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Full Papers

Acutangulosides A−F, Monodesmosidic Saponins from the Bark of *Barringtonia acutangula*

Clive Mills, Anthony R. Carroll, and Ronald J. Quinn*

Natural Product Discovery, Eskitis Institute, Griffith University, Brisbane, Queensland, Australia 4111

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Nine triterpene saponins, acutangulosides A−F (**2**−**7**), and acutanguloside D−F methyl esters (**5a**−**7a**) and a single triterpene aglycone (**1**) were isolated from a water extract of the bark of *Barringtonia acutangula*. Their structures were assigned on the basis of spectroscopic data.

Barringtonia acutangula Gaertn. is a tree or shrub, ranging in size from 2 m to in excess of 25 m, which is widely distributed in the tropical regions of Asia, Malaysia, and the Pacific.[1] Throughout its range *B. acutangula* has been used in a variety of ways by local peoples. These include use as fish poisons,[2−11] in construction (*B. acutangula* was sold under the tradename of Indian Oak),[8,10,12] and as medicines.[4,6,10,13,14] All parts of the plant have been used in applications for both internal and external ailments. So extensive has been the use of seeds from *B. acutangula* that it has been called "nurse fruit".[5] Preparation for application may involve drying and powdering, extraction with hot or cold water, heating, or juicing.[4−8,10,11,13−15]

As fish poisons, *Barringtonia* species, in particular *B. acutangula*,[2] were used extensively in Australia; however it seems that little use was made of *Barringtonia* species as medicines compared with other regions in which the plants are located. In Australia, *B. acutangula* extracts have been used for skin complaints (e.g., wounds, boils, chickenpox), ophthalmia, colic, and parturition and to induce vomiting.[6,8,13,14] More detailed accounts of the uses of *Barringtonia* sp. can be found in the literature.[4,10,14]

Given the widespread use of *B. acutangula* it is somewhat surprising that the chemistry of this tree remains largely unexplored. Most of the publications concerning the chemical constituents of *B. acutangula*, and indeed of many other *Barringtona* species, report the isolation and characterization of sapogenins. The presence of high concentrations of sapogenins has been reported from fruit,[11,16−25] seeds,[16,26,27] leaves,[26,28−30] branch wood,[11,18] heartwood,[11,25,31−33] and bark[26] of *B. acutangula*. It was not until 1991 that the structure of an intact saponin from *B. acutangula* was published. Spectroscopic and chemical data led to the structure being assigned as 2α,3β,19α-trihydroxyolean-12-enedioic acid 28-*O*-β-D-glucopyranoside.[34] Shortly after this publication, the complete structures of three more saponins from the seeds of *B. acutangula*, barringtosides A, B, and C, were published.[35]

There is anecdotal evidence from a group of Aboriginal people living in the Kimberley districts of North Western Australia that the bark of *B. acutangula* has potent analgesic properties. The bark is chewed to form a paste, which is applied externally to wounds, after which pain is said to be relieved quickly and the wound to heal rapidly.

This work reports the isolation and structural assignment of novel monodesmosidic saponins from water extracts of the bark of *B. acutangula* ssp. *acutangula*.

Results and Discussion

A water extract of the dried bark of *B. acutangula* was subjected to preparative and semipreparative HPLC pu-

* To whom correspondence should be addressed. Tel: 61 7 3875 6006. Fax: 61 7 3875 6001. E-mail: R.Quinn@griffith.edu.au.

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rification using C18 and phenyl-bonded silica to give one aglycone and nine new monodesmosidic triterpenoid saponins.

Compound **1** (0.7 mg, amorphous white solid) had a molecular formula of $C_{30}H_{46}O_7$, as determined by negative ion high-resolution electrospray mass spectrometry ((−)-HRESMS) (*m/z* 517.3164, $[M - H]^-$, Δ 0.2 ppm). The structure of **1** was unambiguously assigned using NMR spectroscopy (1D and 2D). Spectroscopic comparison of **1** with 2α,3β,19α-trihydroxy-olean-12-ene-23,28-dioic acid 28-*O*-β-D-glucopyranoside, which was previously isolated from *B. acutangula*,[34] showed that **1** differed only in the lack of a C_{28} glucopyranoside moiety. Therefore compound **1** was assigned as 2α,3β,19α-trihydroxy-olean-12-ene-23,28-dioic acid (**1**).

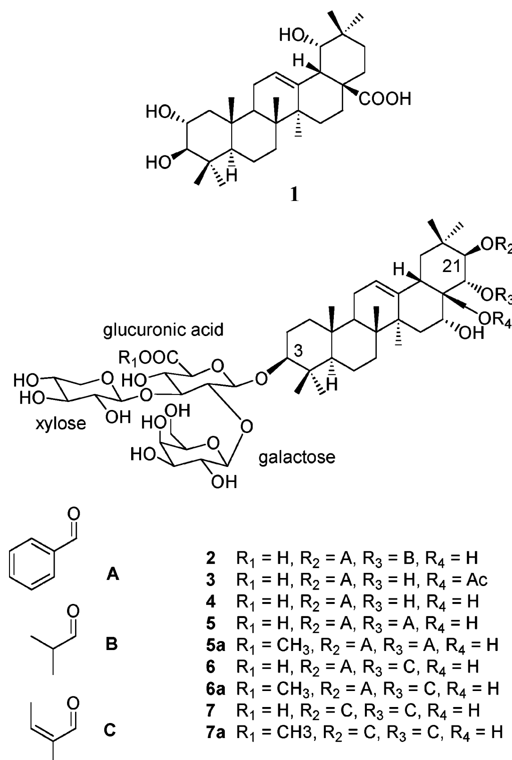
The nine new saponins all possessed the aglycone, barringtogenol C, with three sugars attached at C-3 together with a variety of ester substituents attached directly to the aglycone.

Acutanguloside A (**2**) was isolated as an amorphous white powder. High-resolution negative electrospray mass measurement of the pseudomolecular ion $(M - H^+)^-$ indicated a molecular formula $C_{58}H_{86}O_{22}$ for **2**. The infrared spectrum of **2** had absorption bands between 1740 and 1680, indicating acid and ester carbonyls were present in the molecule. The ^{13}C NMR spectrum of **2** confirmed that three ester or acid carbonyl carbons (175.8, 170.0, and 165.8 ppm) were present. The spectrum also indicated that two olefinic (142.6 and 122.9 ppm), six aromatic (132.6, 130.5, 129.2 (2×C), and 128.4 (2×C) ppm), three anomeric (103.6, 103.1, and 102.5 ppm), and 18 oxygenated carbons were present in the molecule. A DEPT-135 spectrum allowed 47 protonated carbons to be assigned including 9 methyls, 7 aliphatic methylenes, 3 oxygenated methylenes, 4 aliphatic methines, 15 oxygenated methines, 3 anomeric methines, 1 olefinic methine, and 5 aromatic methines. The ^1H NMR spectrum of **2** in DMSO-d_6 was broadly divided into three regions. The most shielded region (δ 0.00−2.20) showed seven methyl singlets (δ 1.06, 1.42, 0.99, 0.90, 0.85, 0.82, and 0.76), two of which integrated for six protons each (δ 0.75 and 0.85), a midfield region (δ 2.20−6.00) that contained three doublets characteristic of anomeric protons (δ 4.67, 4.54, and 4.48), one trisubstituted olefinic proton at δ 5.30 ppm and a large number of oxygenated methylene and methine protons, and finally the third, most deshielded region (δ 7.40−8.00), which contained three aromatic signals at δ 7.88, 7.60, and 7.52, which could be assigned to a monosubstituted phenyl group.

