Cycloartane Triterpenoids from *Astragalus bicuspis*

M. Iqbal Choudhary,* Saleem Jan, Ahmed Abbaskhan, Syed Ghulam Musharraf, Samreen, Samina A. Sattar, and Atta-ur-Rahman

HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

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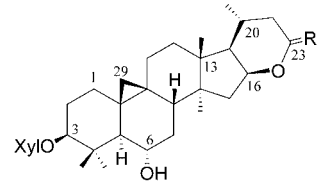
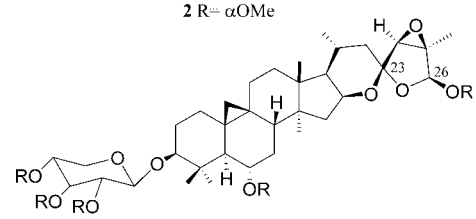
Three new cycloartane glycosides have been isolated from *Astragalus bicuspis.* They were identified as 6α-hydroxy-3-*O*-*β*-xylopyranosyloxy-24,25,26,27-tetranor-9,19-cyclolanosta-16,23-lactone (**1**), 6α -hydroxy-23-methoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-*β*-xyloside (**2**), and 23(*R*),24(*S*),25(*R*),26(*S*)-16*β*/23,23/26,24/25-triepoxy-6α,26-dihydroxy-9,19-cyclolanosta-3-*O*-*β*-xyloside (**3**), on the basis of their spectroscopic data. Two known cycloartane derivatives, **4** and **5**, were also obtained from this plant. Compounds **2**−**5** were tested for leishmanicidal activity against *Leishmania major* promastigotes and for cytotoxicity against 3T3 cancer cells.

Various species of *Astragalus* (Leguminosae) represent old and well-known drugs in traditional medicine used as antiperspirants, diuretics, and tonics,[1] and for the treatment of nephritis, diabetes, leukemia, and uterine cancer.[2] *Astragalus* plants have attracted considerable attention due to their cytotoxic constituents.[3] Some *Astragalus* constituents also stimulate lymphocyte transfer *in vitro,*[4] while some cycloartane-type triterpene glycosides, isolated from the genus *Astragalus*, have exhibited antitumor, immunodepressant, antiviral, and leishmanicidal activities.[5,6]

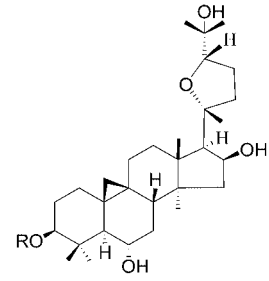
In continuation of our phytochemical studies on pharmacologically interesting natural products, we isolated three new cycloartane triterpene derivatives (**1**−**3**) and two known compounds (**4** and **5**) from a methanolic extract of *Astragalus bicuspis* Fisk. (Leguminosae). Compounds **2**−**5** were also screened for their activity against *Leishmania major* promastigotes and for cytotoxicity against 3T3 cancer cells.



1 R=O
2 R= αOMe



3 R=H
6 R=Ac



4 R=H
5 R=Xyl

Results and Discussion

The whole plant of *A. bicuspis* was extracted with 80% aqueous MeOH. The crude methanolic extract was fractionated by vacuum liquid chromatography (silica gel). The fraction eluted with 30% MeOH in $CHCl_3$ was chromatographed further on silica gel and polyamides to obtain compounds **1**−**5**.

Compound **1** was obtained as an amorphous solid. The $[M + H]^+$ peak in the ESI-QTOF-MS spectrum (*m*/*z* 549.3426) corresponded to the formula $C_{31}H_{49}O_8$. The MS-MS experiment afforded an ion at *m*/*z* 417 consistent with the loss of a pentose sugar. Other significant peaks were at *m*/*z* 399 $[M + H - \text{pentose} - H_2O]^+$ and 381 $[M + H - \text{pentose} - 2H_2O]^+$. The IR spectrum showed OH (3419 cm^{-1}) and lactone (1728 and 1254 cm^{-1}) absorptions.

