Novel Stereoisomeric Triterpene Dimers, Xuxuarines Aα and Aβ, from *Maytenus chuchuhuasca*

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Two novel stereoisomeric triterpene dimers, xuxuarines Aα (1) and Aβ (2), were isolated from a South American medicinal plant, "xuxuá" (*Maytenus chuchuhuasca* Raymond-Hamet et Colas), and their structures and conformations were elucidated by spectroscopic, chemical evidence and MD calculations.

"Xuxuá" and related medicinal plants, belonging to the genus *Maytenus*, have been used for treatment of the rheumatism, and also used as an antitumoral agent for skin cancer by the inhabitants in the Amazonian basin.[12] From a methanol extract of "xuxuá" (*Maytenus chuchuhuasca* Raymond-Hamet et Colas), two novel stereoisomeric triterpene dimers named xuxuarines Aα (1), Aβ (2), were isolated by monitoring the cytotoxicity, in addition to pristimerin, tingenone and 22β-hydroxytingenone.[3-7]

Xuxuarine Aα (1),[8] whose molecular formula was determined as $C_{56}H_{70}O_7$ by FAB-MS spectrum, was suggested by the ^{1}H- and ^{13}C-NMR spectra, to be a triterpene dimer composed of two tingenone type triterpenes, one being in a quinoid form, and the other in an aromatic form. The HMQC and HMBC spectra revealed its partial structures as follows: the quinoid triterpene unit (unit Ta) contained a conjugated ketone system in A − B rings and two oxygenated quaternary carbons at C-3 and C-4, and the aromatic triterpene unit (unit Tb) contained an aromatic ring system in A ring, one conjugated carbonyl group and one double bond on B ring and probably oxygenated C-2' and C-3' on the A ring. No free phenolic hydroxyl group was suggested, since no absorption maximum shift in the UV spectrum was observed on the addition of alkali. The high field shift (Δ 0.08 ppm) of C-3 observed in the ^{13}C-NMR spectrum on addition of D_2O, and the appearance of one amide proton in the ^{1}H-NMR on treatment with trichloroacetyl isocyanate,[9] indicated that the only hydroxyl group of the molecule was attached to C-3, and that the two ether bonds were between C-3, C-4 of the unit-Ta and C-2', C-3' of the Tb. The NOESY spectrum of the methyl derivative of 1 showed the NOE correlations between the protons of the methoxyl group on C-3 and the olefinic proton at C-1',

the olefinic proton at C-6 and the methyl group protons at C-23, the methoxyl group protons and the methyl group protons at C-23. The last mentioned NOE correlation revealed that the 3,4-dioxy bond was in cis configuration. The stereochemistry of the cis 3,4-dioxy bond was cleared by the analysis of CD spectrum: it showed a positive first maximum value at 357 nm, showing that the cis 3,4-dioxy bond of 1 was in α orientation.[10]

Xuxuarine Aβ (2)[11] was shown to have the same molecular formula as 1, and to be consisted of two triterpene units which were the same as in 1. The difference between 1 and 2 was to be in the stereochemistry about the ether linkages between two units. The NMR signals of the protons around the ether bonds of the methyl derivative of 2 were all broad. The NOE data of 2 were essentially identical with those of the methyl derivative of 1. The CD spectrum of 2 showed the negative first Cotton effect at 397 nm, and positive second Cotton effect at 331 nm, which indicated that the cis 3,4-dioxy bond of 2 was in β orientation.

For the purpose of confirming the orientation of the cis 3,4-dioxy bond of 1 and 2, and analyzing complicated conformational features of them, high temperature molecular dynamics (MD) calculations for simulated annealing was tested.[12,13] This simulation which was performed with distance constraints derived from the NOE experiments, gave each snapshot with the lowest energy as a relevant conformation (Figure 2).[14] It is obvious that each conformation is satisfied with the characteristic NOE relationship and is fulfilled for solution conformer.

Biogenetically, xuxuarines Aα and Aβ were assumed to be synthesized from a quinoid type triterpene and the corresponding 2,3-diketone type triterpene in an equilibrium state. By an adduct of the 2,3-diketone type triterpene to a lower or a upper site of the quinoid type one, the stereoisomeric triterpene dimers 1 and 2 would be generated.

