Revised Structures of Cangorosins, Triterpene Dimers from *Maytenus ilicifolia*

Osamu Shirota, Hiroshi Morita, Koichi Takeya, and Hideji Itokawa*

Department of Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy & Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan

Received May 13, 1996[⊗]

Structures of four triterpene dimers, cangorosins, isolated from *Maytenus ilicifolia* Mart., were previously reported to consist of two triterpene units joined by an ether bond formed between the two A rings. On the basis of detailed studies of their chemical and spectroscopic data, including inverse-detected 2D-NMR data, and comparisons with other known triterpene dimers, the structures of the cangorosins have been revised to triterpene dimers linked by two ether bridges between the two A rings for cangorosin B and between the A and the B rings for the cangorosin A series (cangorosin A, isocangorosin A, and 6′,7′-dihydroisocangorosin A).

Previously, four triterpene dimers, cangorosin A, atropcangorosin A, dihydroatropcangorosin A, and cangorosin B, were isolated from a Paraguayan folk medicinal plant "cangorosa", *Maytenus ilicifolia* Mart. (Celastraceae), and these were reported to consist of two triterpenes linked by an ether bond formed between the A rings.[1] Later, stereoisomeric triterpene dimers, xuxuarines, were isolated from Brazilian "xuxuá" (*Maytenus chuchuhuasca* Raymond-Hamet et Colas) and determined to be triterpene dimers composed of one quinoid-type triterpene and one aromatic triterpene linked by two ether linkages between the A rings.[2,3] In our detailed studies of the ^{1}H- and ^{13}C-NMR signal patterns of the xuxuarines, we observed that the two A ring ^{13}C signals had strong similarities to those of cangorosin B, a compound reported to contain one ether link and one epoxide group. We have carried out detailed analyses[4] of the chemical and spectroscopic data, including inverse-detected 2D-NMR, and concluded that the structure of cangorosin B should be revised to **1**. Similar analyses[4] of spectroscopic properties of the other cangorosins (**2**−**4**) resulted in the revised structures and names shown (Chart 1).

Results and Discussion

Cangorosin B (**1**), $C_{58}H_{74}O_8$, was previously shown to be a triterpene dimer composed of one tingenone-type and one pristimerin-type triterpene.[5,6] The pristimerin-type triterpene unit (unit Tb) contains an aromatized A ring and an unsaturated ketone moiety in the B ring, while the tingenone-type triterpene unit (unit Ta) contains a ketone at C-2 conjugated to three double bonds spanning rings A and B.[1] In the previous study,[1] the two quaternary carbons signals at δ 92.01 (s) and 79.53 (s) were assigned to the epoxide carbons (C-3, C-4) of the A ring of the Ta unit of cangorosin B. We then isolated the xuxuarines and investigated their structures. In the xuxuarines,[2,3] the 3,4-dioxy bond ascribed to the ^{13}C signals at δ 92 (s) and 79 (s) and those at δ 91 (s) and 77 (s) were assigned to the xuxuarines of the α type and that of the β type, respectively, on the basis of spectral and chemical evidence (inverse-detected 2D-NMR studies, UV spectra on addition of alkali, ^{13}C-NMR signal shifts by addition of D_2O, and ^{1}H-NMR spectral changes treated with TAI reagent[7]). The close correspondence of these NMR signals with those of C-3 (δ 92.01) and C-4 (δ 79.53) in cangorosin B suggested that the two triterpene units of cangorosin B might be similarly linked by two ether units rather than the one ether unit previously proposed. Mass spectral fragments for **1** at *m*/*z* 420 and 480, generated possibly by a retro Diels−Alder reaction, support this conclusion (Figure 1). The CD spectrum for **1** showed a positive first maxim value at 345 nm similar to the xuxuarines of the α type. Thus, **1** was assigned the α orientation at C-3 and C-4. However, the NOESY spectral pattern of the methyl derivative of **1** was different from the pattern of both the α and β types of xuxuarine. The observed NOE correlations between the introduced methoxy methyl protons on C-3 and the H-23′ methyl protons, and between the H-6 olefinic proton and the H-1′ olefinic proton, revealed that the 3,4-dioxy bond in **1** consisted of C-3−C-3′ and C-4−C-2′ bonds (Figure 1). Thus, **1** was shown to be a reverse conjugated type of xuxuarine. On the basis of these spectroscopic data, the revised structure of cangorosin B was determined to be **1**.