The cycloartane-type skeleton was evident from ^1H NMR signals characteristic of cyclopropyl protons at δ_H 0.53 and 0.18 (each d, $J_{AX} = 3.6$ Hz, CH_2-19) and four tertiary methyls at δ_H 0.97 (CH_3-30), 1.01 (CH_3-18), 1.29 (CH_3-29), and 1.96 (CH_3-28), along with a secondary methyl at δ_H 0.92 (d, $J_{21,20} = 4.2$ Hz, CH_3-21). Two oxygen-bearing methine signals appeared at δ_H 3.61 (dd, $J_{3,2\beta} = 11.7$ Hz, $J_{3,2\alpha} = 3.7$ Hz, H-3) and 3.76 (m, H-6). The NMR data of compound **1** (see Experimental Section) were consistent with a tetranor-cycloartane 3-*O*-*β*-xyloside.[7,8] The ^{13}C NMR spectrum exhibited signals for 31 carbons, 26 of which were assigned to the aglycone moiety and five to a xylose unit. The ^{13}C NMR spectrum supported cyclolanostanol as the aglycone, with signals for the cyclopropane methylene carbon at δ_C 29.0 (C-19), five methyl carbons at δ_C 19.4 (C-18), 21.2 (C-21), 28.6 (C-28), 16.6 (C-29), and 19.9 (C-30), oxygen-bearing methine carbons at δ_C 88.5 (C-3), 67.0 (C-6), and 80.6 (C-16), and a lactone carbonyl carbon at δ_C 173.9 (C-23). Considering the degree of unsaturation and IR absorption bands at 1728 and 1254 cm^{-1}, the oxygenated carbon signal at δ_C 80.6 (C-16) was incorporated into a δ-lactone ring. The presence of a lactone was also deduced from the ^3J correlations of δ_H 2.41 and 2.21 (H-22) with the ester carbonyl carbon and also by ^3J correlation of the methine proton signal at δ_H 4.73 (H-16) with the ester carbonyl carbon. The presence of an OH at C-6 was inferred from its chemical shift (δ_C 67.0) and also from the ^2J correlations of H-5 (δ_H 1.75, d, $J_{5\alpha,\ 6\beta} = 8.1$ Hz) with C-6. The chemical shifts of the 4α-methyl protons CH_3-28 (δ_H 1.96) and C-5 (δ_C 53.7) were also consistent with this inferrence.[9] The

* Corresponding author. Tel: +92-21-4824924-5; 4819010. Fax: + 92-21-4819018-9. E-mail: hej@cyber.net.pk.

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orientation of H-6 was assigned to be β on the basis of a NOE between H-6 and CH_3-29 and between H-6 and H_2-19, indicating the presence of 6α-OH. The α-orientation of H-16 was deduced on the basis of a NOE between H-16, CH_3-30, and H-17α. The NMR spectra of **1** showed an anomeric proton at δ_H 4.90 (d, $J_{1',2'}$ = 7.1 Hz) with corresponding carbon at δ_C 107.6. Comparison of NMR data with those reported in the literature indicated a β-oriented xyloside.[10] Substitution of xylose at C-3 was inferred from the downfield chemical shift of C-3 (δ_C 88.5)[11] and HMBC cross-peaks between anomeric H-1′ and C-3. Thus, compound **1** was deduced to be 6α-hydroxy-3-*O*-β-xylopyranosyloxy-24,25,26,27-tetranor-9,19-cyclolanosta-16,23-lactone and was named bicusposide-A.

Compound **2** was isolated as a white, amorphous powder having the molecular formula $C_{32}H_{51}O_8$ (ESI-QTOF-MS showed a pseudomolecular ion at *m/z* 563.3622 $[M - H]^-$). The CID-MS experiment showed an $[M - H]^-$ ion at *m/z* 563 and a significant peak at *m/z* 431 $[M - H -$ pentose$]^-$, representing the aglycone. The NMR data of compound **2**, except for the lactone ring, were distinctly similar to the NMR spectra of compound **1** (see Experimental Section), except that **2** contained an acetal, instead of a lactone. This was inferred from the absence of lactone absorptions in the IR spectrum and the appearance of additional signals for a methoxy group [δ_H 3.35 (s) and δ_C 54.6] and an acetal methine carbon [δ_H 4.79 (t, $J_{23,22}$ = 7.2 Hz) and δ_C 100.4] in the ^{1}H NMR and ^{13}C NMR spectra. The other chemical shift differences between the two compounds were for C-16 [δ_C 80.6 for **1** and δ_C 70.7 for **2**] and C-23 [δ_C 173.9 for **1** and δ_C 100.4 for **2**]. α-Orientation of the methoxy group at C-23 was deduced from the ROESY spectrum, in which a methine signal at δ_H 4.34 (H-16) showed a cross-peak with the signal at δ_H 3.35 (OCH_3-23).