The structural assignment of acutanguloside A (**2**) began with the carbon at 54.9 ppm, located as a relatively isolated peak in the ^{13}C NMR spectrum, which was directly coupled to a proton at δ 0.72. Long-range coupling was observed in the gHMBC spectrum between this carbon and methyl protons at δ 0.99, 0.76, and 0.90, which were directly coupled to carbons at 26.9, 15.7, and 15.0 ppm, respectively. In addition, cross-peaks were observed in the gHMBC spectrum between the protons at δ 0.99 and 0.76 and carbons at 89.0 and 38.8 ppm and also between the proton at δ 0.90 and carbons at 36.0, 38.1, and 46.1 ppm. The carbon at 46.1 ppm was directly coupled to a methine proton at δ 1.57, while the absence of the carbons at 36.0 and 38.8 ppm in the DEPT-135 spectrum suggested quaternary carbons. The carbon at 89.0 ppm was directly coupled to a proton at δ 3.07 ppm, which in turn showed long-range coupling to a carbon at 103.6 ppm. A cross-peak in the gHSQC spectrum indicated that this carbon was directly coupled to an anomeric proton at 4.48 ppm. Comparison of these chemical shifts with similar compounds reported in the literature[34−36] suggested that the carbon at 89.0 ppm was C-3 of the aglycone and that this carbon was the point of attachment of the sugar moiety. H-3 was coupled to two methylene protons at δ 1.56 and 1.72, which were in turn coupled to two more methylene protons at δ 0.93 and 1.54 in the dqfCOSY spectrum. These proton pairs were directly coupled in the gHSQC spectrum to carbons at 25.3 and 38.1 ppm, respectively. Given the preceding information, it was possible to assign the carbon chemical shifts as C-1 (38.1 ppm), C-2 (25.3 ppm), C-3 (89.0 ppm), C-4 (38.8 ppm), C-5 (54.9 ppm), C-9 (46.1 ppm), C-10 (36.0 ppm), C-23 (26.9), C-24 (15.7 ppm), and C-25 (15.0 ppm). The close proximity of the methyl groups at positions H-23 and H-24 on the aglycone was supported by cross-peaks in the gHMBC spectrum between H-23/C24 and H-24/C23. The proton at δ 0.72 was coupled to two methylene protons at δ 1.24 and 1.44 (H-6a/b), which in turn were coupled to methylene protons at δ 1.26 and 1.50 (H-7a/b). These proton pairs were directly coupled to carbons at 17.2 (C-6) and 32.1 (C-7). The carbon at C-7 was coupled to a methyl proton at δ 0.85 in the gHMBC spectrum, which in turn was directly coupled to a carbon at 16.2 ppm in the gHSQC spectrum. The proton at δ 0.85, which was assigned as H-26, showed additional long-range coupling to carbons at 39.3, 40.9 (quaternary carbons), and 46.1 (methine carbon) ppm. These carbons were assigned as carbons C-8, C-14, and C-9, respectively. H-9 showed a cross-peak in the dqfCOSY spectrum to a proton at δ 1.84 (H-11), which in turn was coupled to the olefinic proton at δ 5.30 (H-12). C-9 showed long-range coupling to H-12, which was directly coupled to a carbon at 122.9 ppm. H-11 was directly coupled to a carbon at 22.7 ppm (C-11) and showed long-range coupling to a quaternary carbon at 142.6 ppm, which was assigned as C-13. H-12 showed long-range coupling to a quaternary carbon at 40.9 ppm (C-14), which in turn showed long-range coupling to two methyl protons at δ 0.85 (H-26) and 1.42 ppm. This latter proton was in turn directly coupled to a carbon at 26.6 ppm and was assigned as the C-27 methyl group. H-27 showed long-range coupling to carbons at 142.6 (C-13), 40.9 (C-14), 39.3 (C-8), and 33.5 ppm. The carbon at 33.5 ppm was directly coupled to protons at δ 1.25 and 1.63. These protons were in turn coupled to a methine proton at δ 3.98, which in turn was directly coupled to a carbon at 66.9 ppm, the low field suggesting an oxygen-bearing carbon. A cross-peak in the dqfCOSY spectrum between this methine proton and an exchangeable proton at δ 4.83 suggested a hydroxyl substituent. This allowed the assignment of C-15 (33.5 ppm) and C-16 (66.9 ppm). Proton H-12 showed additional long-range coupling to carbons at 46.1 (C-9) and 38.9 ppm, which was directly coupled to a proton at δ 2.56. This proton (δ 2.56) gave cross-peaks in the gHMBC spectrum to carbons at 122.9 (C-12), 142.6 (C-13), and 46.2 ppm and was directly coupled to a carbon at 38.9 ppm. Additionally, cross-peaks between this proton at δ 2.56 and methylene protons at δ 1.16 and 2.60 were observed in the dqfCOSY spectrum. In turn, these methylene protons were directly coupled to a carbon at 46.2 ppm. Given this information, the carbons at 38.9 and 46.2 ppm were assigned as C-18 and C-19, respectively. The protons at δ 1.16/2.60 showed long-range coupling in the gHMBC spectrum to methyl carbons at 29.0 and 19.2 ppm and also to a methine carbon at 79.0 ppm. These carbons were in turn coupled to protons at δ 0.82, 1.06, and 5.98, respectively. In addition the methyl protons at δ 0.82 and 1.06 showed long-range coupling to carbons at 35.6 and 79.0 ppm. This allowed the