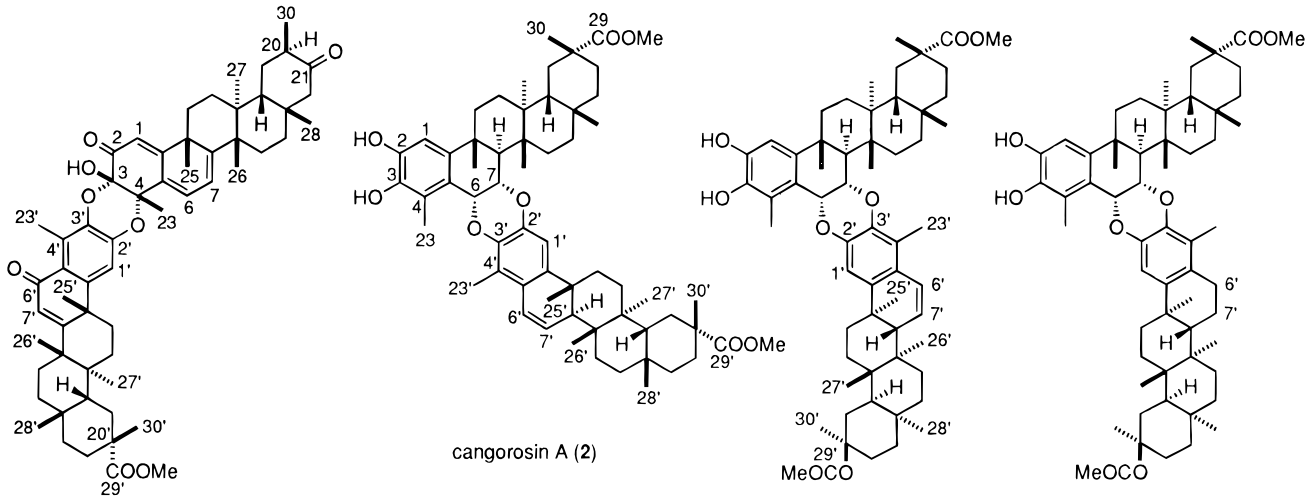
Cangorosin A (**2**), $C_{60}H_{84}O_9$, was a triterpene dimer composed of two pristimerin-type triterpenes having aromatized A rings. One triterpene unit (unit Ta) possessed α cis dioxy groups at C-6 and C-7, and the other (unit Tb) possessed a Δ^6 double bond, as evidenced by proton coupling and NOE correlations in the ^{1}H NMR.[1] In the present experiment, formation of two amide proton signals was observed in the ^{1}H-NMR spectrum when **2** was treated with trichloroacetyl isocyanate (TAI).[7] Furthermore, high-field shifts (Δ0.03−0.14 ppm) of A ring carbons in unit Ta were observed in the ^{13}C-NMR spectrum upon addition of D_2O (Figure 2). These observations indicated that the two free hydroxyl groups of **2**, giving the IR absorptions at 3602 and 3554 cm^{-1}, should be at C-2 and C-3 rather than at C-6 and C-7 in the Ta unit. Thus, it appeared that the α *cis* dioxy position of the B ring in unit Ta and the dioxy position of the A ring, at C-2′ and C-3′ in unit Tb, should form the linkages between the two units. To confirm the positions of hydroxy groups and the linkages between these two units, an HMBC spectrum of a dimethyl derivative of **2** was measured. In this spectrum, the introduced methyl protons gave cross-peaks with C-2 and C-3, respectively. In addition, the H-7

* To whom correspondence should be addressed. Phone: +81-426-76-3007. FAX: +81-426-77-1436.

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.

S0163-3864(96)00448-X CCC: $14.00

Chart 1



methine proton of unit Ta gave a cross-peak with the C-2′ aromatic carbon of unit Tb. Therefore, the two hydroxy groups were placed at C-2 and C-3 in unit Ta, and the linkages between the two units were confirmed to be between C-6 and C-3′, and C-7 and C-2′. Considering these spectroscopic evidence, cangorosin A was reassigned structure **2**.

Compound **3**, previously named atropcangorosin A, had the same molecular formula and consisted of the same triterpene units as cangorosin A (**2**). Treatment of **3** with CH_3I resulted in the introduction of methyl groups at C-2 and C-3 in unit Ta. The difference between **2** and **3** was noted only in the connection pattern of the two units. The linkage pattern between the two units in **3** was examined by HMBC of its dimethyl derivative: a cross-peak between the H-7 methine proton of unit Ta and the C-3′ aromatic carbon of unit Tb was observed, indicating that in **3** the two triterpene units were joined by two ether linkages between C-6 and C-2′, and C-7 and C-3′. Thus, the structure of isocangorosin A was reassigned to be **3**.

Compound **4**, previously named dihydroatropcangorosin A, was a dihydro derivative of **3** as evidenced by HPLC analysis of the hydrogenation product of **3** and the mass fragmentation peaks at *m*/*z* 468 and 466.[1] The position of hydrogenation was assigned to be at C-6′ and C-7′ by analysis of 2D-NMR spectra. Therefore, the structure of 6′,7′-dihydroisocangorosin A was revised to **4**.

Possible routes for the biosynthesis of cangorosins (**1**−**4**) and the xuxuarines include a 2,3-diketone-type triterpene (the front or the reverse side) approaching the counterpart triterpene molecule to form Diels−Alder adducts. Reactions of this type could also account for the formation of geometrical isomer triterpene dimers such as cangorosin A (**2**) and isocangorosin A (**3**).