Thus, **2** was deduced to be 6α-hydroxy-23-methoxy-16β,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*-β-xyloside and was given the trivial name bicusposide-B.

Compound **3** was obtained as a colorless powder with a $[M - H]^-$ ion at *m/z* 633.3662 ($C_{35}H_{53}O_{10}$). The MS-MS showed a significant aglycone peak at *m/z* 501 $[M - H -$ pentose$]^-$. The IR spectrum showed an OH band at 3427 cm^{-1}. Cyclopropane methylene signals were observed in the ^{1}H NMR spectrum of compound **3** at δ_H 0.21 and 0.52 (each 1H, d, J_{AX} = 3.8 Hz), with a corresponding resonance at δ_C 29.7 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum also showed five tertiary methyl singlets at δ_H 1.18, 1.72, 1.94, 1.27, and 0.90, with corresponding carbons at δ_C 19.5, 13.2, 28.7, 16.6, and 20.2, respectively, in the ^{13}C NMR spectrum. A signal at δ_H 0.94 (CH_3-21, d, $J_{21,20}$ = 5.1 Hz) indicated a secondary methyl resonating at δ_C 20.6 in the ^{13}C NMR spectrum. Compound **3** had 16/23-, 23/26-, and 24/25-epoxy moieties (H-16: δ_H 4.57; C-16: δ_C 74.5; C-23: δ_C 105.9; H-26: δ_H 5.80; C-26: δ_C 97.7; C-23: δ_C 105.9; H-24: δ_H 3.82; C-24: δ_C 64.1; C-25: δ_C 63.7), hemiacetal moiety (H-26: δ_H 5.80; C-26: δ_C 97.7; C-23: δ_C 105.9), and a β-xylopyranosyl moiety (H-1′: δ_H 4.88, d, $J_{1',2'}$ = 7.3 Hz; C-1: δ_C 107.6, other signals: δ_H 3.71−4.34; δ_C 67.0−78.5).

Acetylation of compound **3** gave a penta-acetate (**6**, $C_{45}H_{64}O_{15}$), indicating that **3** had five OH groups: three of the xylose and two of the aglycone. A multiplet at δ_H 3.70 in the ^{1}H NMR spectrum of compound **3** displayed a shift to δ_H 4.97 in the penta-acetate (**6**), so the fourth OH was assigned at C-6. Similarly, a peak at δ_H 6.56 was due to the hemiacetal proton.

The relative configuration of **3** was determined on the basis of NOE experiments. The signal at δ 4.57 (H-16) showed cross-peaks with the signals at δ 0.90 (H-30) and 1.65 (H-17α) in the ROESY spectrum, indicating β-orientation of the 16-OH. The β-orientation of H-6 was deduced from NOEs between H-6 and 29-CH_3 and between H-6 and H_2-19. The methine proton at δ_H 3.82 (H-24) showed cross-peaks with the signals at δ_H 1.72 (CH_3-27), 1.18 (CH_3-18), 1.94 (H-20), and 5.80 (H-26) in the ROESY spectrum of compound **3**. Thus, the configurations at C-23, C-24, C-25, and C-26 were tentatively assigned as *R*, *S*, *R*, and *S*, respectively, by comparing with CH_3-18 and H-20 and assuming the same configuration at CH_3-18 and H-20 as in other compounds in this series based on biogenetic considerations. This assumption was supported by NOE difference spectra, in which irradiation of 18-CH_3 resulted in an increase in the signal intensity of the H-24.[12] On the basis of these observations, the structure of compound **3** was deduced to be 23(*R*),24(*S*),25(*R*),26(*S*)-16β/23,23/26,24/25-triepoxy-6α,26-dihydroxy-9,9-cyclolanosta-3-*O*-β-xyloside and was named bicusposide-C.

