Five New Triterpene Dimers from *Maytenus chuchuhuasca*

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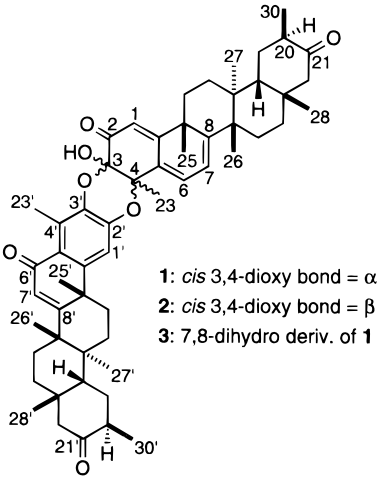
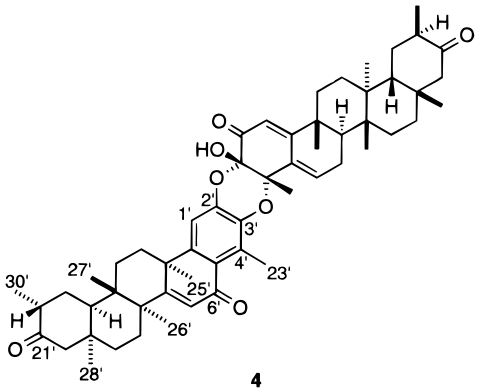
Five new triterpene dimers, named isoxuxuarines Aα (**1**), Aβ (**2**), 7,8-dihydroisoxuxuarine Aα (**3**), 7,8-dihydroxuxuarine Aα (**4**), and xuxuarine Eβ (**5**), were isolated from the South American medicinal plant "xuxuá" (*Maytenus chuchuhuasca*). Their structures, representative of regiochemical and stereochemical isomers, were determined on the basis of spectroscopic evidence including CD spectral studies.

As a part of our studies on medicinal plants belonging to the genus *Maytenus* (Celastraceae),[1−13] which are widely used as folk medicines in South America,[14,15] we have previously reported nine novel triterpene dimers,[10,11] named xuxuarines, from the Brazilian medicinal plant "xuxuá" (*Maytenus chuchuhuasca* Raymond−Hamet et Colas).[15,16] Triterpene dimers of this class have been isolated from only five plants in the Celastraceae, *Rzedowskia tolantonguensis*,[17] *Maytenus ilicifolia*,[1,13] *M. umbellata*,[18] *M. chuchuhuasca*,[10,11] and *M. scutiodes*,[19] and have been studied by only two groups, González *et al.*[17−19] and our own group.[1,10,11,13] Most of these compounds were found to be composed of one quinoid unit and one aromatic unit, of triterpenes derived from pristimerin, tingenone, and/or their congeners, and joined by two ether linkages formed between the two A rings, like the xuxuarines.[10,11]

To obtain additional examples of this compound class, we further investigated the remaining fractions of *M. chuchuhuasca* bark containing minor constituents; these efforts resulted in the isolation of five additional triterpene dimers, isoxuxuarines Aα (**1**), Aβ (**2**), 7,8-dihydroisoxuxuarine Aα (**3**), 7,8-dihydroxuxuarine Aα (**4**), and xuxuarine Eβ (**5**). In the present paper, we report the isolation and the structure elucidation of these five new triterpene dimers, consisting of regiochemical and stereochemical isomers, using several spectroscopic methods.





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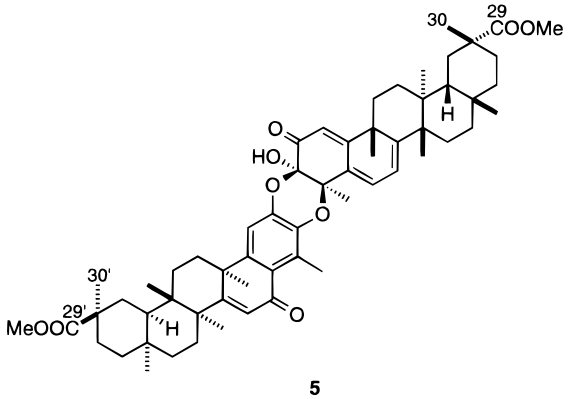

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Results and Discussion

From the methylene chloride-soluble portion of a MeOH extract of *M. chuchuhuasca* (bark; 5 kg), 12 fractions were derived by Si gel column chromatography using a CH_2Cl_2−EtOAc gradient system (1:0−0:1). Purification ODS−HPLC of the remaining portion of fractions V and VI, from which the α and β types of xuxuarines A−D had been previously isolated,[10,11] led to the isolation of four triterpene dimers, isoxuxuarines Aα (**1**: 0.0020% w/w), Aβ (**2**: 0.0003% w/w), 7,8-dihydroisoxuxuarine Aα (**3**: 0.0016% w/w) and 7,8-dihydroxuxuarine Aα (**4**: 0.0001% w/w). Furthermore, fraction IV was separated by MPLC on Si gel, and the derived fractions were further purified by ODS−HPLC to obtain the triterpene dimer, xuxuarine Eβ (**5**: 0.0003% w/w).