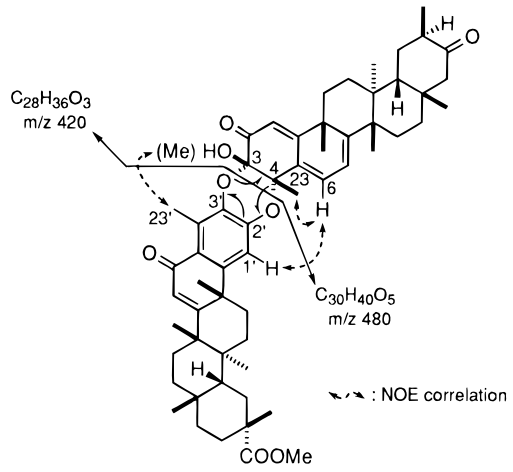


Figure 1. Major MS fragments and NOE correlations of cangorosin B (**1**).

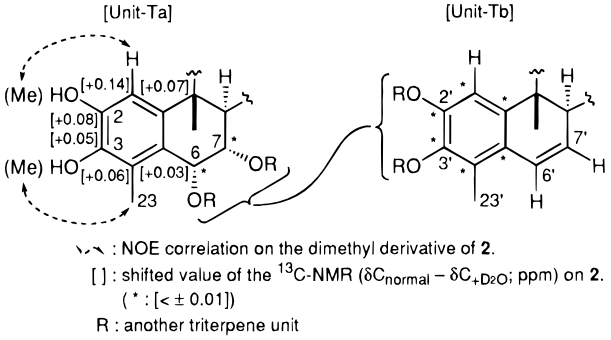


Figure 2. A and B rings in units Ta and Tb of cangorosin A (**2**).

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and were 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 obtained 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 Si 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} plates (Art. 5715; Merck), and the spots were detected by heating after spraying with 10% H_2SO_4. 1D and 2D, ^{1}H 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 600 ms. The value of the delay to optimize one-bond correlations in the HMQC spectrum and to suppress them in the HMBC spectrum was 155 ms, and the evolution delay for long-range couplings in the HMBC spectrum was set to 50 ms. The NMR coupling constants (*J*) are given in Hz.

Plant Material. Reddish to orange brown root bark of *M. ilicifolia* Mart., commonly known as "cangorosa" among Indian tribes,[8] was purchased at Asuncion, Paraguay, in 1987. The botanical identification was made by Dr. Tanaka (Asuncion University, Paraguay). A voucher specimen has been deposited in the herbarium of the Tokyo University of Pharmacy & Life Science.

Extraction and Isolation. Root bark (1140 g) of *M. ilicifolia* was crushed and extracted with hot MeOH to give an extract (364 g) that was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble fraction (62.3 g) was subjected to Si gel column chromatography (CC) using a *n*-hexane−ethyl acetate gradient system (1:0−0:1) to give 17 fractions. Fraction 4 was cytotoxic and subjected further to Si gel column chromatography with *n*-hexane−ethyl acetate (7:3) and then to ODS medium-pressure liquid chromatography (MPLC) with a MeOH−H_2O solvent system to give **1**−**4** and pristmerin[4] (major cytotoxic constituent), all as amorphous solids. These compounds were further purified by Sephadex LH-20 CC with MeOH, followed ODS HPLC with MeOH−H_2O, EtOH−H_2O, acetonitrile−H_2O, or MeOH−EtOH solvent systems.

Cangorosin B (1): yellow amorphous solid (25 mg); $[\alpha]_D$ +483.28° (*c* 0.25, $CHCl_3$); UV (EtOH) λ max (log ϵ) 251 (4.16), 298 (4.05), 378 (3.85) nm; CD (EtOH) λ max ($\Delta\epsilon$) 345 (+19.6), 302 (+21.5), 253 (−31.3), 224 (−4.5) nm; IR ($CHCl_3$) ν max 3480, 1708, 1671, 1644, 1596, 1582, 1556 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.00 (1H, s), 6.33 (1H, dd, J = 1.4, 6.5 Hz), 6.21 (1H, s), 6.12 (1H, d, J = 1.4 Hz), 5.98 (1H, d, J = 6.5 Hz), 3.57 (3H, s, COOMe), 2.84 (1H, d, J = 14.3 Hz, H-22α), 2.48 (3H, s, Me-23′), 2.48 (1H, m, H-20), 1.58 (3H, s, Me-23), 1.55 (3H, s, Me-25′), 1.49 (3H, s, Me-25), 1.30 (3H, s, Me-26′), 1.27 (3H, s, Me-26), 1.19 (3H, s, Me-30′), 1.11 (3H, s, Me-28′), 0.99 (3H, s, Me-30), 0.98 (3H, d, J = 6.4 Hz, Me-30), 0.97 (3H, s, Me-28), 0.63 (3H, s, Me-27′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.30 (s, C-21), 190.50 (s, C-2), 173.17 (s, C-10), 160.26 (s, C-8), 131.14 (s, C-5), 126.17 (d, C-6), 116.35 (d, C-7), 116.24 (d, C-1), 92.01 (s, C-3), 79.53 (s, C-4), 52.58 (t, C-22), 44.38 (s, C-14), 43.65 (d, C-18), 41.96 (d, C-20), 41.61 (s, C-9), 39.66 (s, C-13), 38.82 (s, C-17), 35.73 (q, C-25), 35.64 (t, C-16), 33.54 (t, C-11), 32.64 (q, C-28), 32.30 (t, C-19), 29.97 (t, C-12), 28.76 (t, C-15), 22.45 (q, C-26), 22.21 (q, C-23), 20.12 (q, C-30), 15.14 (q, C-27); EIMS/HRMS *m*/*z* [MTa + H]$^+$ 480 (54, calcd for $C_{30}H_{40}O_5$ 480.2873, found 480.2859), 465 (22), [MTb + H]$^+$ 420 (54, calcd for $C_{28}H_{36}O_3$ 420.2662, found 420.2650), 405 (10), 267 (14), 253 (32), 241 (78), 202 (85), 201 (100); FABMS (positive) *m*/*z* [M + H]$^+$ 899 (7.1), (positive, added KI) *m*/*z* [M + K]$^+$ 937 (9.8), [M + H]$^+$ 899 (13.2).