assignment of carbons at 29.0, 19.2, 35.6, and 79.0 ppm as C-29, C-30, C-20, and C-21, respectively. The methine proton at δ 5.98 (H-21) showed correlations in the gHMBC spectrum to a carbonyl carbon at 165.8 ppm, which in turn showed long-range coupling to a proton at δ 7.88. This proton showed a cross-peak in the dqfCOSY spectrum to a proton at δ 7.52, which in turn was coupled to a proton at δ 7.60. These protons were directly coupled to carbons at 129.2, 128.4, and 132.6 ppm, respectively. Similarly coupling in the gHMBC spectrum was observed between the proton at δ 7.52 and carbons at 132.6, 130.5, and 128.4 and also between the proton at δ 7.60 and carbons at 129.2 and 128.4 ppm. This information enabled the assignment of the carbons at 165.8, 130.5, 129.2, 128.4, and 132.6 ppm as C-1, C-2, C-3/C-7, C-4/C-6, and C-5 of a benzoate ester. The proton at δ 5.98 (H-21) showed additional long-range correlations to carbons at 46.2, 35.6, 29.0, 19.2, and 72.1 ppm in the gHMBC spectrum and also to a second methine proton at δ 5.60 in the dqfCOSY spectrum. This last proton was directly coupled to the carbon at 72.1 ppm and showed a long-range correlation to a second carbonyl carbon at 175.8 ppm. Protons at δ 2.24, 0.86, and 0.75 also showed long-range gHMBC correlations to this carbonyl carbon. The methyl protons at δ 0.86 and 0.75 were directly coupled to carbons at 18.4 and 18.1 ppm and showed cross-peaks in the dqfCOSY spectrum to a proton at δ 2.24. This proton presented as a septet in the ^{1}H spectrum, suggesting close proximity to the two methyl protons, and was also directly coupled to a methine carbon at 33.2 ppm. Given this information, the carbons at 175.8, 33.2, 18.4, and 18.1 ppm were assigned as C-1, C-2, C-3, and C-4 of an isobutyrate ester. Additional long-range correlations from proton H-22 were observed in the gHMBC spectrum to carbons at 66.9, 46.8, 38.9, 79.0 (C-21), and 62.8 ppm. The carbon at 62.8 ppm was directly coupled to methylene protons at 2.86 and 3.08 ppm, the low field suggesting that the carbon was also attached to a hydroxyl group. These carbons were assigned as C-16 (66.9 ppm), C-17 (46.8 ppm), C-18 (38.9 ppm), C-21 (79.0 ppm), and C-28 (62.8 ppm). Therefore the aglycone of acutanguloside A (**2**) was assigned as a C-21/C-22-substituted barringtogenol.

The relative configuration of the aglycone was assigned by analysis of coupling constants and correlations in the ROESY spectrum. The chemical shifts of the angular methyl groups at C-23/C-24 and C-29/C-30 are characteristic; the equatorial methyl in both cases is less shielded than the axial methyl.[36] Therefore, C-23/C-29 (26.9/29.0 ppm) and C-24/C-30 (15.7/19.2 ppm) were assigned as having equatorial and axial orientations, respectively. Correlations in the ROESY spectrum between H-24 and H-25 also suggest an axial orientation of the methyl at C-25. This correlation was only possible if the A/B ring junction was *trans*-fused, which is consistent with X-ray data of similar compounds.[37-39] Furthermore, correlations between H-30 and H-18, H-18, and H-28a suggested a *cis* fusion between rings D and E of the aglycone. This is also supported in the literature.[37-39] The hydroxyl substituent at C-3 is commonly reported as having a β-orientation. Correlations in the ROESY spectrum from H-3 to H-23 and also to H-5 supported this orientation for **2**. Further analysis of the ROESY spectrum showed coupling from H-5 to H-9 and from H-9 to H-27, which suggested an axial orientation for the C-27 methyl group. The low field of H-27 was characteristic of 16α-hydroxyoleananes.[40] Correlations between H-16 and H-28a/b in the ROESY spectrum also supported the α-orientation of the C-16 hydroxyl function.



The doublets assigned as H-21 and H-22 show large coupling constants (9.4 Hz) associated with axial−axial interactions. Cross-peaks observed in the ROESY spectrum from H-22 to H-28a/b and H-16 support an α-axial H-22 orientation.

The aglycone moiety for **2** was thus assigned as 21-*O*-benzoyl-22-*O*-isobutyrylbarringtogenol C.

Acutanguloside A (**2**) contained three sugar units, as shown by the presence of three anomeric carbons in the ^{13}C spectrum at 102.5, 103.1, and 103.6 ppm, which correlated to protons at δ 4.67 [d, J = 7.2 Hz], 4.54 [d, J = 7.5 Hz], and 4.48 [d, J = 7.2 Hz], respectively, in the gHSQC spectrum.

Coupling in the tnTOCSY spectrum from the most shielded anomeric proton (H-1′) at δ 4.48 showed that the spin system associated with this proton contained five more protons at chemical shifts of δ 3.42, 3.58, 3.64, 3.74, and 4.45. Furthermore, correlations in the dqfCOSY spectrum allowed the assignment of these chemical shifts as protons H-4′, H-2′, H-3′, and H-5′ respectively. A second correlation from H-4′ to an exchangeable hydroxy proton at δ 4.45 was also observed in the dqfCOSY spectrum. A broad singlet centered at δ 12.75 suggested an acidic proton. The carbon chemical shifts were assigned by gHSQC as being 77.5 (C2′), 83.8 (C3′), 69.5 (C4′), and 74.6 (C5′) ppm, while multiplicities were confirmed by DEPT-135. Long-range coupling between a proton at δ 3.42 (H-4′) and a carbonyl carbon at 170.0 ppm in the gHMBC spectrum enabled the assignment of C-6′, while other correlations in the gHMBC spectrum added support for the remaining assignments. Correlations in the ROESY spectrum were observed from H-1′ to H-3′, H-1′ to H-5′, and H-2′ to H-4′, suggesting an axial−axial relationship between these proton pairs. The 7.2 Hz coupling constant for H-1′ was consistent with a β-anomeric configuration and also suggested an axial−axial relationship with H-2′. This, in conjunction with the strong correlations observed in the dqfCOSY spectrum, indicated that all protons were axially orientated. These data were consistent with the assignment of this sugar as β-glucuronic acid. A correlation from H-3 of the aglycone to C-1′

and from H-1′ to C-3 in the gHMBC spectrum indicated that the glucuronic acid was attached directly to the aglycone at C-3.

Correlations in the tnTOCSY spectrum from the anomeric proton (H-1″) at 4.67 ppm identified a spin system comprising a further six protons at chemical shifts of δ 3.25, 3.26, 3.30, 3.42, 3.56, and 3.68. Analysis of the cross-peaks observed in the dqfCOSY spectrum enabled assignment of these protons as H-3″, H-2″, H-5″, H-6a″, H-6b″, and H-4″, respectively. No exchangeable protons were observed in the dqfCOSY spectrum for this sugar. The chemical shifts of carbons attached to each of these protons were assigned by gHSQC as being 71.2 (C-2″), 73.5 (C-3″), 67.8 (C-4″), 74.6 (C-5″), and 59.6 (C-6″). These assignments were supported by long-range coupling in the gHMBC spectrum. The coupling constant for H-1″ (7.2 Hz) suggested an axial−axial relationship between H-1″ and H-2″. Analysis of the ROESY spectrum showed correlations between H-1″−H-3″, H-1″−H-5″, and H-2″−H-4″, also suggesting axial−axial relationships between these proton pairs. This information was consistent with sugar 2 being assigned as β-galactose.