Compound **4** was identical in all respects to cyclosiversigenin, while compound **5** was identified as 3-*O*-β-D-xylocyclosiversigenin, both reported earlier from *Astragalus* species.[13]

Compounds **2**−**5** were evaluated for their leishmanicidal and cytotoxic potential. Amphtericine B (IC_{50} 0.54 ± 0.02 μmol) and pentamidine (IC_{50} 4.32 ± 0.09 μmol) were used as standard drugs in the leishmanicidal assay. Compounds **3** and **5** showed only modest leishmanicidal activity (IC_{50} 64.35 ± 0.60 μmol for **3** and 56.51 ± 0.28 μmol for **5**). These compounds were also screened for cytotoxicicty against 3T3 fibroblast cells, and moderate cytotoxicity was exhibited by compound **5** (IC_{50} 19.51 ± 5.3 μmol), as compared to the standard drug, cycloheximide (IC_{50} 0.912 μmol).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a digital JASCO DIP-360 polarimeter in MeOH. IR spectra were recorded as KBr discs on a JASCO A-302 spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AV-300 spectrometer operating at 300 (^{1}H NMR). The ^{13}C NMR spectra were recorded on Bruker AV-300 and AV-400 spectrometers operating at 75 and 100 MHz, respectively. HMQC and HMBC spectra were recorded on a Bruker AV-400 spectrometer. Chemical shifts are reported in δ (ppm), referenced with respect to the residual solvent signal of C_5H_5N, and coupling constants (*J*) were measured in Hz. Mass spectra were recorded on a Q-STAR XL (Applied Biosystem). Each compound (4 μg/mL, dissolved in acetonitrile/0.1% $HCOOH_{aq}$ (2:1)) was directly infused into the mass spectrometer at a flow rate of 3 μL/min to acquire full scan and product ion mass spectra. The electrospray voltage at the spraying needle was optimized at 5200 and −5200 V for positive and negative modes of ionization, respectively. Low-energy collision-induced dissociation (CID) experiments were performed by using nitrogen (CID gas valve set to 4) as collision gas, and a collision energy of 10−40 eV was used. TLC was performed on precoated silica gel plates (DC-Alugram 60 UV_{254}, E. Merck), and the spots were observed, first under UV light (254 nm) and then stained with cerium(IV) sulfate spray reagent and heated until coloration developed. Polyamide-6 DF (Riedel-De Haen AG) and silica gel (E. Merck, 160−200 μm mesh) were used as stationary phases in column chromatography (CC).

Plant Material. The whole plant of *A. bicuspis* was collected in Khaltaran-Haramosh, Gilgit (Pakistan), in July 2003. A voucher specimen (#67854) was deposited at the Herbarium of the Department of Botany, University of Karachi, Karachi.

Extraction and Isolation. The collected plant material was kept in the dark and air-dried for 3 days. The air-dried plant material (2 kg) was chopped into thick pieces and extracted with 80% aqueous MeOH (3 × 20 L, each soaking was continued for 1 week). The resulting extract was filtered and concentrated. The crude methanolic extract (120 g) was subjected to vacuum liquid chromatography (VLC) on silica gel (1000 g, 160−200 μm). Elution was carried out with solvents of increasing polarity: 30% $CHCl_3$/hexane (5 L × 3), 50% $CHCl_3$/hexane (5 L × 2), 70% $CHCl_3$/hexane (5 L × 3), $CHCl_3$ (5 L × 3), 30% MeOH/$CHCl_3$ (10 L × 3), 50% MeOH/$CHCl_3$ (10 L × 2), 100% MeOH (15 L). Seven main fractions (1−7) were obtained. Fraction 5 (40 g, eluted with 30% MeOH/$CHCl_3$), rich in saponins, was separated by CC on silica gel eluted successively with acetone/hexane (20:80; 30:70; 40:60; 50:50; 60:40; 80:20; 100:0) to afford seven subfractions (A−G). Subfraction A (3 g, eluted with 20% acetone/hexane) was subjected to silica gel CC (MeOH/$CHCl_3$, 2:98), which afforded compound **4**. Subfraction E (9 g, eluted with 60% acetone/hexane) was subjected to polyamide CC with $CHCl_3$/MeOH as eluting solvents in a gradient manner, which yielded nine fractions (1′−9′). Fraction 5′ (4 g, eluted with 2% MeOH/$CHCl_3$), after silica gel CC (MeOH/$CHCl_3$, 1:10), yielded compound **5**. Two fractions, 8′ (3 g, eluted with 3%

MeOH/$CHCl_3$) and 9′ (3 g, eluted with 3% MeOH/$CHCl_3$), were combined on the basis of similar TLC behavior and rechromatographed over silica gel (MeOH/$CHCl_3$, 1:10) to obtain compounds **1**−**3**.