Preparation of Methyl Cangorosin B. 1 (1.2 mg) was dissolved in 1 mL of diethyl ether and treated with an excess amount of diazomethane for 6 h at room temperature. The reaction mixture was concentrated, and then it was subjected to Si gel MPLC with *n*-hexane−ethyl acetate (7:3) to give 0.8 mg of methyl cangorosin B; yellow amorphous solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, s), 6.25 (1H, dd, J = 1.5, 6.4 Hz, H-6), 6.23 (1H, s), 5.99 (1H, dd, J = 1.5, 6.4 Hz), 5.94 (1H, d, J = 6.4 Hz), 5.30 (1H, s), 3.62 (3H, s), 3.57 (3H, s), 2.58 (3H, s), 1.60 (3H, s), 1.55 (3H, s), 1.46 (3H, s), 1.30 (3H, s), 1.26 (3H, s), 1.19 (3H, s), 1.11 (3H, s), 0.99 (3H, s), 0.98 (3H, d, J = 6.7 Hz), 0.97 (3H, s), 0.62 (3H, s).

Cangorosin A (2): colorless amorphous solid (45 mg); $[\alpha]_D$ +237.4° (*c* 0.25, $CHCl_3$); UV (EtOH) λ max (log ϵ) 207 (4.51), 232 (4.32), 284 (3.90), 301 (3.60), 312 (3.52) nm; CD (EtOH) λ max ($\Delta\epsilon$) 313 (+4.9), 302 (+7.2), 285 (+23.0), 277 (+23.0), 235 (+41.7), 218 (+30.8); IR ($CHCl_3$) ν max 3602, 3554, 1724, 1603, 1578 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.68 (1H, s, H-1), 6.66 (1H, dd, J = 2.9, 10.0 Hz, H-6′), 6.46 (1H, s, H-1′), 5.89 (1H, dd, J = 2.7, 10.0 Hz, H-7′), 4.97 (1H, dd, J = 2.9, 11.3 Hz, H-7), 4.88 (1H, d, J = 2.9 Hz, H-6), 3.62 (3H, s, 29′-COOMe), 3.58 (3H, s, 29-COOMe), 2.49 (1H, br s, H-8′), 2.38 (1H, d, J = 11.3 Hz, H-8), 2.33 (3H, s, Me-23), 2.18 (3H, s, Me-23′), 1.34 (3H, s, Me-26), 1.30 (3H, s, Me-25), 1.20 (3H, s, Me-30), 1.20 (3H, s, Me-30′), 1.12 (3H, s, Me-28), 1.12 (3H, s, Me-28′), 1.08 (3H, s, Me-26′), 1.03 (3H, s, Me-25′), 0.84 (3H, s, Me-27′), 0.77 (3H, s, Me-27); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.60 (s, C-29′), 179.37 (s, C-29), 144.41 (s, C-2), 143.76 (s, C-10′), 143.66 (s, C-10), 141.60 (s, C-2′), 140.68 (s, C-3), 139.07 (s, C-3′), 128.56 (d, C-7′), 124.69 (s, C-5), 124.29 (s, C-6′), 124.13 (s, C-5′), 123.17 (s, C-4), 122.01 (s, C-4′), 108.30 (d, C-1), 107.97 (d, C-1′), 74.49 (d, C-7), 71.49 (d, C-6), 51.62 (q, 29-COOMe), 51.43 (q, 29′-COOMe), 45.81 (d, C-8′), 45.07 (d, C-8), 44.51 (d, C-18′), 43.56 (d, C-18), 40.66 (s, C-20), 40.63 (s, C-20′), 40.32 (s, C-9), 39.99[a] (s, C-14), 39.67[a] (s, C-13), 39.13[b] (s, C-14′), 38.95[b] (s, C-13′), 37.52 (s, C-9′), 37.14[c] (t, C-22), 36.61[c] (t, C-22′), 36.17[d] (t, C-16), 36.08[d] (t, C-16′), 34.77 (t, C-11), 31.98[e] (q, C-28), 31.98[f] (q, C-30), 31.85[e] (q, C-28′), 31.67 (t, C-15), 31.67 (t, C-11′), 31.56[g] (t, C-21), 31.56[g] (t, C-21′), 30.57[f] (q, C-30′), 30.51[h] (s, C-17′), 30.42 (t, C-12), 30.42 (t, C-15′), 30.03[i] (t, C-19), 30.03 (t, C-12′), 29.83[h] (s, C-17), 29.45[i] (t, C-19′), 28.03 (q, C-25), 22.34 (q, C-25′), 18.31 (q, C-27), 17.85 (q, C-26), 17.32 (q, C-27′), 17.24 (q, C-26′), 11.14 (q, C-23), 10.77 (q, C-23′) ([a−i]assignments for values bearing the same superscript may be reversed); EIMS/HRMS *m*/*z* [$M_{1/2}$ + H]$^+$ 466 (20, calcd for $C_{30}H_{42}O_4$ 466.3083, found 466.3056), [$M_{1/2}$ − O + H]$^+$, 450 (8, calcd for $C_{30}H_{42}O_3$ 450.3134, found 450.3113), [$M_{1/2}$ − COOMe]$^+$ 406 (5, calcd for $C_{28}H_{38}O_2$ 406.2872, found 406.2874), 298 (7), 283 (6), 269 (12), 253 (10), 239 (16), 227 (22), 202 (64), 188 (100); FABMS (positive, added KI) *m*/*z* [M + K]$^+$ 969 (0.7), [M + H]$^+$ 931 (1.3), [M]$^+$ 930 (1.9).