Xuxuarine Aα (1) showed a moderate cytotoxicity on cultured tumor cells (L1210: IC_{50} = 9.4 × 10^{-2} mol/l; P388: IC_{50} = 5.9 × 10^{-2} mol/l), but Aβ (2) did not show any appreciable activity.
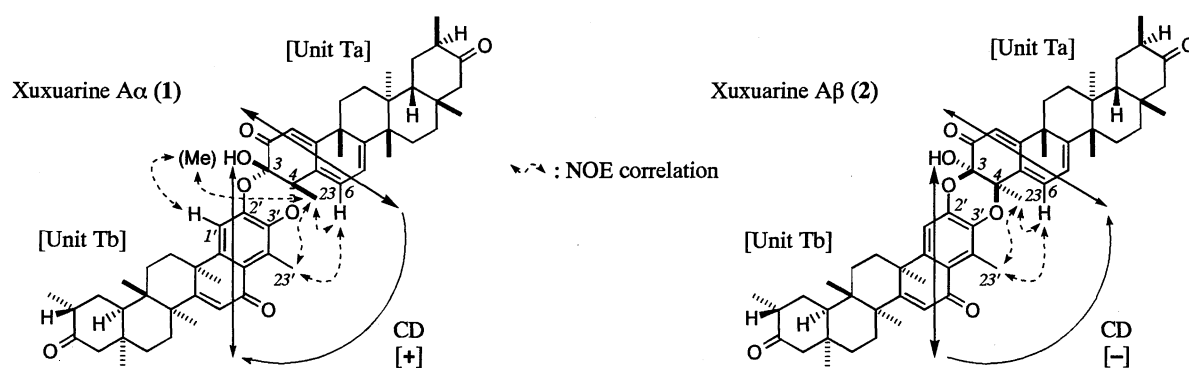

Figure 1. Structures of xuxuarines Aα (1) and Aβ (2). NOE correlations and CD spectral data were illustrated in the structures.
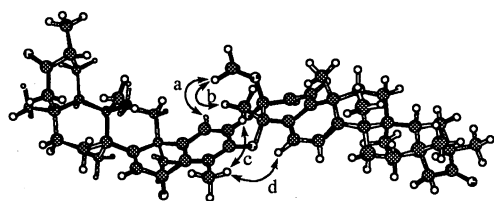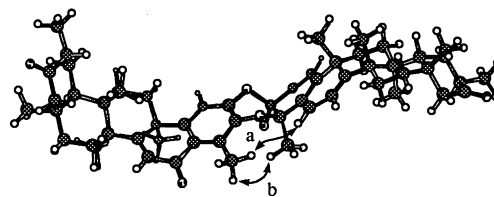
Methyl xuxuarine Aα (1) a = 2.900, b = 2.564, c = 2.850, d = 2.926 Å   Xuxuarine Aβ (2) a = 2.970, b = 2.829 Å

Figure 2. Prespective views of the lowest energy conformers of the methyl derivative of **1** and **2**.
The values of a – d represent the distances between two protons indicated by arrows.

References and Notes

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7   Cytotoxic CH_2Cl_2-soluble fraction of the MeOH extract was subjected to Silica-gel cc using a CH_2Cl_2 – EtOAc gradient system. Active fractions were further subjected to ODS MPLC with CH_3CN – H_2O gradient system to give three known triterpenes as active principles, and then elution with CH_3CN gave **1** (0.0026% from dry material) and **2** (0.0046%).