Bicusposide A (1): amorphous solid (3 mg); $[\alpha]^{25}_D$ −4.0 (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1} 3419 (OH), 1728, 1254 (lactone); ESI-QTOF-MS-MS on *m/z* 549 $[M + H]^+$ (ce 15 eV) *m/z* (%) 549 (18), 531 (2), 417 (65), 399 (100), 381 (79), 363 (7), 277 (6), 123 (10); ^1H NMR (300 MHz, C_5D_5N) δ_H 4.73 (1H, q, $J_{16,15\text{ and }17}$ = 7.0 Hz, H-16), 3.76 (1H, m, H-6), 3.61 (1H, dd, $J_{3,2\beta}$ = 11.7 Hz, $J_{3,2\alpha}$ = 3.7 Hz, H-3), 2.41 (1H, ovlp, H-2a), 2.41 (1H, ovlp, H-22a), 2.21 (1H, ovlp, H-22b), 2.01 (1H, m, H-15a), 2.00 (1H, ovlp, H-2b), 1.96 (3H, s, H-28), 1.92 (1H, ovlp, H-17), 1.91 (1H, m, H-20), 1.87 (1H, ovlp, H-8), 1.82 (1H, m, H-11a), 1.77 (1H, m, H-7a), 1.75 (1H, d, $J_{5\alpha,6\beta}$ = 8.1 Hz, H-5), 1.69 (1H, m, H-15b), 1.64 (1H, m, H-1a), 1.59 (1H, m, H-7b), 1.49 (1H, m, H-12a), 1.38 (1H, m, H-12b), 1.29 (3H, s, H-29), 1.25 (1H, m, H-11b), 1.22 (1H, m, H-1b), 1.01 (3H, s, H-18), 0.97 (3H, s, H-30), 0.92 (3H, d, $J_{21,20}$ = 4.2 Hz, CH_3-21), 0.18, 0.53 (1H each, d, J_{AX} = 3.6 Hz, H-19a and 19b, respectively); sugar moiety δ_H 4.90 (1H, d, $J_{1',2'}$ = 7.1 Hz, H-1′), 4.36 (1H, dd, $J_{5'a,4'}$ = 10.9 Hz, $J_{5'a,5'b}$ = 4.8, H-5′a), 4.22 (1H, ovlp, H-4′), 4.15 (1H, t, $J_{3',2'\text{ and }4'}$ = 8.9 Hz, H-3′), 4.07 (1H, br t, H-2′), 3.69 (1H, ovlp, H-5′b); ^{13}C NMR (100 MHz, C_5D_5N) δ_C 173.9 (C, C-23), 88.5 (CH, C-3), 80.6 (CH, C-16), 67.0 (CH, C-6), 54.1 (CH, C-17), 53.7 (CH, C-5), 46.6 (C, C-13), 45.7 (CH, C-8), 44.8 (C, C-14), 43.4 (CH_2, C-15), 42.6 (C, C-4), 38.8 (CH_2, C-22), 37.8 (CH_2, C-7), 32.3 (CH_2, C-1), 32.3 (CH_2, C-12), 30.2 (CH_2, C-2), 29.1 (C, C-10), 29.0 (CH_2, C-19), 28.6 (CH_3, C-28), 27.3 (CH, C-20), 26.0 (CH_2, C-11), 21.2 (CH_3, C-21), 21.1 (C, C-9), 19.9 (CH_3, C-30), 19.4 (CH_3, C-18), 16.6 (CH_3, C-29); sugar moiety δ_C 107.6 (CH, C-1′), 78.6 (CH, C-3′), 75.6 (CH, C-2′), 71.3 (CH, C-4′), 67.1 (CH_2, C-5′); ESI-QTOF-MS, *m/z* 549.3426 $[M + H]^+$ ($C_{31}H_{49}O_8$, calcd for 549.3477).