Reaction of Cangorosin A with TAI (Trichloroacetyl Isocyanate). One drop of TAI was added to **2** (less than 1 mg) in an NMR sample tube and mixed by shaking at once. As the result of reaction between the hydroxyl groups in **2** and the isocyanate group of TAI, two amide proton signals in the ^1H-NMR gradually appeared, which was clearly confirmed after about 8 h. No obvious change in the spectral profile of this reaction mixture was observed even after 1 week.

Preparation of Dimethyl Cangorosin A. 2 (3.6 mg) was dissolved in 1 mL of acetone and treated with

excess CH_3I and excess K_2CO_3 for 12 h at 70 °C. The reaction mixture was added to cold H_2O, and the mixture was extracted with $CHCl_3$. Then the $CHCl_3$ extract was subjected to Si gel MPLC with *n*-hexane−ethyl acetate (8:2) to give 3.3 mg of dimethyl cangorosin A as a colorless amorphous solid: UV (EtOH) λ max (log ϵ) 208 (4.78), 233 (4.62), 279 (4.14), 299 (3.86), 312 (3.78) nm; CD (EtOH) λ max ($\Delta\epsilon$) 311 (+5.8), 301 (+8.2), 284 (+22.7), 277 (+24.3), 239 (+39.5), 227 (+32.7), 215 (+32.0) nm; IR ($CHCl_3$) ν max 1733 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.69 (1H, s), 6.67 (1H, dd, J = 3.1, 9.9 Hz), 6.47 (1H, s), 5.89 (1H, dd, J = 2.7, 9.9 Hz), 4.98 (1H, dd, J = 3.0, 11.3 Hz), 4.88 (1H, d, J = 3.0 Hz), 3.87 (3H, s), 3.81 (3H, s), 3.63 (3H, s), 3.58 (3H, s), 2.49 (1H, br s), 2.41 (1H, d, J = 11.3 Hz), 2.37 (3H, s), 2.19 (3H, s), 1.35 (3H, s), 1.34 (3H, s), 1.20 (3H, s) × 2, 1.12 (3H, s), 1.11 (3H, s), 1.07 (3H, s), 1.03 (3H, s), 0.84 (3H, s), 0.79 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.58 (s), 179.38 (s), 153.53 (s), 146.96 (s), 145.93 (s), 143.68 (s), 141.65 (s), 139.15 (s), 132.48 (s), 128.66 (d), 124.31 (d), 124.15 (s), 123.31 (s), 122.08 (s), 108.02 (d), 105.60 (d), 74.01 (d), 71.53 (d), 60.37 (q), 55.75 (q), 51.63 (q), 51.51 (q), 45.77 (d), 44.98 (d), 44.55 (d), 43.63 (d), 40.85 (s), 40.70 (s), 40.64 (s), 40.11 (s), 39.69 (s), 39.14 (s), 39.01 (s), 37.56 (s), 37.13 (t), 36.62 (t), 36.22 (t), 36.12 (t), 34.74 (t), 31.58 (t) × 2, 30.78 (t) × 2, 32.12 (q), 30.08 (t) × 2, 32.05 (q), 31.91 (q), 30.78 (q), 30.55 (s), 30.39 (t), 29.88 (s), 29.53 (t), 28.44 (t), 28.07 (q), 22.41 (q), 18.43 (q), 17.83 (q), 17.36 (q), 17.22 (q), 11.35 (q), 10.87 (q); EIMS *m/z* $[M]^+$ 958 (27), 492 (47), 478 (23), 466 (36), 450 (24), 216 (100), 201 (75).