Compounds **1** and **2** were each obtained as yellow amorphous solids, with $[\alpha]_D$ +520.5° (*c* 0.16, $CHCl_3$) for **1** and $[\alpha]_D$ −514.5° (*c* 0.12, $CHCl_3$) for **2**. The FABMS of both **1** and **2** gave $[M + H]^+$ ion peaks at *m/z* 855,

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Table 1. Typical ^{1}H-NMR Chemical Shifts (ppm, Multiplicity, and *J*/Hz) for Compounds **1**−**5**[a]

position	**1**		**2**		**3**		**4**		**5**	
H-1	6.13	(d, 1.6)	6.14	(d, 1.7)	6.01	(s)	6.01	(s)	6.08	(d, 1.5)
H-6	6.28	(dd, 1.6, 6.5)	6.61	(dd, 1.7, 7.0)	6.30	(br s)	6.31	(br s)	6.52	(dd, 1.5,7.0)
H-7	5.92	(d, 6.5)	6.13	(d, 7.0)	2.05	(m)	2.18	(m)	6.08	(d, 7.0)
					2.19	(m)	2.24	(m)		
H-19α									2.39	(d, 15.6)
H-20	2.48	(m)	2.49	(m)	2.50	(m)	2.52	(m)		
H-22α	2.83	(d, 14.3)	2.89	(d, 14.3)	2.87	(d, 14.4)	2.87	(d, 14.0)		
Me-23	1.59	(s)	1.58	(s)	1.49	(s)	1.51	(s)	1.58	(s)
Me-25	1.48	(s)	1.49	(s)	1.12	(s)	1.13	(s)	1.38	(s)
Me-26	1.26	(s)	1.29	(s)	1.04	(s)	1.06	(s)	1.17	(s)
Me-27	0.99	(s)	0.98	(s)	1.23	(s)	1.27	(s)	0.53	(s)
Me-28	0.97	(s)	0.99[b]	(s)	0.95	(s)	0.99	(s)	1.06[b]	(s)
Me-30	0.98	(d, 7.0)	1.00	(d, 7.0)	0.99	(d, 6.4)	1.00	(d, 6.7)	1.16[c]	(s)
COOMe									3.59	(s)
H-1′	7.03	(s)	6.98	(s)	6.99	(s)	6.84	(s)	6.74	(s)
H-7′	6.25	(s)	6.25	(s)	6.26	(s)	6.29	(s)	6.21	(s)
H-19′α									2.39	(d, 15.6)
H-20′	2.51	(m)	2.49	(m)	2.50	(m)	2.49	(m)		
H-22′α	2.92	(d, 14.4)	2.91	(d, 14.5)	2.91	(d, 14.4)	2.91	(d, 14.3)		
Me-23′	2.50	(s)	2.45	(s)	2.55	(s)	2.73	(s)	2.73	(s)
Me-25′	1.61	(s)	1.62	(s)	1.61	(s)	1.58	(s)	1.48	(s)
Me-26′	1.39	(s)	1.37	(s)	1.39	(s)	1.37	(s)	1.26	(s)
Me-27′	1.07	(s)	1.00	(s)	1.06	(s)	1.00	(s)	0.55	(s)
Me-28′	1.02	(s)	1.12[b]	(s)	1.02	(s)	1.01	(s)	1.09[b]	(s)
Me-30′	0.99	(d, 6.2)	1.00	(d, 7.0)	0.99	(d, 6.4)	0.99	(d, 6.7)	1.17[c]	(s)
COOMe									3.48	(s)

[a] All measurements were performed in $CDCl_3$ at 400 MHz, 300 K. [b,c] Assignments for values in each compound bearing the same superscript may be reversed.

Table 2. ^{13}C-NMR Chemical Shifts (ppm and multiplicity) for Compounds **1**−**5**[a]