Bicusposide B (2): amorphous solid (6 mg); $[\alpha]^{25}_D$ −4.4 (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1} 3418 (OH); ESI-QTOF-MS-MS on *m/z* 563 $[M - H]^-$ (ce −20 eV) *m/z* (%): 563 (13), 431 (3), 413 (100), 395 (13), 89 (13); ^1H NMR (300 MHz, C_5H_5N) δ_H 4.79 (1H, t, $J_{23,22}$ = 7.2 Hz, H-23), 4.34 (1H, ovlp, H-16), 3.75 (1H, m, H-6), 3.62 (1H, dd, $J_{3,2\beta}$ = 11.4 Hz, $J_{3,2\alpha}$ = 4.4 Hz, H-3), 3.35 (3H, s, OCH_3), 2.39 (1H, ovlp, H-2a), 2.00 (1H, ovlp, H-2b), 1.96 (3H, s, H-28), 1.92 (1H, dd, $J_{8\beta,7\alpha}$ = 10.6 Hz, $J_{8\beta,7\beta}$ = 4.5 Hz, H-8), 1.92 (1H, m, H-15a), 1.89 (1H, m, H-11a), 1.89 (1H, m, H-20), 1.84 (1H, ovlp, H-22a), 1.73 (1H, d, $J_{5\alpha,6\beta}$ = 8.9 Hz, H-5), 1.69 (1H, m, H-7a), 1.68 (1H, m, H-7b), 1.64 (1H, m, H-1a), 1.62 (m, H-17), 1.61 (1H, ovlp, H-22b), 1.59 (1H, m, H-15b), 1.49 (1H, m, H-12a), 1.30 (3H, s, H-29), 1.24 (1H, m, H-11b), 1.24 (1H, m, H-12b), 1.21 (1H, m, H-1b), 1.13 (3H, s, H-18), 0.96 (3H, s, H-30), 0.85 (3H, d, $J_{21,20}$= 6.3 Hz, H-21), 0.22, 0.54 (1H each, d, J_{AX} = 4.0 Hz, H-19a and 19b, respectively); sugar moiety δ_H 4.90 (1H, d, $J_{1',2'}$ = 7.2 Hz, H-1′), 4.36 (1H, dd, $J_{5'a,4'}$ = 11.2 Hz, $J_{5'a,5'b}$ = 5.2 Hz, H-5′a), 4.21 (1H, ovlp, H-4′), 4.14 (1H, t, $J_{3',2'\text{ and }4'}$ = 8.5 Hz, H-3′), 4.05 (1H, br t, H-2′), 3.71 (1H, ovlp, H-5′b); ^{13}C NMR (75 MHz) δ_C 100.4 (C, C-23), 88.6 (CH, C-3), 70.7 (CH, C-16), 67.4 (CH, C-6), 56.6 (CH, C-17), 54.6 (OCH_3), 53.9 (CH, C-5), 46.2 (CH, C-8), 46.1 (C, C-13), 44.8 (C, C-14), 43.5 (CH_2, C-15), 42.7 (C, C-4), 38.5 (CH_2, C-22), 37.9 (CH_2, C-7), 33.3 (CH_2, C-12), 32.4 (CH_2, C-1), 30.3 (CH_2, C-2), 29.6 (CH_2, C-19), 29.2 (C, C-10), 28.7 (CH_3, C-28), 26.2 (CH_2, C-11), 25.6 (CH, C-20), 21.2 (C, C-9), 20.5 (CH_3, C-21), 20.2 (CH_3, C-30), 19.5 (CH_3, C-18), 16.6 (CH_3, C-29); sugar moiety δ_C 107.6 (CH, C-1′), 78.5 (CH, C-3′), 75.6 (CH, C-2′), 71.3 (CH, C-4′), 67.1 (CH_2, C-5′); ESI-QTOF-MS, *m/z* 563.3622 $[M - H]^-$ ($C_{32}H_{51}O_8$, calcd for 563.3589).