Isocangorosin A (3): colorless amorphous solid (15 mg); $[\alpha]_D$ +76.4° (*c* 0.28, $CHCl_3$); UV (EtOH) λ max (log ϵ) 209 (4.67), 229 (4.51), 283 (4.10), 303 (3.76), 313 (3.70) nm; CD (EtOH) λ max ($\Delta\epsilon$) 315 (+0.8), 300 (+1.4), 283 (+7.4), 274 (+9.9), 235 (+46.9), 210 (+15.0) nm; IR ($CHCl_3$) ν max 3605, 3555, 1724, 1602, 1579 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.68 (1H, s, H-1), 6.66 (1H, dd, J = 2.9, 9.9 Hz, H-6′), 6.56 (1H, s, H-1′), 5.90 (1H, dd, J = 2.6, 9.9 Hz, H-7′), 5.06 (1H, dd, J = 3.0, 11.2 Hz, H-7), 4.87 (1H, d, J = 3.0 Hz, H-6), 3.66 (3H, s, 29-COOMe), 3.58 (3H, s, 29′-COOMe), 2.49 (1H, br s, H-8′), 2.39 (1H, d, J = 11.2 Hz, H-8), 2.33 (3H, s, Me-23), 2.18 (3H, s, Me-23′), 1.35 (3H, s, Me-26), 1.32 (3H, s, Me-25), 1.20 (3H, s, Me-30′), 1.19 (3H, s, Me-30), 1.12 (3H, s, Me-28), 1.12 (3H, s, Me-28′), 1.07 (3H, s, Me-26′), 0.98 (3H, s, Me-25′), 0.88 (3H, s, Me-27′), 0.79 (3H, s, Me-27); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.47 (s, C-29′), 179.39 (s, C-29), 144.44 (s, C-2), 143.91 (s, C-10), 142.08 (s, C-2′), 141.93 (s, C-10′), 140.59 (s, C-3), 138.04 (s, C-3′), 128.86 (d, C-7′), 125.69 (s, C-5′), 124.68 (s, C-5), 124.41 (d, C-6′), 122.96 (s, C-4), 121.05 (s, C-4′), 108.18 (d, C-1), 108.18 (d, C-1′), 74.25 (d, C-7), 71.34 (d, C-6), 51.62 (q, 29-COOMe), 51.55 (q, 29′-COOMe), 45.93 (d, C-8′), 44.96 (d, C-8), 44.54 (d, C-18′), 43.91 (d, C-18), 40.69 (s, C-20), 40.63 (s, C-20′), 40.28 (s, C-9), 39.95[a] (s, C-14), 39.61[a] (s, C-13), 39.15[b] (s, C-14′), 39.01[b] (s, C-13′), 37.62 (s, C-9′), 36.90[c] (t, C-22), 36.67[d] (t, C-22′), 36.11[c] (t, C-16), 36.11[d] (t, C-16′), 34.62 (t, C-11), 32.11[e] (q, C-30′), 31.85[f] (q, C-28), 31.85[f] (q, C-28′), 31.30[e] (q, C-30), 31.11 (t, C-11′), 30.74[g] (d, C-19), 30.74[h] (t, C-19′), 30.48 (s, C-17′), 30.29 (t, C-12), 30.04 (t, C-12′), 30.04[h] (t, C-21′), 29.89 (s, C-17), 29.89 (t, C-15′), 29.68[g] (t, C-21), 28.47 (t, C-15), 28.25 (q, C-25), 22.25 (q, C-25′), 18.43 (q, C-27), 17.60 (q, C-27′), 17.13 (q, C-26), 17.13 (q, C-26′), 11.13 (q, C-23), 11.04 (q, C-23′) ([a−g]assignments for values bearing the same superscript may be reversed); EIMS/HRMS *m/z* $[M_{1/2} + H]^+$ 466 (27, calcd for $C_{30}H_{42}O_4$ 466.3083, found 466.3104), 464 (16), $[M_{1/2} - O + H]^+$ 450 (10, calcd for $C_{30}H_{42}O_3$ 450.3134, found 450.3124), $[M_{1/2} - COOMe]^+$ 406 (2, calcd for $C_{28}H_{38}O_2$ 406.2872, found 406.2859), 298 (9), 283 (5), 269 (10), 253 (11), 241 (16), 227 (21), 215 (12), 201 (58), 188 (100); FABMS (positive, added KI) *m/z* $[M + K]^+$ 969 (1.7), $[M + H]^+$ 931 (7.9), $[M]^+$ 930 (9.5), (positive) *m/z* $[M + H]^+$ 931 (20.3), $[M]^+$ 930 (27.7), (negative) *m/z* $[M]^+$ 930 (3.2), $[M - H]^+$ 929 (4.8).