	1				**2**				**3**				**4**				**5**			
position	quinoid		aromatic		quinoid		aromatic		quinoid		aromatic		quinoid		aromatic		quinoid		aromatic	
C-1	115.9	(d)	110.4	(d)	115.2	(d)	110.7	(d)	113.1	(d)	110.4	(d)	112.6	(d)	111.4	(d)	114.7	(d)	110.6	(d)
C-2	190.3	(s)	144.5	(s)	189.6	(s)	144.3	(s)	191.4	(s)	144.5	(s)	191.4	(s)	144.6	(s)	189.4	(s)	145.2	(s)
C-3	91.9	(s)	138.4	(s)	90.7	(s)	138.5	(s)	91.3	(s)	138.3	(s)	91.5	(s)	137.7	(s)	91.0	(s)	137.5	(s)
C-4	79.4	(s)	129.5	(s)	77.1[j]	(s)	128.4[b]	(s)	79.5	(s)	129.4	(s)	79.5	(s)	127.8	(s)	77.3[j]	(s)	128.3	(s)
C-5	130.7	(s)	123.2	(s)	132.4	(s)	124.0	(s)	134.3	(s)	123.3	(s)	133.6	(s)	124.6	(s)	131.8	(s)	123.8	(s)
C-6	125.9	(d)	187.2	(s)	128.4[b]	(d)	187.6	(s)	133.7	(d)	187.0	(s)	134.4	(d)	187.7	(s)	128.8	(d)	187.4	(s)
C-7	116.1	(d)	126.1	(d)	116.9	(d)	126.1	(d)	24.1	(t)	126.2	(d)	24.3	(t)	126.2	(d)	117.2	(d)	126.1	(d)
C-8	160.3	(s)	170.4	(s)	163.6	(s)	170.4	(s)	41.1	(d)	170.1	(s)	41.5	(d)	170.7	(s)	164.4	(s)	171.2	(s)
C-9	41.4	(s)	39.9	(s)	43.9	(s)	39.8	(s)	37.3	(s)	39.9	(s)	37.5	(s)	39.8	(s)	43.9	(s)	40.0	(s)
C-10	173.0	(s)	151.8	(s)	173.1	(s)	151.1	(s)	169.6	(s)	151.8	(s)	170.4	(s)	150.4	(s)	173.2	(s)	151.1	(s)
C-11	33.2	(t)	34.3	(t)	33.1	(t)	34.3	(t)	30.5	(t)	34.4	(t)	30.5	(t)	34.4	(t)	32.8	(t)	34.0	(t)
C-12	29.7	(t)	30.1	(t)	29.9	(t)	30.3	(t)	29.3	(t)	30.1	(t)	29.4	(t)	30.2	(t)	29.8[b]	(t)	29.8[b]	(t)
C-13	39.4	(s)	40.2	(s)	39.9	(s)	40.2	(s)	40.0[b]	(s)	40.1[b]	(s)	40.1[b]	(s)	40.2[b]	(s)	38.6	(s)	39.0	(s)
C-14	44.2	(s)	44.3	(s)	44.0	(s)	44.3	(s)	40.2[b]	(s)	44.3	(s)	40.2[b]	(s)	44.4	(s)	44.4	(s)	44.7	(s)
C-15	28.2	(t)	28.3	(t)	28.5	(t)	28.4	(t)	27.9	(t)	28.4	(t)	28.0	(t)	28.4	(t)	28.6[c]	(t)	28.5[c]	(t)
C-16	35.4[b]	(t)	35.4[b]	(t)	35.5[c]	(t)	35.6[c]	(t)	35.3	(t)	35.5	(t)	35.3	(t)	35.6	(t)	36.4	(t)	36.4	(t)
C-17	38.2	(s)	30.2	(s)	38.2[d]	(s)	38.2[d]	(s)	38.1	(s)	38.2	(s)	38.1[e]	(s)	38.2[e]	(s)	30.5[d]	(s)	30.5[d]	(s)
C-18	43.4	(d)	43.3	(d)	43.5	(d)	43.5	(d)	43.8	(d)	43.4	(d)	43.9	(d)	43.5	(d)	44.2	(d)	44.2	(d)
C-19	32.1	(t)	32.0	(t)	31.9[e]	(t)	32.0[e]	(t)	31.8[c]	(t)	32.0[c]	(t)	31.8	(t)	32.0	(t)	30.9[e]	(t)	30.8[e]	(t)
C-20	41.8[c]	(d)	41.9[c]	(d)	41.9[f]	(d)	41.9[f]	(d)	42.2	(d)	41.9	(d)	42.3	(d)	41.9	(d)	40.5[f]	(s)	40.4[f]	(s)
C-21	213.8	(s)	213.8	(s)	213.7	(s)	213.6	(s)	213.9	(s)	213.6	(s)	213.9	(s)	213.6	(s)	29.8[b]	(t)	29.5[b]	(t)
C-22	52.3	(t)	52.6	(t)	52.4	(t)	52.6	(t)	53.4	(t)	52.6	(t)	53.5	(t)	52.6	(t)	35.0[g]	(t)	34.7[g]	(t)
C-23	22.0	(q)	13.2	(q)	24.2	(q)	12.8	(q)	22.7	(q)	13.4	(q)	22.8[d]	(q)	13.0	(q)	24.6	(q)	13.2	(q)
C-25	35.5	(q)	38.6	(q)	40.0	(q)	38.9	(q)	22.8	(q)	38.7	(q)	22.8[d]	(q)	38.5	(q)	39.2	(q)	37.7	(q)
C-26	22.3	(q)	20.7	(q)	22.4	(q)	20.9	(q)	15.7	(q)	20.8	(q)	15.7	(q)	20.8	(q)	22.3	(q)	20.9	(q)
C-27	19.8	(q)	20.0	(q)	19.7[g]	(q)	19.7[g]	(q)	18.1	(q)	19.8	(q)	18.2	(q)	19.7	(q)	18.2	(q)	18.4	(q)
C-28	32.4	(q)	32.5	(q)	32.5[h]	(q)	32.6[h]	(q)	32.6[d]	(q)	32.7[d]	(q)	32.7	(q)	32.6	(q)	31.6[h]	(q)	31.5[h]	(q)
C-29																	179.1[i]	(s)	178.8[i]	(s)
C-30	15.0[d]	(q)	15.0[d]	(q)	15.14	(q)	15.14	(q)	15.1[e]	(q)	15.2[e]	(q)	15.2	(q)	15.1	(q)	32.9	(q)	32.7	(q)
COOMe																	51.6	(q)	51.4	(q)