Cross-peaks in the tnTOCSY spectrum from the anomeric proton (H-1‴), δ 4.54, indicated a further six protons in this spin system. Analysis of the dqfCOSY spectrum allowed the assignment of these protons as δ 3.04 (H-2‴), 3.10 (H-3‴), 3.33 (H-4‴), 3.11 (H-5a‴), and 3.76 (H-5b‴). Carbons attached to these protons were assigned by gHSQC at chemical shifts of 73.5 (C-2‴), 76.5 (C-3‴), 69.6 (C-4‴), and 65.6 (C-5‴) ppm. A cross-peak in the dqfCOSY spectrum between an exchangeable hydroxy proton at δ 4.40 and H-2‴ was also observed. Confirmation of the carbon multiplicities was by DEPT-135. The coupling constant for H-1‴ was 7.5 Hz, which again suggested an axial−axial relationship between H-1‴ and H-2‴. Correlations between H-1‴ and H-3‴, H-1‴ and H-5‴, and H-2‴ and H-4‴ observed in the ROESY spectrum again allow assignment of axial−axial relationships between these proton pairs. This information was consistent with the third sugar being assigned as β-xylose.

The chemical shifts of C-2′ and C-3′ (77.5 and 83.8 ppm, respectively) of the glucuronic acid moiety were consistent with glycosidic linkages at these positions.[35,42] Long-range coupling was observed in the gHMBC spectrum from H-2′ (δ 3.58) to 102.5 ppm (C-1″) and from H-3′ (δ 3.64) to 103.1 (C-1‴). Furthermore, gHMBC correlations were present from the protons at δ 4.67 (H-1″) and 4.54 (H-1‴) to carbons at 77.5 (C-2′) and 83.8 (C-3′) ppm, respectively. This information suggested that xylose and galactose were attached to C-2′ and C-3′ of glucuronic acid, respectively. This was further supported by correlations observed from H-2′ to H-1″ and from H-3′ to H-1‴ in the ROESY spectrum. The D-configuration of the sugars within the C-3 carbohydrate moiety was assumed in keeping with the assertion of Massiot and Lavaud regarding the monosaccharides commonly found in saponins.[43] This has been cited elsewhere[40,44] and is consistent with the D-configuration of similar carbohydrate moieties isolated from the same[35] and related (*B. asiatica*) species.[40] Therefore, the complete carbohydrate moiety was assigned as a 3-*O*-[β-D-xylopyranosyl(1→3)]-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranosyl moiety. This is supported in the negative ion LR-ESMS, where an *m*/*z* value of 687 ([M − (Gal-Xyl-GlcA) + 1 + Na]$^+$) was consistent with the loss of the complete sugar moiety from the aglycone and the peak at 493 ([Gal-Xyl-GlcA-moiety + Na]$^+$) was consistent with the entire sugar moiety itself.

The preceding information allowed the assignment of acutanguloside A (**2**) as 3-*O*-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranosyl-21-*O*-benzoyl-22-*O*-isobutyrylbarringtogenol C.

The remaining eight saponins possessed spectroscopic properties very similar to those of acutanguloside A.

High-resolution mass measurement of the pseudomolecular ion in the high-resolution negative electrospray mass spectrum of acutanguloside B (**3**) indicated that the compound had the molecular formula $C_{56}H_{82}O_{22}$. The ^{1}H and ^{13}C NMR spectra of **3** were almost identical to those of **2**. The only major differences observed in the ^{1}H NMR spectrum were the addition of an acetate methyl signal at δ 2.03, the absence of the signals associated with the isobutyrate, and the 1.71 ppm upfield shift of H-22 and the downfield shift of H-28a (Δ 0.78 ppm) and H-28b (Δ 0.70 ppm) compared to **2**. A gHMBC correlation from H-28a and H-28b and the acetate methyl proton to an ester carbonyl carbon at 169.3 ppm indicated that **3** was the 22-deisobutyryl-28-acetyl derivative of **2**.

Acutanguloside C (**4**) had a molecular mass 70 Da smaller than acutanguloside A. The molecular formula, $C_{54}H_{80}O_{21}$, derived from mass measurement of the pseudomolecular ion in the negative HRESMS suggested that **4** was the deisobutryl derivative of **2**. Analysis of the ^{1}H and ^{13}C NMR spectra confirmed that compound **4** was the deisobutyryl derivative of **2**. The spectra were almost identical with those of **2** except for the lack of signals associated with the isobutyryl moiety and the upfield shift of H-22 (4.03 ppm, shifted upfield by 1.57 ppm).

The ^{1}H NMR spectrum of acutanguloside D (**5**) contained signals for an additional benzoate and lacked signals for the isobutyrate when compared to **2**; the remaining signals were almost identical. The molecular formula derived from (−)-HRESMS indicated the molecular formula $C_{61}H_{84}O_{22}$ for **5**. The combination of these data suggested that **5** was the 22-benzoate-22-deisobutyrate derivative of **2**. Two-dimensional NMR analysis supported this assignment.

The molecular formula for acutanguloside D methyl ester (**5a**) (derived from (−)-HRESMS) indicated that **5a** possessed an additional methyl group when compared with compound **5**. A three-proton singlet at 3.68 ppm which was directly coupled to a carbon at 51.8 ppm suggested a methyl ester was present in the molecule. A correlation in the gHMBC spectrum from the methoxyl proton to the glucuronate carbonyl carbon 169.1 ppm confirmed that compound **5a** was the glucuronic acid methyl ester of acutanguloside D (**5**).

Acutanguloside E (**6**) had the molecular formula $C_{59}H_{86}O_{22}$ from analysis of the (−)-HRESMS data. The NMR data for **6** were very similar to those of **2**. The only differences were associated with the lack of signals for the isobutyrate and the addition of signals assigned to a tigloyl or angeloyl ester. Proton H-22 in **6** (δ 5.64) showed long-range coupling in the gHMBC spectrum to a carbonyl carbon at 166.8 ppm. Additional correlations in the gHMBC spectrum were observed between this carbonyl carbon and protons at δ 6.60 and 1.51, which in turn were directly coupled to carbons at 136.0 and 11.7 ppm, respectively. Long-range coupling was observed in the gHMBC spectrum from the proton at δ 6.60 to carbons at 13.9 and 11.7 ppm and also from protons at δ 1.51 and 1.58 to carbons at 136.0 and 130.2 ppm. The proton at δ 1.58 was directly coupled to a carbon at 13.9 ppm. This allowed the assignment of a tigloyl or angeloyl ester attached to C-22 in **6**. The methyl carbons of angelic acid tend to be more deshielded (ca. 16

Table 1. ^{13}C NMR Data of the Aglycone Portion of Acutangulosides A−F (**2**−**7**)