Bicusposide C (3): amorphous solid (10 mg); $[\alpha]^{25}_D$ −4.8 (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1} 3427 (OH); ESI-QTOF-MS-MS on *m/z* 633 $[M - H]^-$ (ce 40 eV) *m/z* (%) 633 (17), 549 (94), 501 (23), 483 (23), 417 (88), 401 (20), 131 (23), 111 (26), 83 (64), 71 (100); ^1H NMR (300 MHz, C_5H_5N) δ_H 5.80 (1H, s, H-26), 4.57 (1H, q, $J_{16,15\text{ and }17}$ = 7.4 Hz, H-16), 3.82 (1H, s, H-24), 3.70 (1H, m, H-6), 3.59 (1H, dd, $J_{3,2\beta}$ = 11.5 Hz, $J_{3,2\alpha}$ = 4.6 Hz Hz, H-3), 2.40 (1H, ovlp, H-2a), 2.19 (1H, ovlp, H-22a), 1.98 (1H, m, H-15a), 1.95 (1H, ovlp, H-2b), 1.94 (3H, s, H-28), 1.94 (1H, m, H-20), 1.88 (1H, m, H-11a), 1.87 (1H, dd, $J_{8\beta,7\alpha}$ = 11.4 Hz, $J_{8\beta,7\beta}$ = 5.2 Hz, H-8), 1.75 (1H, m, H-7a), 1.72 (3H, s, H-27), 1.72 (1H, ovlp, H-5), 1.65 (1H, m, H-17), 1.64 (1H, ovlp, H-22b), 1.62 (1H, m, H-1a), 1.58 (1H, m, H-15b), 1.57 (1H, m, H-7b), 1.50 (1H, m, H-12a), 1.27 (3H, s, H-29), 1.22 (1H, m, H-12b), 1.21 (1H, m, H-1b), 1.20 (1H, m, H-11b), 1.18 (3H, s, H-18), 0.94 (3H, d, $J_{21,20}$ = 5.1 Hz, H-21), 0.90 (3H, s, H-30), 0.21, 0.52 (1H each, d, J_{AX} = 3.8 Hz, H-19a and 19b, respectively); sugar moiety δ_H 4.88 (1H, d, $J_{1',2'}$ = 7.3 Hz, H-1′), 4.34 (1H, dd, $J_{5'a,4'}$ = 10.9 Hz, $J_{5'a,5'b}$ = 4.8 Hz, H-5′a), 4.20 (1H, ovlp, H-4′), 4.14 (1H, t, $J_{3',2'\text{ and }4'}$ = 8.4 Hz, H-3′), 4.05 (1H, br t, H-2′), 3.71 (1H, ovlp, H-5′b); ^{13}C NMR (75 MHz, C_5D_5N) δ_C 105.9 (C, C-23), 97.7 (CH, C-26), 88.6 (CH, C-3), 74.5 (CH, C-16), 67.4 (CH, C-6), 64.1 (CH, C-24), 63.7 (C, C-25), 56.4 (CH, C-17), 53.7 (CH, C-5), 46.2 (C, C-13), 46.1 (CH, C-8), 44.7 (C, C-14), 43.7 (CH_2, C-15), 42.6 (C, C-4), 42.5 (CH_2, C-22), 38.2 (CH_2, C-7), 33.2 (CH_2, C-12), 32.4 (CH_2, C-1), 30.3 (CH_2, C-2), 29.7 (CH_2, C-19), 29.2 (C, C-10), 28.7 (CH_3, C-28), 26.2 (CH, C-20), 26.1 (CH_2, C-11), 21.1 (C, C-9), 20.6 (CH_3, C-21), 20.2 (CH_3, C-30), 19.5 (CH_3, C-18), 16.6 (CH_3, C-29), 13.2 (CH_3, C-27); sugar moiety δ_C 107.6 (CH, C-1′), 78.5 (CH, C-3′), 75.6 (CH, C-2′), 71.3 (CH, C-4′), 67.0 (CH_2, C-5′); ESI-QTOF-MS, *m/z* 633.3662 $[M - H]^-$ ($C_{35}H_{53}O_{10}$, calcd for 633.3644).