[a] All measurements were performed in $CDCl_3$ at 100 MHz, 300 K. [b−i] Assignments for values in each compound bearing the same superscript may be reversed. [j] Signals bearing this superscript were superimposed on solvent signals.

and their identical molecular formulas were shown to be $C_{56}H_{70}O_7$, based on HRFABMS analysis. The IR absorptions at 3443 cm^{-1} for **1** and 3445 cm^{-1} for **2** were attributed to one free hydroxyl group in each isomer. Their ^{1}H- and ^{13}C-NMR spectra suggested that these two compounds were triterpene dimers each composed of two tingenone-type triterpenes, one in the quinoid form and the other in the aromatic form, resembling xuxuarines Aα and A*β*.[10,11] Analysis of their HMQC and HMBC spectra enabled assignments of the signals of each quinoid and aromatic triterpene unit, including the signals at C-3 (δ_C 91.9 for **1**, 90.7 for **2**) and C-4 (δ_C 79.4 for **1**, 77.1 for **2**) in the 3-hydroxy-4-methyl-3,4-dioxy part of the quinoid unit, as shown in Tables 1 and 2. Structural differences between **1** and **2** and the xuxuarine A series appeared in the type of connection of the two triterpene units. It was suspected that the 3,4-dioxy bond in both **1** and **2** consisted of C-3−C-3′ and C-4−

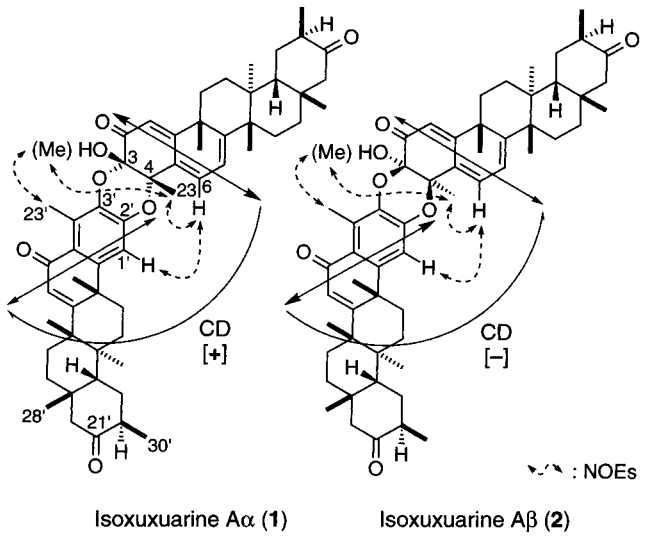

Figure 1. NOE correlations and CD exciton couplings for isoxuxuarins Aα (**1**) and A*β* (**2**).

C-2′ bonds, differing from the α and *β* types of the xuxuarines that incorporate C-3−C-2′ and C-4−C-3′ bonds. The NOESY spectrum of their methyl derivative showed NOE correlations between the introduced methoxy methyl protons at C-3 and the H-23′ methyl protons, and between the methoxy methyl protons and H-23 methyl protons (Figure 1). Although these spectral patterns were different from those of the α and *β* types of the xuxuarines, they were similar to that of cangorosin B, which has C-3−C-3′ and C-4−C-2′ bonds.[13] Thus, both **1** and **2** were shown to represent the reverse conjugated types of xuxuarines Aα and A*β*. The CD spectrum of **1** showed a positive first maximum value at 341 nm, similar to the α type of xuxuarines and cangorosin B, while that of **2** showed a negative first maximum value at 394 nm and a positive second maximum value at 338 nm, similar to the *β* type of xuxuarines.[10,11,13] Considering this spectroscopic evidence, compounds **1** and **2** were assigned as isoxuxuarines Aα and A*β*, respectively.

