28}H_{36}O_5$: 452.2563; found :

452.2581), 437 (13), 420 (M_{Ta}^+ + H, 38, calc. for $C_{28}H_{36}O_3$: 420.2664; found : 420.2711), 406 (11), 253 (11), 241 (30), 227 (20), 215 (16), 202 (100), 201 (87); FAB-MS m/z(%), 871 (M^+ + H, 28); IR ν max ($CHCl_3$) cm^{-1}, 3472, 1706, 1647, 1598, 1583, 1564; UV λ max (MeOH) nm (log ε), 206 (4.57), 221 (4.18), 252 (4.26), 297 (4.13), 384 (4.07); ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

7',8'-dihydroxyxuxuarine Aβ (9)

Yellow amorphous powder; $[\alpha]_D$ -562.1° (c 0.29, $CHCl_3$); CD λ max (MeOH) nm (Δε), 395 (-14.1), 318 (+11.7), 254 (-39.5), 232 (+24.4), 213 (-15.4); EI-MS / HR-MS m/z (%), 438 (M_{Tb}^+ + H, 39, calc. for $C_{28}H_{38}O_4$: 438.2770; found : 438.2767), 421 (M_{Ta}^+ + 2H, 39), 420 (M_{Ta}^+ + H, 100, calc. for $C_{28}H_{36}O_3$: 420.2664; found : 420.2663), 253 (22), 241 (55), 201 (63); FAB-MS m/z(%), 856 (M^+ + H, 20); IR ν max ($CHCl_3$) cm^{-1}, 3469, 1704, 1666, 1635, 1596; UV λ max (MeOH) nm (log ε), 208 (4.42), 233 (4.33), 276 (4.16), 312 (3.85), 383 (4.04); ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 2.

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