C	**2**	**3**	**4**	**5**	**6**	**7**
1	38.1	38.1	38.2	38.2	38.2	38.2
2	25.3	25.5	25.6	25.6	25.6	25.6
3	89.0	88.8	88.9	88.9	88.9	88.8
4	38.8	38.6	38.6	38.7	38.6	38.6
5	54.9	54.9	55.0	55.0	55.0	55.0
6	17.2	17.6	17.7	17.7	17.7	17.7
7	32.1	32.2	32.3	32.3	32.3	32.3
8	39.3	40.3	40.3	40.4	40.3	40.3
9	46.1	45.9	46.0	46.0	46.0	46.0
10	36.0	36.0	36.1	36.1	36.1	36.1
11	22.7	22.9	22.9	23.0	23.0	22.9
12	122.9	122.9	122.3	123.0	122.9	122.8
13	142.6	141.7	142.5	141.8	141.8	141.8
14	40.9	40.7	40.8	40.7	40.7	40.6
15	33.5	33.5	33.3	33.5	33.5	33.5
16	66.9	66.4	66.6	67.3	67.4	67.3
17	46.8	46.2	46.7	46.6	46.5	46.4
18	38.9	38.6	38.6	38.7	38.6	38.6
19	46.2	46.2	46.2	46.4	46.4	46.3
20	35.6	35.9	35.5	35.7	35.6	35.4
21	79.0	80.7	81.4	79.2	79.2	78.2
22	72.1	69.2	70.2	69.4	72.5	72.6
23	26.9	26.6	26.9	27.3	27.3	27.2
24	15.7	15.8	15.9	15.9	15.7	15.8
25	15.0	15.2	15.3	15.3	15.3	15.3
26	16.2	16.3	16.4	16.4	16.4	16.3
27	26.6	26.6	26.9	26.9	26.9	26.8
28	62.8	64.9	63.4	62.9	62.4	62.4
$C_{28}O\mathit{C}OCH_3$		169.7				
$C_{28}OCO\mathit{C}H_3$		20.5				
29	29.0	29.2	29.3	29.1	29.0	29.0
30	19.2	19.4	19.7	19.5	19.4	19.4

and 21 ppm) than those of tiglic acid, and therefore this ester moiety was assigned as tiglic acid.[41]

Compound **6a** was shown to be the glucuronic acid methyl ester of **6** from analysis of the (−)-HRESMS and NMR data.

Acutanguloside F (**7**) had the molecular formula $C_{57}H_{88}O_{22}$ from analysis of (−)-HRESMS data. The NMR data indicated that in **7** a tiglic acid ester had replaced the benzoate at C-21 of the aglycone when compared with **6**. All of the remaining NMR data were identical with those of **6**.

MS and NMR analyses indicated that compound **7a** was the methyl glucuronate of acutanguloside F (**7**).

Experimental Section

General Experimental Procedures. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), and 2D-NMR spectra were recorded in DMSO-d_6 on a Varian Unity Inova 600 spectrometer. Shigemi NMR tubes (DMSO matched) were used where only small sample weights could be isolated. Standard Varian pulse sequences were used for all experiments. Spectral analysis was performed using MestReC software.[45] LR-ESMS was performed on a Fisons VG Platform LCMS with MassLynx software (v 1.0). HR-ESMS was performed using a Bruker BioApex 47e FTMS. Optical rotations were measured in MeOH on a Jasco P-1020 polarimeter. HPLC was performed using a Waters 600 chromatography system fitted with a 996 PDA detector and 717 autosampler. Chromatograms were recorded using Millenium 32 software. Semipreparative chromatography was performed using C18 (Dynamax 8 μm 10 × 250 mm, 4 mL/min) and phenyl (Dynamax 3 μm 10 × 50 mm, 4 mL/min) bonded silica. Preparative chromatography was performed using a 26 × 230 mm glass column (Büchi, 12.5 mL/min), which was packed with C18 bonded silica (Davisil). All solvents were of AR grade and were distilled as required.

Table 2. ^{13}C NMR Data of the Side Chain Portion of Acutangulosides A−F (**2**−**7**)

C	**2**	**3**	**4**	**5**	**6**	**7**
21-O-ester						
1	165.8	165.6	165.8	165.4	165.5	166.7
2	130.5	130.8	130.9	130.0	130.2	128.3
3/7	129.2	129.0	129.0	128.9	128.9	135.9
4/6	128.4	128.4	128.5	128.3	128.4	13.9
5	132.6	132.6	132.7	132.8	132.8	11.8
22-O-ester						
1	175.8			165.3	166.8	166.9
2	33.2			130.2	130.2	128.3
3	18.4			129.0	136.0	135.9
4	18.1			128.4	13.9	13.9
5				132.7	11.7	11.8
6				128.4		
7				129.0		
glucuronic acid						
1	103.6	103.6	103.7	103.7	103.7	103.7
2	77.5	77.7	77.8	77.7	77.7	77.7
3	83.8	83.9	84.0	84.0	84.0	84.0
4	69.5	69.6	69.6	69.7	69.7	69.7
5	74.6	74.7	74.6	74.8	74.8	74.8
6	170.0	169.9	169.8	169.8	169.8	169.7
xylose						
1	103.1	103.1	103.2	103.2	103.2	103.1
2	73.5	73.4	73.4	73.4	73.4	73.4
3	76.5	76.3	76.4	76.6	76.6	76.6
4	69.3	69.3	69.4	69.4	69.4	69.4
5	65.6	65.7	65.8	65.8	65.8	65.8
galactose						
1	102.5	102.5	102.5	102.6	102.6	102.5
2	71.2	71.6	71.5	71.7	71.7	71.7
3	73.5	73.3	73.5	73.6	73.4	73.4
4	67.8	67.6	67.6	67.7	67.7	67.7
5	74.6	74.7	74.7	74.8	74.8	74.8
6	59.6	59.5	59.6	59.7	59.7	59.7

Plant Material. The identity of the tree was confirmed as *Barringtonia acutangula* var. *acutangula*, and a voucher (#AQ595349) was deposited with the Queensland Herbarium. The samples were collected from the Fitzroy River in the Kimberley district of Western Australia in July of 1989 and 1994.

Extraction and Isolation. The dried and powdered bark of *B. acutangula* was extracted with distilled water (dH_2O) and the extract freeze-dried. A portion of the dried water extract (10 g) was redissolved in dH_2O and centrifuged (12000*g*, 20 min) and the supernatant applied to a C18 preparative column (0−100% MeOH in dH_2O, 10% steps).

The fraction eluting at 70% MeOH (F70) was further subjected to C18 gradient chromatography (70−80%: 20 min, 80−100%: 5 min, MeOH/1% TFA in dH_2O), which resulted in five fractions (F70.1−F70.5). The second fraction (F70.2) was reapplied to a C18 column and eluted isocratically (40% MeCN/1% TFA in dH_2O) to yield a further eight fractions (F70.2.1−F70.2.8). Repeated chromatography of F70.2.3 and F70.2.5 using identical conditions resulted in acutanguloside C (**4**) and **1**.

Fraction F70.3 was applied to a C18 column using a MeCN/1% TFA in dH_2O gradient (45% MeCN: 15 min, 45%−50% MeCN: 20 min) to yield seven fractions (F70.3.1−F70.3.7). Repeated chromatography of F70.3.2 and F70.3.4 under identical conditions resulted in acutangulosides B (**3**) and A (**2**).