Compounds **3** and **4** were obtained as pale yellow amorphous solids with $[\alpha]_D$ +308.8° (*c* 0.44, $CHCl_3$) for **3** and $[\alpha]_D$ +226.4° (*c* 0.11, $CHCl_3$) for **4**. Both **3** and **4** exhibited $[M + H]^+$ ion peaks at *m*/z 857 in the FABMS, and their identical molecular formula, $C_{56}H_{72}O_7$, was established by HRFABMS. The presence of one free hydroxyl group in each molecule was suggested by their IR spectra. Their ^{1}H- and ^{13}C-NMR spectra showed that both **3** and **4** were triterpene dimers composed of two tingenone-type units, but were different from those of the xuxuarine A and isoxuxuarine A types. The breadth of the signal assignable to the H-6 methine proton (δ_H 6.30 for **3**, 6.31 for **4**) and the disappearance of the H-7 methine proton signal from the lowfield region were observed in the ^{1}H-NMR spectra of **3** and **4**. Correspondingly, the disappearance of two olefinic carbons and the appearance of substituted signals (δ_C 24.13, t, and 41.14, d, for **3**; 24.26, t, and 41.46, d, for **4**) were observed in their ^{13}C-NMR spectra. These results suggested that the conjugated ketone system in the A and B rings of the quinoid triterpene unit in each molecule was partially saturated between C-7 and C-8. The triterpene units were finally shown to be identical for **3** and **4** from the analysis of their HMQC and HMBC spectra. Accordingly, the structural differences between **3** and **4** refer to the conjugated form of their constituting triterpene units. The chemical shift value of the signal assignable to H-23′ (δ_H 2.55) of **3** did not match that of **4** (δ_H 2.73), but it was closely comparable to data for **1**, **2**, and cangorosin B (δ_H 2.5) with C-3−C-3′ and C-4−C-2′ linkages, while the value of **4** agreed with data for xuxuarines A−D (δ_H 2.7) with C-3−C-2′ and C-4−C-3′ linkages. Furthermore, the chemical shift of signals assignable to C-4, C-23, and H-6, appeared at δ_C 79.5 (s), 22.7 (q), and δ_H 6.30 (br s) for **3** and δ_C 79.5 (s), 22.8 (q), and δ_H 6.31 (br s) for **4**, and resembled those of **1** and the α type of xuxuarines (δ_C 79 for C-4, δ_C 22 for C-23, and δ_H 6.3 for H-6), rather than those of **2** and the *β* type of xuxuarines (δ_C 77 for C-4, δ_C 24 for C-23, and δ_H 6.5 for H-6). These diagnostic NMR chemical shifts were authenticated by the NOESY (or ROESY) spectra of the methyl derivatives and CD spectra. In the NOESY spectrum of *O*-methylated **3**, NOE correlations between the introduced methoxy methyl protons on C-3 and the H-23′ methyl protons permitted the assignment of the configuration of the connection between two units to be the isoxuxuarine type (reverse-conjugated type with C-3−C-3′ and C-4−C-2′ bonds). Also, the ROESY spectrum of *O*-methylated **4** showed correlations between the introduced methoxy methyl protons on C-3 and the H-1′ methine proton, which resulted from linkages of the C-3−C-2′ and C-4−C-3′ bonds. In addition, the CD spectra of both **3** and **4** showed positive first Cotton effects (326 nm, sh, for **3**; 321 nm, sh, for **4**), proving an α orientation for the *cis* 3,4-dioxy bond like that of **1** and the α type of xuxuarines, enabling the conclusion to be made that compounds **3** and **4** were 7,8-dihydroxyisoxuxuarine Aα and 7,8-dihydroxuxuarine Aα, respectively.

Compound **5** was a yellow amorphous solid with $[\alpha]_D$ −352.9° (*c* 0.14, $CHCl_3$), with the molecular formula of $C_{60}H_{78}O_9$ established by HRFABMS. The ^{1}H- and ^{13}C-NMR spectra of **5** revealed that it was a triterpene dimer composed of two pristimerin-type units. The chemical shift value of signals assignable to H-6 (δ_H 6.52), C-4 (δ_C 77.3), and C-23 (δ_C 24.6) suggested a *β* orientation about the *cis* 3,4-dioxy bond, and the chemical shift of H-23′ (δ_H 2.73) suggested the presence of xuxuarine-type linkages (C-3−C-2′ and C-4−C-3′ bonds) as in the case of **4**. The ROESY spectrum of the methyl derivative of **5**, in which the introduced methoxy methyl protons showed a correlation with the H-1′ aromatic methine proton, and the CD spectrum of **5**, in which a negative first Cotton effect was observed at 401 nm and a positive second Cotton effect at 332 nm, confirmed these inferences. Thus, structure **5** was assigned to be xuxuarine E*β*.

Complete assignments of the ^{1}H- and ^{13}C-NMR signals of new isolates (**1**−**5**) are shown in Tables 1 and 2.

Previously, we have proposed possible routes for the biosynthesis of triterpene dimers found in species of the Celastraceae.[11] Thus, a 2,3-diketone-type triterpene, which is in an equilibrium state with its quinoid form, approaches from the front or the reverse direction to the counterpart triterpene molecule from the upper or the lower side to form Diels−Alder-type adducts. These may account for the formation of the geometric and stereochemical isomer triterpene dimers, xuxuarines α and *β*, and the isoxuxuarine α and *β* types.