Acetylation of 3. Compound **3** was acetylated with Ac_2O/pyridine at room temperature. The crude product (8 mg) was subjected to CC on silica gel with hexane/EtOAc (90:10) to yield compound **6** (3 mg): colorless powder, $C_{45}H_{64}O_{15}$, $[\alpha]^{25}_D$ −14.4 (*c* 0.125, MeOH); IR ν_{max} (KBr) cm^{-1} 3408 (OH), 1728 (ester); ^1H NMR (600 MHz, C_5H_5N) δ_H 6.56 (1H, s, H-26), 4.97 (1H, m, H-6), 4.44 (1H, q, $J_{16,15\text{ and }17}$ = 7.4 Hz, H-16), 3.87 (1H, s, H-24), 3.34 (1H, dd, $J_{3,2\beta}$ = 11.6 Hz, $J_{3,2\alpha}$ = 4.4 Hz, H-3), 1.51 (3H, s, H-27), 1.05 (3H, s, H-28), 1.03 (3H, s, H-18), 1.00 (3H, s, H-29), 0.96 (3H, d, $J_{21,20}$ = 6.0 Hz, CH_3-21), 0.89 (3H, s, H-30), 2.13, 2.10, 2.08, 2.03, 1.96 (3H, each, s, 5 × OAc), 0.14, 0.47 (1H each, d, J_{AX} = 4.5 Hz, H-19a and 19b, respectively); sugar moiety δ_H 5.72 (1H, t, $J_{3',2'\text{ and }4'}$ = 9.1 Hz, H-3′), 5.46 (1H, t, $J_{2',1'\text{ and }3'}$ = 8.4 Hz, H-2′), 5.32 (1H, m, H-4′), 4.88 (1H, d, $J_{1',2'}$ = 7.4, H-1′), 4.33 (1H, dd, $J_{5'a,4'}$ = 11.5 Hz, $J_{5'a,5'b}$ = 5.2 Hz, H-5′a), 3.70 (1H, ovlp, H-5′b); ^{13}C NMR (600 MHz, C_5D_5N) δ_C 103.4 (C, C-23), 96.1 (CH, C-26), 87.8 (CH, C-3), 74.7 (CH, C-16), 62.8 (CH, C-24), 62.5 (CH, C-6), 62.0 (C, C-25), 55.7 (CH, C-17), 49.4 (CH, C-5), 45.9 (C, C-13), 44.7 (C, C-14), 43.9 (CH, C-8), 43.0 (CH_2, C-15), 41.8 (C, C-4), 40.9 (CH_2, C-22), 32.9 (CH_2, C-7), 32.5 (CH_2, C-12), 31.4 (CH_2, C-1), 29.4 (CH_2, C-2), 29.2 (C, C-10), 27.0 (CH_2, C-19), 26.1 (CH_3, C-28), 25.9 (CH, C-20), 25.8 (CH_2, C-11), 21.1 (C, C-9), 20.6 (CH_3, C-21), 19.6 (CH_3, C-18), 19.1 (CH_3, C-30), 16.1 (CH_3, C-29), 12.3 (CH_3, C-27); sugar moiety δ_C 103.4 (CH, C-1′), 72.5 (CH, C-3′), 72.2 (CH, C-2′), 69.8 (CH, C-4′), 62.4 (CH_2, C-5′), 170.3, 170.2, 170.0, 169.9, 169.6 (5 × acetoxy carbonyls), 25.9, 21.7, 20.5, 20.4, 20.4 (5 × acetoxy methyls).

***In Vitro* Leishmanicidal Activity.** *Leishmania major* (DESTO) promastigotes were grown at 22−25 °C in RPMI-1640 medium[14] (Sigma) containing 10% heat-inactivated fetal bovine serum (FBS). Logrithmic phase of growth was maintained, and the final concentration of parasites was adjusted to 1 × 10^6 cells/mL. The test compound (1 mg) was dissolved in 50 μL of DMSO. Then the volume was adjusted to 1 mL with complete media. In a 96-well microtiter plate, 180 μL of medium was added in different wells. Then 20 μL of the test compound was added in the medium and serially diluted. A total of 100 μL of parasite suspension was added into each well of the 96-well plates. Plates were incubated at 21−22 °C for 72 h. Cell viability was examined microscopically on an improved Neubauer counting chamber, and IC_{50} values of compounds possessing antileishmanial activity were calculated by Ezfit 5.03 (Perrella Scientific software). All assays were run in duplicate. Compound **1** was not screened due to insufficient quantity.

Cytotoxicity. Cytoxicity of compounds was evaluated in 96-well flat-bottom microplates using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay.[15,16] For this purpose, 3T3 cells (mouse fibroblasts) were cultured in Dulbecco's modified Eagle's medium, supplemented with 5% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin by using a 25 cm^2 flask, in a 5% CO_2 incubator at 37 °C. Exponentially growing cells were harvested, counted with a hemocytometer, and diluted with a particular medium. Cell cultures with a concentration of 3 × 10^4 cells/mL were prepared and were plated (100 μL/well) onto 96-well plates. After overnight incubation, medium was removed and 200 μL of fresh medium was added with different concentrations of compounds (1−100 μmol). After 72 h, 50 μL of MTT (2 mg/mL) was added to each well and incubation was continued for 4 h. Subsequently, 100 μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measurement of the absorbance at 540 nm using a microplate ELISA reader. Cytotoxicity was recorded as the concentration causing 50% growth inhibition.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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