8   Xuxuarine Aα (**1**) : yellow amorphous solid; $[\alpha]_D$ +645.2° (c 0.61, $CHCl_3$); CD λ max (MeOH) nm (Δε), 357 (+19.4), 246 (–31.6); HR-MS m/z (%), 436 (M_{Tb}^+ + H, 67, calcd. for $C_{28}H_{36}O_4$: 436.2614; found : 436.2610), 420 (M_{Ta}^+ + H, 63, calcd. for $C_{28}H_{36}O_3$: 420.2664; found : 420.2638); 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), δ 6.06 (1H, d, J = 1.2 Hz) / H-1, 6.23 (1H, dd, J = 1.2, 6.6 Hz) / H-6, 5.94 (1H, d, J = 6.6 Hz) / H-7, [a]2.44 (1H, m) / H-20, [b]2.85 (1H, d, J = 14.3 Hz) / H-22α, 1.56 (3H, s) / Me-23, 1.44 (3H, s) / Me-25, 1.22 (3H, s) / Me-26, [c]0.96 (3H, s) / Me-27, [c]0.96 (3H, s) / Me-28, [d]0.92 (3H, d, J = 6.5 Hz) / Me-30, 6.76 (1H, s) / H-1', 6.23 (1H, s) / H-7', [a]2.42 (1H, m) / H-20', [b]2.80 (1H, d, J = 14.4 Hz) / H-22'α, 2.70 (3H, s) / Me-23', 1.50 (3H, s) / Me-25', 1.31 (3H, s) / Me-26', [c]0.95 (3H, s) / Me-27', [c]0.95 (3H, s) / Me-28', [d]0.94 (3H, d, J = 6.5 Hz) / Me-30', (a, b, c, d: The assigment of each set of values may be interchanged.); ^{13}C-NMR ($CDCl_3$, 100 MHz), δ 115.53 (d) / C1, 190.09 (s) / C2, 91.96 (s) / C3, 79.25 (s) / C4, 130.20 (s) / C5, 126.35 (d) / C6, 116.09 (d) / C7, 160.23 (s) / C8, 41.52 (s) / C9, 173.35 (s) / C10, 33.14 (t) / C11, 29.67 (t) / C12, 39.30 (s) / C13, 44.12 (s) / C14, 28.18 (t) / C15, [a]35.45 (t) / C16, [b]38.04 (s) / C17, [c]43.39 (d) / C18, [d]31.94 (t) / C19, 41.69 (d) / C20, 213.34 (s) / C21, [e]52.41 (t) / C22, 22.14 (q) / C23, 35.45 (q) / C25, 22.14 (q) / C26, [f]19.79 (q) / C27, [g]32.42 (q) / C28, 14.94 (q) / C30, 111.21 (d) / C1', 144.68 (s) / C2', 137.58 (s) / C3', 127.65 (s) / C4', 124.17

(s) / C5', 187.41 (s) / C6', 125.96 (d) / C7', 170.46 (s) / C8', 39.64 (s) / C9', 150.34 (s) / C10', 34.17 (t) / C11', 30.03 (t) / C12', 40.07 (s) / C13', 44.18 (s) / C14', 28.29 (t) / C15', [a]35.31 (t) / C16', [b]38.01 (s) / C17', [c]43.25 (d) / C18', [d]31.85 (t) / C19', 41.69 (d) / C20', 213.34 (s) / C21', [e]52.31 (t) / C22', 12.82 (q) / C23', 38.37 (q) / C25', 20.65 (q) / C26', [f]19.52 (q) / C27', [g]32.40 (q) / C28', 14.94 (q) / C30', (a, b, c, d, e, f, g: The assigment of each set of values may be interchanged.).

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11 Xuxuarine Aβ (**2**) : yellow amorphous solid; $[\alpha]_D$ –512.6° (c 0.40, $CHCl_3$); CD λ max (MeOH) nm (Δε), 397 (–12.2), 261 (–60.0); EI-MS m/z (%), 436 (M_{Tb}^+ + H, 95), 421 (M_{Ta}^+ + 2H, 100), 420 (M_{Ta}^+ + H, 58); FAB-MS / HR-MS m/z (%), 855 (M^+ + H, 36, calcd. 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), δ 6.06 (1H, d, J = 1.3) / H-1, 6.52 (1H, dd, J = 1.3, 6.9 Hz) / H-6, 6.10 (1H, d, J = 6.9 Hz) / H-7, [a]2.44 (1H, m) / H-20, 2.84 (1H, d, J = 14.4 Hz) / H-22α, 1.57 (3H, s) / Me-23, 1.36 (3H, s) / Me-25, 1.21 (3H, s) / Me-26, 0.96 (3H, s) / Me-27, 0.96 (3H, s) / Me-28, [b]0.92 (3H, d, J = 6.6 Hz) / Me-30, 6.73 (1H, s) / H-1', 6.20 (1H, s) / H-7', [a]2.42 (1H, m) / H-20', 2.84 (1H, d, J = 14.4 Hz) / H-22'α, 2.70 (3H, s) / Me-23', 1.51 (3H, s) / Me-25', 1.32 (3H, s) / Me-26', 0.93 (3H, s) / Me-27', 0.93 (3H, s) / Me-28', [b]0.94 (3H, d, J = 6.6 Hz) / Me-30', (a, b: The assigment of each set of values may be interchanged.).

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13 Computer modeling and all calculations were performed using the molecular modeling software SYBYL 6.03 (Tripos Associates, St. Leuis, MO) on an IRIS 4-D workstation.

14 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.[15]

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