Experimental Section

General Experimental Procedures. Si gel open column chromatography was performed on Si gel 60 (Merck). Medium-pressure liquid chromatography (MPLC) was performed with a CIG column system (22 mm i.d. × 300 mm or 22 mm i.d. × 100 mm; Kusano Scientific Co., Tokyo) packed with 10 μm or 5 μm of Si gel and/or octadecyl Si gel (ODS). HPLC was performed with an Inertsil PREP-ODS column (5 mm i.d. × 250 mm for analysis, 20 mm i.d. × 250 mm for preparative; GL Science Inc., Tokyo) packed with 10 μm of ODS. TLC was conducted on precoated Si gel 60 F_{254} (Merck) and/or RP-18 F_{254} (Merck), and the spots were detected by heating after spraying with 10% H_2SO_4. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^{2}g^{-1}. UV and IR spectra were taken with a Hitachi U-2000 spectrophotometer and a JASCO FT/IR-5300 spectrophotometer, respectively. 1D and 2D ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer (AM 400) or a Varian spectrometer (Unity Plus 400) at 300 K using Bruker or Varian standard pulse sequences. NMR coupling constants (J) are given in Hz. Phase-sensitive NOESY experiments were conducted with a mixing time of 500 ms, and phase-sensitive ROESY experiments were conducted with a mixing time of 300 ms. A 150-ms delay was used to optimize one-bond correlation in HMQC and HSQC spectra and suppress them in HMBC spectra, and the evolution delay for long-range couplings in HMBC spectra was set to 63 ms. FABMS and HRFABMS spectra were obtained on a JEOL AX-505H spectrometer.

Plant Material. The dark reddish brown stem bark of *M. chuchuhuasca*, commonly known as "xuxuá", was 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 University of Pharmacy and Life Science.

Extraction and Isolation. The crushed bark (5 kg) was 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 Si gel column chromatography using a CH_2Cl_2−EtOAc gradient system (1:0−0:1) followed by MeOH to give 12 fractions. From fractions V and VI, nine triterpene dimers, xuxuarines A−D series, have been previously isolated.[10,11] Continuous ODS-HPLC purification (85% CH_3CN) of the remaining portion of fractions V and VI led to the isolation of four triterpene dimers, isoxuxuarines Aα (**1**: 0.0016% w/w), Aβ (**2**: 0.0003% w/w), 7,8-dihydroisoxuxuarine Aα (**3**: 0.0016% w/w), and 7,8-dihydroxuxuarine Aα (**4**: 0.0001% w/w). Fraction IV was separated by Si-MPLC using a *n*-hexane−EtOAc gradient system (8:2−7:3), and the fractions derived were further purified by ODS−HPLC (100% CH_3CN) to obtain a triterpene dimer, xuxuarine Eβ (**5**: 0.0003% w/w).

Isoxuxuarine Aα (1): a yellow amorphous solid; $[\alpha]_D$ +520.5° (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 251 (4.31), 299 (4.17), 374 (3.95) nm; CD λ (MeOH) max ($\Delta\epsilon$), 341 (+22.5), 301 (+24.0), 253 (−36.3) nm; IR (KBr) ν_{max} 3443, 2951, 1709, 1676, 1649, 1595, 1458, 1379, 1306, 1202, 1150, 1067, 1020, 858 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Mz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 Mz) data, see Table 2; FABMS *m/z* [M + H]$^+$ 855 (80), 437 (3), 420 (15); HRFABMS *m/z* [M + H]$^+$ found 855.5223, calcd for $C_{56}H_{71}O_7$ 855.5199.

Isoxuxuarine Aβ (2): a yellow amorphous solid; $[\alpha]_D$ −514.5° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 253 (4.24), 299 (4.12), 382 (4.04) nm; CD λ (MeOH) max ($\Delta\epsilon$), 394 (−11.9), 338 (+4.5), 262 (−31.1) nm; IR (KBr) ν_{max} 3445, 2955, 1711, 1651, 1595, 1539, 1458, 1379, 1306, 1206, 1152, 1069, 1020, 841 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Mz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 Mz) data, see Table 2; FABMS *m/z* [M + H]$^+$ 855 (55), 437 (3), 420 (16); HRFABMS *m/z* [M + H]$^+$ found 855.5227, calcd for $C_{56}H_{71}O_7$ 855.5119.

7,8-Dihydroisoxuxuarine Aα (3): a pale yellow amorphous solid; $[\alpha]_D$ +308.8° (*c* 0.44, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 250 (4.20), 296 (4.25) nm; CD λ (MeOH) max ($\Delta\epsilon$), 326 (sh, +15.7), 294 (+26.1), 253 (−12.6) nm; IR (KBr) ν_{max} 3464, 2948, 1711, 1647, 1595, 1456, 1381, 1306, 1202, 1144, 1030, 872 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Mz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 Mz) data, see Table 2; FABMS *m/z* [M + H]$^+$ 857 (60), 435 (3), 423 (5); HRFABMS *m/z* [M + H]$^+$ found 857.5355, calcd for $C_{56}H_{73}O_7$ 857.5356.

7,8-Dihydroxuxuarine Aα (4): a pale yellow amorphous solid; $[\alpha]_D$ +226.4° (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 250 (4.27), 293 (4.27) nm; CD λ (MeOH) max ($\Delta\epsilon$), 321 (sh, +11.5), 287 (+28.4), 255 (−18.3) nm; IR (KBr) ν_{max} 3434, 2932, 1709, 1649, 1584, 1458, 1381, 1306, 1196, 1146, 1047, 1020, 870 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Mz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 Mz) data, see Table 2; FABMS *m/z* [M + H]$^+$ 857 (22), 437 (3), 423 (4); HRFABMS *m/z* [M + H]$^+$ found 857.5359, calcd for $C_{56}H_{73}O_7$ 857.5356.