Reaction of Isocangorosin A with TAI. By the same procedure described above for **2**, a small amount of **3** was reacted in an NMR sample tube with one drop of TAI. Two amide proton signals were produced by TAI, which gradually appeared in the ^1H-NMR spectrum.

Preparation of Dimethyl Isocangorosin A. 3 (3.9 mg) was methylated using the same procedure described above for **2**. The dimethyl derivative of **3** (2.8 mg) was purified by Si gel MPLC with *n*-hexane−ethyl acetate (8:2): colorless amorphous solid; UV (EtOH) λ max (log ϵ) 209 (4.80), 230 (4.69), 280 (4.24), 303 (3.90), 313 (3.85) nm; CD (EtOH) λ max ($\Delta\epsilon$) 283 (+7.5), 276 (+8.3), 232 (+41.4), 208 (+12.9) nm; IR ($CHCl_3$) ν max 1733 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.68 (1H, s), 6.66 (1H, dd, J = 3.0, 9.9 Hz), 6.55 (1H, s), 5.90 (1H, dd, J = 2.6, 9.9 Hz), 5.07 (1H, dd, J = 3.0, 11.3 Hz), 4.87 (1H, d, J = 3.0 Hz), 3.86 (3H, s), 3.80 (3H, s), 3.65 (3H, s), 3.58 (3H, s), 2.50 (1H, br s), 2.37 (3H, s), 2.30 (1H, d, J = 13.3 Hz), 2.15 (3H, s), 1.35 (3H, s), 1.34 (3H, s), 1.19 (3H, s), 1.19 (3H, s), 1.11 (3H, s) × 2, 1.06 (3H, s), 0.97 (3H, s), 0.87 (3H, s), 0.79 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.51 (s),179.40 (s), 153.47 (s), 146.90 (s), 145.88 (s), 142.06 (s), 141.86 (s), 138.0 (s), 132.42 (s), 128.92 (d), 125.61 (d), 124.34 (s), 123.00 (s), 120.98 (s), 108.19 (d), 105.35 (d), 74.15 (d), 71.26 (d), 60.31 (q), 55.72 (q), 51.66 (q), 51.59 (q), 45.76 (d), 44.74 (d), 44.51 (d), 43.89 (d), 40.71 (s), 40.62 (s), 40.58 (s), 39.99 (s), 39.50 (s), 39.07 (s), 38.98 (s), 37.56 (s), 36.78 (t), 36.56 (t), 36.07 (t) × 2, 34.51 (t), 32.20 (q), 31.87 (q), 31.82 (s), 31.82 (q), 31.32 (t), 31.24 (t), 30.69 (t) × 2, 30.69 (q), 30.43 (s), 30.19 (t), 30.02 (t), 29.84 (t), 29.84 (s), 29.66 (t), 28.21 (q), 22.24 (q), 18.43 (q) × 2, 17.53 (q), 16.99 (q), 11.29 (q), 11.04 (q); EIMS *m/z* $[M]^+$ 958 (25), 943 (2), 494 (62), 478 (36), 231 (52), 216 (100).

6′,7′-Dihydroisocangorosin A (4): colorless amorphous solid (10 mg); $[\alpha]_D$ +87.3° (*c* 0.45, $CHCl_3$); UV (EtOH) λ max (log ϵ) 211 (4.73), 233 (4.24), 251 (3.92), 260 (3.92), 287 (3.89), 313 (3.22) nm; CD (EtOH) λ max ($\Delta\epsilon$) 308 (+4.1), 259 (−4.5), 226 (+15.8), 214 (+50.9) nm; IR ($CHCl_3$) ν max 3605, 3556, 1724, 1603 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.69 (1H, s, H-1), 6.55 (1H, s, H-1′), 4.95 (1H, dd, J = 3.0, 11.2 Hz, H-7), 4.89 (1H, d, J = 3.0 Hz, H-6), 3.62 (3H, s, 29′-COOMe), 3.58 (3H, s, 29-COOMe), 2.73 (1H, dd, J = 5.8, 16.9 Hz, H-6′b), 2.57 (1H, m, H-6′a), 2.37 (1H, d, J = 11.2 Hz, H-8), 2.32 (3H, s, Me-23), 2.04 (3H, s, Me-23′), 1.32 (3H, s, Me-26), 1.30 (3H, s, Me-25), 1.21 (3H, s, Me-25′), 1.20[a] (3H, s, Me-30′), 1.19[a] (3H, s, Me-30), 1.12 (3H, s, Me-28′), 1.11 (3H, s, Me-28), 1.10 (3H, s, Me-27′), 0.95 (3H, s, Me-26′), 0.76 (3H, s, Me-27) ([a]assignments for values bearing the same superscript may be reversed); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.84 (s, C-29′), 179.38 (s, C-29), 145.07