Fraction F70.4 was applied to a C18 column (70% MeOH/1% TFA in dH_2O) to give three fractions (F70.4.1−F70.4.3). F70.4.2 was chromatographed using phenyl bonded silica (35% MeCN/1% TFA in dH_2O) to give four peaks (F70.4.2.1−F70.4.2.4). Repeated chromatography of peaks F70.4.2.3 and F70.4.2.4 under identical conditions resulted in acutangulosides E (**6**) and D (**5**). F70.4.3 was applied to a phenyl column and eluted using a MeCN/1% TFA gradient (35−40%: 10 min),

Table 3. ^{1}H NMR Data for the Aglycone Portion of Acutangulosides A–F (**2**–**7**) (multiplicity, *J* in Hz)

H	**2**	**3**	**4**	**5**	**6**	**7**
1a	0.93 (m)	0.89 (m)	0.89 (m)	0.92 (m)	0.90 (m)	0.90 (m)
1b	1.54 (m)	1.53 (m)	1.52 (m)	1.54 (m)	1.53 (m)	1.52 (m)
2a	1.56 (m)	1.55 (m)	1.56 (m)	1.56 (m)	1.55 (m)	1.54 (m)
2b	1.72 (m)	1.71 (m)	1.71 (m)	1.71 (m)	1.72 (m)	1.71 (m)
3	3.07 (br, dd)	3.06 (dd 7.6, 15.6)	3.05 (br,dd)	3.06 (br, dd)	3.06 (br, dd)	3.06 (br, dd)
5	0.72 (s)	0.71 (m)	0.72 (m)	0.72 (m)	0.72 (m)	0.71 (s)
6a	1.24 (m)	1.31 (m)	1.32 (m)	1.31 (m)	1.32 (m)	1.31 (m)
6b	1.44 (br, s)	1.49 (m)	1.51 (m)	1.50 (m)	1.49 (m)	1.49 (br, s)
7a	1.26 (m)	1.28 (m)	1.26 (m)	1.26 (m)	1.28 (m)	1.24 (m)
7b	1.50 (m)	1.50 (m)	1.50 (m)	1.51 (m)	1.46 (m)	1.49 (m)
9	1.57 (m)	1.56 (m)	1.56 (m)	1.59 (m)	1.60 (m)	1.57 (m)
11	1.84 (br, d)	1.81 (br, d)	1.84 (br, d)	1.86 (br, d)	1.83 (br d)	1.82 (br, s)
12	5.30 (br t, 3.6)	5.26 (br t, 3.6)	5.27 (br t, 3.6)	5.34 (br t, 3.3)	5.30 (br t, 3.1)	5.27 (br t, 3.2)
15a	1.25 (m)	1.29 (m)	1.26 (m)	1.28 (m)	1.26 (m)	1.24 (m)
15b	1.63 (m)	1.65 (m)	1.64 (m)	1.66 (m)	1.61 (m)	1.62 (m)
16	3.98 (t-like, s)	4.05 (s)	4.02 (t-like, s)	4.13 (t-like, s)	3.96 (t-like, s)	3.91 (t-like, s)
$C_{16}OH$	4.83 (br s)			5.06 (d, 3.8)		4.78 (br s)
18	2.56 (br dd)	2.46 (dd, 14.1, 3.6)	2.46 (dd, 13.5, 2.9)	2.63 (br dd)	2.59 (m)	2.54 (br dd)
19a	1.16 (br dd)	1.12 (m)	1.08 (dd, 11.5, 1.6)	1.21 (m)	1.17 (m)	1.08 (br dd)
19b	2.60 (br s)	2.59 (t, 13.4)	2.53 (t, 13.2)	2.65 (m)	2.60 (s)	2.55 (m)
21	5.98 (d, 10.1)	5.76 (d, 9.6)	5.71 (d, 9.6)	6.12 (d, 10.2)	6.02 (d 10.1)	5.80 (d, 10.0)
22	5.60 (d, 10.1)	3.89 (d, 9.6)	4.03 (d, 10.2)	5.84 (d, 9.8)	5.64 (d 10.1)	5.48 (d, 10.0)
23	0.99 (s)	0.99 (s)	0.98 (s)	0.99 (s)	0.99 (s)	1.00 (s)
24	0.76 (s)	0.76 (s)	0.76 (s)	0.76 (s)	0.75 (s)	0.76 (s)
25	0.90 (s)	0.89 (s)	0.89 (s)	0.89 (s)	0.89 (s)	0.89 (s)
26	0.85 (s)	0.85 (s)	0.85 (s)	0.86 (s)	0.84 (s)	0.83 (s)
27	1.42 (s)	1.40 (s)	1.39 (s)	1.47 (s)	1.43 (s)	1.41 (s)
28a	2.86 (d, 10.2)	3.64 (m)	3.04 (d, 2.4)	2.91 (d, 10.2)	2.82 (br d)	2.79 (d, 10.1)
28b	3.08 (d)	3.78 (m)	3.15 (d, 1.8)	3.12 (d)	3.07 (m)	3.06 (m)
$C_{28}OH$	4.52 (m)			4.41 (m)		4.35 (m)
$C_{28}OCOCH_3$		2.03 (s)				
29	0.82 (s)	0.80 (s)	0.78 (s)	0.84 (s)	0.82 (s)	0.75 (s)
30	1.06 (s)	1.06 (s)	1.04 (s)	1.13 (s)	1.08 (s)	0.98 (s)

resulting in six fractions (F70.4.3.1–F70.4.3.6). Repeated chromatography of F70.4.3.2 resulted in compound **7a**.

The fraction eluting at 80% MeOH (F80) was applied to a C18 column (70% MeOH/1% TFA in dH_2O) to give six fractions (F80.1–F80.6). F80.6 was applied to a phenyl column and eluted with 40% MeCN in 1% TFA in dH_2O to give seven fractions (F80.6.1–F80.6.7). Repeated chromatography of fractions F80.6.2, F80.6.6, and F80.6.7 gave acutanguloside F (**7**), **6a**, and **5a**.