Xuxuarine Eβ (5): a yellow amorphous solid; $[\alpha]_D$ −352.9° (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 253 (4.26), 297 (4.14), 384 (4.06) nm; CD λ (MeOH) max ($\Delta\epsilon$), 401 (−6.3), 332 (+9.3), 262 (−38.9) nm; IR (KBr) ν_{max} 3447, 2946, 1732, 1651, 1597, 1464, 1379, 1308, 1204, 1152, 1020, 1005, 841 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Mz) data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 Mz) data, see Table 2; FABMS *m/z* [M + H]$^+$ 943 (100), 481 (34), 465 (28); HRFABMS *m/z* [M + H]$^+$ found 943.5709, calcd for $C_{60}H_{79}O_9$ 943.5724.

Preparation of *O*-Methyl Derivatives. Each compound (5−10 mg) was dissolved in 0.5 mL of CH_3CN−MeOH (9:1) and treated with 2 drops of TMS−CHN_2 (2.0 M, *n*-hexane solution) and 2 drops of *N*,*N*-diisopropylethylamine for 6−18 h at room temperature.[20] The reaction mixture was partitioned between CH_2Cl_2 and H_2O, and the organic layer was concentrated. The residue was then purified by HPLC eluted with 97% or 100% MeOH to give each methyl derivative (60−80% yield).

***O*-Methylisoxuxuarine Aα (*O*-methyl derivative of 1):** a yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.00 (1H, s, H-1′), 6.27 (1H, s, H-7′), 6.19 (1H, dd, J = 1.7, 6.6 Hz, H-6), 5.99 (1H, d, J = 1.7 Hz, H-1), 5.87 (1H, d, J = 6.6 Hz, H-7), 3.63 (3H, s, 3-OMe), 2.92 (1H, d, J = 14.3 Hz, H-22′α), 2.83 (1H, d, J = 14.5 Hz, H-22α), 2.60 (3H, s, H-23′), 2.50 (1H, m, H-20′), 2.49 (1H, m, H-20), 1.62 (3H, s, H-25′), 1.61 (3H, s, H-23), 1.45 (3H, s, H-25), 1.39 (3H, s, H-26′), 1.25 (3H, s, H-26),

1.03 (3H, s, H-27′), 1.00 (3H, s, H-28′), 1.00 (3H × 2, s, H-27 and H-28), 0.98 (3H, d, J = 6.3 Hz, H-30′), 0.98 (3H, J = 6.8 Hz, H-30); FABMS m/z $[M + H]^+$ 869 (66), 434 (4), 406 (13).

***O*-Methylisoxuxuarine Aβ (*O*-methyl derivative of 2):** a yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (1H, br s, H-1′), 6.54 (1H, br d, J = 6.8 Hz, H-6), 6.26 (1H, s, H-7′), 6.08 (1H, d, J = 6.8 Hz, H-7), 5.95 (1H, br s, H-1), 3.55 (3H, s, 3-OMe), 2.91 (1H × 2, d, J = 14.5 Hz, H-22α and H-22′α), 2.67 (3H, br s, H-23′), 2.51 (1H, m, H-20), 2.49 (1H, m, H-20′), 1.59 (3H, s, H-25′), 1.56 (3H, s, H-23), 1.50 (3H, s, H-25), 1.37 (3H, s, H-26′), 1.29 (3H, s, H-26), 1.01 (3H × 4, br s, H-30, H-30′, H-28 and H-28′), 1.00 (3H × 2, s, H-27, H-27′); FABMS m/z $[M + H]^+$ 869 (49), 434 (3), 406 (15).

***O*-Methyl-7,8-dihydroisoxuxuarine Aα (*O*-methyl derivative of 3):** a pale yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.97 (1H, s, H-1′), 6.27 (1H, s, H-7′), 6.21 (1H, br s, H-6), 5.87 (1H, d, J = 1.1 Hz, H-1), 3.62 (3H, s, 3-OMe), 2.91 (1H, d, J = 14.5 Hz, H-22′α), 2.86 (1H, d, J = 14.3 Hz, H-22α), 2.65 (3H, s, H-23), 2.52 (1H, m, H-20′), 2.51 (1H, m, H-20), 1.61 (3H, s, H-25′), 1.52 (3H, s, H-23), 1.39 (3H, s, H-26′), 1.23 (3H, s, H-27), 1.09 (3H, s, H-25), 1.06 (3H, s, H-27′), 1.02 (3H × 2, s, H-26 and H-28′), 0.99 (3H × 2, d, J = 6.3 Hz, H-30 and H-30′), 0.95 (3H, s, H-28); FABMS m/z $[M + H]^+$ 871 (66), 437 (7), 408 (15).