(s, C-10′), 144.29 (s, C-2), 143.67 (s, C-10), 140.61 (s, C-3), 140.12 (s, C-2′), 138.86 (s, C-3′), 125.60 (s, C-4′), 124.59 (s, C-5), 123.63 (s, C-5′), 123.21 (s, C-4), 109.48 (d, C-1′), 108.21 (d, C-1), 73.69 (d, C-7), 71.37 (d, C-6), 51.64 (q, 29′-COOMe), 51.35 (q, 29′-COOMe), 44.71 (d, C-8), 44.45 (d, C-18′), 44.15 (d, C-8′), 43.59 (d, C-18), 40.63[a] (s, C-20), 40.59[a] (s, C-20′), 40.25 (s, C-9), 39.98[b] (s, C-14), 39.58[b] (s, C-13), 39.42[c] (s, C-14′), 39.02[c] (s, C-13′), 37.04[d] (t, C-22), 36.86 (s, C-9′), 36.64[e] (t, C-22′), 36.30[e] (t, C-16′), 36.13[d] (t, C-16), 34.67 (t, C-11), 34.34 (t, C-11′), 31.95 (q, C-28), 31.86 (q, C-28′), 31.75[f] (q, C-30), 31.36 (t, C-15), 30.77[f] (q, C-30′), 30.68[g] (t, C-21), 30.68[g] (t, C-21′), 30.37 (t, C-12), 30.30 (t, C-12′), 30.24 (s, C-17′), 29.95[g] (d, C-19), 29.77 (s, C-17), 29.47[g] (t, C-19′), 29.14 (t, C-15′), 28.05 (q, C-25), 28.05 (t, C-6′), 27.24 (q, C-25′), 18.47 (t, C-7′), 18.31 (q, C-27), 17.58 (q, C-26), 17.26 (q, C-27′), 16.09 (q, C-26′), 11.13 (q, C-23), 10.90 (q, C-23′) ([a−g]assignments for values bearing the same superscript may be reversed); EIMS/HRMS *m/z* $[MTb + H]^+$ 468 (41, calcd for $C_{30}H_{44}O_4$ 468.3240, found 468.3237), $[MTa + H]^+$ 466 (80, calcd for $C_{30}H_{42}O_4$ 466.3083, found 466.3084), $[MTb - O]^+$ 451 (5, calcd for $C_{30}H_{43}O_3$ 451.3212, found 451.3246), $[MTb - COOMe + H]^+$ 407 (2, calcd for $C_{28}H_{39}O_2$ 407.2950, found 407.2919), 298 (27), 283 (23), 269 (47), 241 (49), 215 (38), 201 (98), 188 (100); FABMS (positive, added KI) *m/z* $[M + K]^+$ 971 (1.1), $[M + H]^+$ 933 (5.2), $[M]^+$ 932 (7.8), (positive) *m/z* $[M]^+$ 932 (5.0), (negative) *m/z* $[M]^+$ 932 (8.3), $[M - H]^+$ 931 (7.3).

Reductions of 2 and 3. Compounds **2** and **3** each (ca. 1 mg) were dissolved in 0.5 mL of $CHCl_3$ and separately reacted with H_2 in the presence of PtO_2 (ca. 1 mg) for 12 h at room temperature. Each reaction mixture was filtered, and then the filtrate was subjected to HPLC using a YMC R-ODS-5 S-5 120A ODS column (5 × 250 mm, ODS, 10 mm; YMC Inc., Tokyo) and MeOH−EtOH (1:1) at 1 mL/min. Retention times of the dihydro derivatives of **2**, and **3** and compound **4** were 23.0, 13.5, and 13.5 min, respectively: the retention times of the dihydro derivative of **3** and that of compound **4** are identical.

References and Notes

(1) Itokawa, H.; Shirota, O.; Morita, H.; Takeya, K.; Tomioka, N.; Itai, A. *Tetrahedron Lett.* **1990**, *31*, 6881−6882.
(2) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *Chem. Lett.* **1995**, 101−102.
(3) Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *Tetrahedron* **1995**, *51*, 1107−1120.
(4) All studies were done on the original compounds isolated in the previous work (see ref 1), and the detailed isolation procedure is described in the Experimental Section of this paper.
(5) Harada, R.; Kakisawa, H.; Kobayashi, S.; Musya, M.; Nakanishi, K.; Takahashi, Y. *Tetrahedron Lett.* **1962**, 603−607.
(6) Nakanishi, K.; Gullo, V. P.; Miura, I.; Govindachari, T. R.; Viswanathan, N. *J. Am. Chem. Soc.* **1973**, *95*, 6473−6475.
(7) Goodlett, V. W. *Anal. Chem.* **1965**, *37*, 431−432.
(8) Gonzales, D. M. In *Catálogo de Plantas Medicinales Usadas en Paraguay*: Asuncion: Paraguay, 1970; p 111.

NP9604487