Compound 1 (F70.2.5), 2α,3*β*,19α-trihydroxy-olean-12-ene-23,28-dioic acid: amorphous white powder (0.7 mg); $[\alpha]_D^{24.8}$ +29.8° (*c* 0.042, MeOH); UV (MeOH) λ_{max} (log ϵ) 201.9 (3.95) nm; IR ν_{max} (thin film) 3395, 2942, 1688, 1201 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6) δ 0.80 (1H, t, J = 12.0 Hz, H1a), 1.83 (1H, dd, J = 12.6, 4.2 Hz, H1b), 3.95 (1H, m, H2), 2.78 (1H, d, J = 9.6 Hz, H3), 0.98 (1H, d, J = 11.4 Hz, H5), 1.64 (1H, m, H6a), 1.71 (1H, br m, H6b), 1.26 (1H, m, H7a), 1.41 (1H, dt, J = 12.9, 3.6 Hz, H7b), 1.64 (1H, m, H9), 1.88 (2H, br dd, H11), 5.24 (1H, br t, J = 3.4 Hz, H12), 0.92 (1H, br m, H15a), 1.52 (1H, m, H15), 1.50 (1H, m, H16a), 2.19 (1H, m, H16b), 2.92 (1H, br s, H18), 3.12 (1H, br s, H19), 0.91 (1H, m, H21a), 1.63 (1H, m, H21b), 1.54 (1H, m, H22a), 1.63 (1H, m, H22b), 1.30, 0.85, 0.68, 1.25, 0.85, 0.88 (each 3H, s, Me of C23, C25, C26, C27, C29, and C30); ^{13}CNMR (150 MHz, DMSO-d_6) δ 47.2 (C1), 66.5 (C2), 82.2 (C3), 48.9 (C4), 55.2 (C5), 19.8 (C6), 32.5 (C7), 38.9 (C8), 46.7 (C9), 38.0 (C10), 23.3 (C11), 122.1 (C12), 143.4 (C13), 41.2 (C14), 27.8 (C15), 27.2 (C16), 44.7 (C17), 43.1 (C18), 80.0 (C19), 34.8 (C20), 28.5 (C21), 32.2 (C22), 24.1 (C23), 177.5 (C24), 14.3 (C25), 16.7 (C26), 23.9 (C27), 179.0 (C28), 28.0 (C29), 24.5 (C30); (–)-LRESMS 517 ([M – H]$^-$), 1035 ([2M – H]$^-$); (+)-LRESMS 541 ([M + Na]$^+$), 1059 ([2M + Na]$^+$), 1578 ([3M + Na]$^+$); (–)-HRESMS 517.3164 ([M – H]$^-$) (calcd for $C_{30}H_{45}O_7$ 517.3165).

Acutanguloside A (2) (F70.3.4), 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21-*O*-benzoyl-22-*O*-isobutyrylbarringtogenol C: amorphous white powder (0.7 mg); $[\alpha]_D^{23.5}$ –6.6° (*c* 0.780, MeOH); UV (MeOH) λ_{max} (log ϵ) 199.5 (4.41), 226.1 (4.04) nm; IR ν_{max} (thin film) 3386, 2929, 1740–1680, 1276, 1072, 1042 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6), see Tables 3 and 4; ^{13}CNMR (150 MHz, DMSO-d_6), see Tables 1 and 2; (–)-LRESMS 1133 ([M – H]$^-$); (+)-LRESMS 1157 ([M + Na]$^+$), 687 ([M – (Gal-Xyl-GlcA) + H + Na]$^+$), 493 ([Gal-Xyl-GlcA-moiety + Na]$^+$); (–)-HRESMS 1133.5549 ([M – H]$^-$) (calcd for $C_{58}H_{85}O_{22}$ 1133.5533).

Acutanguloside B (3) (F70.3.2), 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21-*O*-benzoyl-28-*O*-acetylbarringtogenol C: amorphous white powder (3.1 mg); $[\alpha]_D^{24.9}$ +30.2° (*c* 0.440, MeOH); UV (MeOH) λ_{max} (log ϵ) 201.9 (4.43), 223.7 (4.15), 272.3 (3.14) nm; IR ν_{max} (thin film) 3404, 2947, 1745–1640, 1279, 1073, 1042 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6), see Tables 3 and 4; ^{13}CNMR (150 MHz, DMSO-d_6), see Tables 1 and 2; (–)-LRESMS 1106 ([M – H]$^-$), 1048 ([M – 59]$^-$), 975 ([M – Xyl + H]$^-$); (+)-LRESMS 1151 ([M – H + 2Na]$^+$), 1130 ([M + Na]$^+$), 660 ([M – (Gal-Xyl-GlcA) + H]$^+$), 493 ([Gal-Xyl-GlcA + Na]$^+$); (–)-HRESMS 1105.5284 ([M – H]$^-$) (calcd for $C_{56}H_{81}O_{22}$ 1105.5220).

Acutanguloside C (4) (F70.2.3), 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21-*O*-benzoylbarringtogenol C: amorphous white powder (3.2 mg); $[\alpha]_D^{24.8}$ +12.1° (*c* 0.087, MeOH); UV (MeOH) λ_{max} (log ϵ) 201.9 (4.39), 225.0 (4.08), 269.8 (3.11), 280.0 (3.04) nm; IR ν_{max} (thin film) 3392, 2929, 1730–1580, 1280, 1074, 1043 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6), see Tables 3 and 4; ^{13}CNMR (150 MHz, DMSO-d_6), see Tables 1 and 2; (–)-LRESMS 1063 ([M – H]$^-$); (+)-LRESMS 1109 ([M – H + 2Na]$^+$), 1087 ([M + Na]$^+$), 617 ([M – (Gal-Xyl-GlcA-moiety) + Na]$^+$); (–)-HRESMS 1063.5094 ([M – H]$^-$) (calcd for $C_{54}H_{79}O_{21}$ 1063.5114).

Acutanguloside D (5) (F70.4.2.4), 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21,22-*O*-dibenzoylbarringtogenol C: amorphous white powder (2.1 mg); $[\alpha]_D^{23.3}$ –19.3° (*c* 0.327 MeOH); UV

Table 4. ^{1}H NMR Data for the Side Chain Portion of Acutangulosides A−F (**2**−**7**) (multiplicity, *J* in Hz)