***O*-Methyl-7,8-dihydroxuxuarine Aα (*O*-methyl derivative of 4):** a pale yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, s, H-1′), 6.30 (1H, s, H-7′), 6.21 (1H, br s, H-6), 5.86 (1H, s, H-1), 3.64 (3H, s, 3-OMe), 2.91 (1H, d, J = 14.8 Hz, H-22′α), 2.87 (1H, d, J = 15.6 Hz, H-22α), 2.72 (3H, s, H-23′), 2.53 (1H, m, H-20), 2.49 (1H, m, H-20′), 1.59 (3H, s, H-25′), 1.53 (3H, s, H-23), 1.38 (3H, s, H-26′), 1.24 (3H, s, H-27), 1.10 (3H, s, H-25), 1.04 (3H, s, H-26), 1.03 (3H, s, H-28′), 1.00 (3H, d, J = 6.4 Hz, H-30), 0.97 (3H, d, J = 6.6 Hz, H-30′). 0.97 (3H × 2, s, H-28 and H-27′); FABMS m/z $[M + H]^+$ 871 (8).

***O*-Methylxuxuarine Eβ (*O*-methyl derivative of 5):** a yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.02 (1H, br s, H-1′), 6.50 (1H, br d, J = 6.6 Hz, H-6), 6.22 (1H, s, H-7′), 6.04 (1H, d, J = 6.6 Hz, H-7), 5.86 (1H, s, H-1), 3.60 (3H, s, 3-OMe), 3.54 (3H × 2, s, 20-COOMe and 20′-COOMe), 2.50 (3H, br s, H-23′), 2.43 (1H, d, J = 15.6 Hz, H-19α), 2.42 (1H, d, J = 15.2 Hz, H-19′α), 1.56 (3H, s, H-23), 1.52 (3H, s, H-25′), 1.42 (3H, s, H-25), 1.28 (3H, s, H-26′), 1.20 (3H, s, H-26), 1.18 (3H, s, H-30), 1.17 (3H, s, H-30′), 1.09 (3H, s, H-28), 1.08 (3H, s, H-28′), 0.61 (3H, s, H-27′), 0.55 (3H, s, H-27).

References and Notes

(1) Itokawa, H.; Shirota, O.; Morita, H.; Takeya, K.; Tomioka, N.; Itai, A. *Tetrahedron Lett.* **1990**, *31*, 6881−6882.
(2) Itokawa, H.; Shirota, O.; Morita, H.; Takeya, K.; Iitaka, Y. *Phytochemistry* **1991**, *30*, 3713−3716.
(3) Itokawa, H.; Shirota, O.; Morita, H.; Takeya, K. *Heterocycles* **1992**, *34*, 885−889.
(4) Itokawa, H.; Shirota, O.; Morita, H.; Takeya, K.; Iitaka, Y. *J. Chem. Soc., Perkin Trans. I* **1993**, 1247−1254.
(5) Itokawa, H.; Shirota, O.; Ichitsuka, K.; Morita, H.; Takeya, K. *J. Nat. Prod.* **1993**, *56*, 1479−1485.
(6) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *Heterocycles* **1994**, *38*, 383−389.
(7) Itokawa, H.; Shirota, O.; Ichitsuka, K.; Morita, H.; Takeya, K. *J. Nat. Prod.* **1994**, *57*, 460−470.
(8) Shirota, O.; Otsuka, A.; Morita, H.; Takeya, K.; Itokawa, H. *Heterocycles* **1994**, *38*, 2219−2229.
(9) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1994**, *57*, 1675−1681.
(10) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *Chem. Lett.* **1995**, 101−102.
(11) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *Tetrahedron* **1995**, *51*, 1107−1120.
(12) Shirota, O.; Tamemura, T; Morita, H.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1996**, *59*, 1072−1075.
(13) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *J. Nat. Prod.* **1997**, *60*, 111−115.
(14) Flores, A. F. In *Advances in Economic Botany, Vol. 1: Ethnobotany in the Neotropics*; Prance, G. T., Kallunti, J. A., Ed. The New York Botanical Garden: Bronx, NY, 1984; pp 1−8.
(15) Gonzalez, G. J.; Monache, D. G.; Monache, D. F.; Marini-Bettolo, B. G. *J. Ethnopharmacol.* **1982**, *5*, 73−77.
(16) Martinod, P.; Paredes, A.; Monache, D. F.; Marini-Bettolo, B. G. *Phytochemistry* **1976**, *15*, 562−563.
(17) González, A. G.; Mendoza, J. J.; Luis, J. G.; Ravelo, A. G.; Bazzocchi, I. L. *Tetrahedron Lett.* **1989**, *30*, 863−866.
(18) González, A. G.; Jiménez, J. S; Moujir, L. M.; Ravelo, A. G.; Luis, J. G.; Bazzocchi, I. L.; Gutiérrez, A. M. *Tetrahedron* **1992**, *48*, 769−774.
(19) González, A. G.; Alvarenga, N. L.; Estévez-Braum, A.; Ravelo, A. G.; Bazzocchi, I. L.; Moujir, L. M. *Tetrahedron* **1996**, *52*, 9597−9608.
(20) Aoyama, T.; Terasawa, S.; Sudo, K.; Shioiri, T. *Chem. Pharm. Bull.* **1984**, *32*, 3759−3760.

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