H	**2**	**3**	**4**	**5**	**6**	**7**
21-*O*-ester						
3/7	7.88 (d, 7.7)	7.99 (dd, 7.3, 1.0)	7.89 (dd, 7.8, 1.0)	7.79 (dd, 7.2, 1.1)	7.89 (d, 7.6)	6.64 (q, 6.9)
4/6	7.52 (t, 7.6)	7.53 (t, 7.7)	7.52 (t, 7.4)	7.42 (t, 7.8)	7.52 (t, 7.6)	1.68 (m)
5	7.60 (t, 7.7)	7.63 (tt, 7.4, 1.0)	7.63 (t, 7.3)	7.53 (tt, 7.3, 1.1)	7.62 (t, 7.3)	1.65 (s)
22-*O*-ester						
2	2.24 (sept, 6.9)					
3	0.86 (m)			7.83 (dd, 7.2, 1.1)	6.60 (q, 6.8)	6.61 (q, 6.9)
4	0.75 (d, 6.9)			7.38 (t, 7.8)	1.58 (d, 6.8)	1.68 (m)
5				7.51 (t, 7.3)	1.51 (s)	1.65 (s)
6				7.40 (tt, 7.4, 1.3)		
7				7.83 (dd, 7.2, 1.1)		
glucuronic acid						
1	4.48 (d, 7.2)	4.47 (d, 7.4)	4.47 (d, 7.4)	4.48 (d, 7.4)	4.48 (d, 7.3)	4.47 (d, 7.0)
2	3.58 (m)	3.57 (m)	3.57 (m)	3.58 (m)	3.58 (m)	3.58 (m)
3	3.64 (m)	3.65 (m)	3.64 (m)	3.66 (m)	3.66 (m)	3.65 (m)
4	3.42 (m)	3.43 (m)	3.44 (m)	3.45 (m)	3.43 (m)	3.43 (m)
C_4-O*H*	4.45 (d, 7.2)	4.34 (d, 7.5)				
5	3.74 (m)	3.74 (m)	3.74 (m)	3.74 (m)	3.73 (m)	3.73 (m)
C_6OO*H*	12.75 (br s)	12.75 (br s)	12.70 (br s)	12.75 (br s)	12.75 (br s)	12.75 (br s)
galactose						
3-*O*-C_1'	4.67 (d, 7.2)	4.67 (d, 7.4)	4.68 (d, 7.4)	4.67 (d, 7.3)	4.68 (d, 7.3)	4.67 (d, 7.3)
3-*O*-C_2'	3.26 (m)	3.28 (m)	3.28 (m)	3.29 (m)	3.31 (m)	3.28 (m)
3-*O*-C_2O*H*	4.42 (br s)	4.41 (d, 7.6)	4.40 (d, 7.2)			4.92 (d, 6.2)
3-*O*-C_3'	3.25 (m)	3.25 (m)	3.26 (m)	3.26 (m)	3.25 (m)	3.26 (m)
3-*O*-C_3O*H*						5.00 (d 4.6)
3-*O*-C_4'	3.68 (m)	3.67 (m)	3.67 (m)	3.61 (m)	3.72 (m)	3.67 (m)
3-*O*-C_4O*H*						
3-*O*-C_5'	3.30 (m)	3.30 (m)	3.29 (m)	3.29 (m)	3.30 (m)	3.29 (m)
3-*O*-C_6a'	3.56 (m)	3.42 (m)	3.57 (m)	3.56 (m)	3.58 (m)	3.42 (m)
3-*O*-C_6b'	3.42 (m)	3.57 (m)	3.43 (m)	3.43 (m)	3.42 (m)	3.57 (m)
3-*O*-C_6O*H*		4.45 (m)		4.46 (m)		4.37 (m)
xylose						
3-*O*-C_1''	4.54 (d, 7.5)	4.52 (d, 7.6)	4.53 (d, 7.6)	4.53 (d, 7.7)	4.53 (d, 7.5)	4.52 (d, 7.5)
3-*O*-C_2''	3.04 (m)	3.05 (m)	3.04 (m)	3.06 (m)	3.07 (m)	3.06 (m)
3-*O*-C_2O*H*	4.40 (m)	4.21 (d, 7.8)				5.61 (d, 4.9)
3-*O*-C_3''	3.10 (m)	3.11 (m)	3.11 (m)	3.11 (m)	3.12 (m)	3.11 (m)
3-*O*-C_3O*H*		5.24 (m)	5.18 (d, 7.4)			5.06 (d, 3.9)
3-*O*-C_4''	3.33 (m)	3.32 (m)	3.34 (m)	3.32 (m)	3.33 (m)	3.33 (m)
3-*O*-C_4O*H*	4.35 (d, 7.7)	4.34 (d, 7.5)				5.00 (d, 4.6)
3-*O*-C_5a''	3.11 (m)	3.10 (m)	3.09 (m)	3.11 (m)	3.10 (m)	3.10 (m)
3-*O*-C_5b''	3.76 (m)	3.76 (m)	3.76 (m)	3.78 (m)	3.77 (m)	3.77 (m)

(MeOH) λ_{max} (log ϵ) 200.5 (4.48), 228.3 (4.28), 272.0 (3.18) nm; IR ν_{max} (thin film) 3396, 2923, 1740−1620, 1286, 1071, 1043 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6), see Tables 3 and 4; ^{13}CNMR (150 MHz, DMSO-d_6), see Tables 1 and 2; (−)-LRESMS 1167 ([M − H]$^-$), 1035 ([M − Xyl]$^-$); (+)-LRESMS 1191 ([M + Na]$^+$), 1169 ([M + H]$^+$), 1060 ([M − Xyl + Na]$^+$), 1037 ([M − Xyl + H]$^+$), 721 ([M − (Gal-Xyl-GlcA) + Na]$^+$), 494 ([(Gal-Xyl-GlcA) + H + Na]$^+$); (−)-HRESMS 1167.5380 (calcd for $C_{61}H_{83}O_{22}$ 1167.5376).

Acutanguloside D methyl ester (5a) (F80.6.7), 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-methylglucuronopyranosyl-21,22-*O*-dibenzoyl-barringtogenol C: amorphous white powder (1.3 mg); $[\alpha]_D^{25.0}$ −8.4° (0.253); UV (MeOH) λ_{max} (log ϵ) 201.9 (4.45), 226.2 (4.22), 272.3 (3.12) nm; IR ν_{max} (thin film) 3402, 2926, 1735−1620, 1284, 1071, 1042, 1026 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6) (identical to **5** except for 3-*O*-glucuronic ester) δ 4.53 (1H, d, J = 7.6, H-1), 3.68 (1H, s, C_6OOCH_3) ppm; ^{13}CNMR (150 MHz, DMSO-d_6) 3-*O*-glucuronic ester 103.6 (C-1), 51.8 (C_6OOCH_3) ppm; (−)-LRESMS 1181 ([M − H]$^-$); (+)-LRESMS 1205 ([M + Na]$^+$), 1183 ([M + H]$^+$), 961 ([M − Gal + H − benzoate + 2Na]$^+$), 698 ([M − (Gal-Xyl-GlcA) + H]$^+$); (−)-HRESMS 1181.5587 (calcd for $C_{62}H_{85}O_{22}$ 1181.5533).

Acutanguloside E (6) (F70.4.2.3), 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21-*O*-benzoyl-22-*O*-tigloylbarringtogenol C: amorphous white powder (7.8 mg); $[\alpha]_D^{25.1}$ −14.9° (*c* 0.090, MeOH); UV (MeOH) λ_{max} (log ϵ) 201.9 (4.37), 222.4 (4.26), 272.3 (2.97) nm; IR ν_{max} (thin film) 3389, 2925, 1740−1680, 1282, 1072, 1042 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6), see Tables 3 and 4; ^{13}CNMR (150 MHz, DMSO-d_6), see Tables 1 and 2; (−)-LRESMS 1146 ([M − H]$^-$), 1014 ([M − Xyl]$^-$); (+)-LRESMS 1192 ([M − H + 2Na]$^+$), 1170 ([M + Na]$^+$), 1148 ([M + H]$^+$), 699 ([M − (Gal-Xyl-GlcA) + Na]$^+$), 494 ([(Gal-Xyl-GlcA) + H + Na]$^+$); (−)-HRESMS 1145.5502 (calcd for $C_{59}H_{85}O_{22}$ 1145.5533).

Acutanguloside E methyl ester (6a) (F80.6.6), 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-methylglucuronopyranosyl-21-*O*-benzoyl-22-*O*-tigloyl-barringtogenol C: amorphous white powder (2.4 mg); $[\alpha]_D^{24.8}$ −7.0° (0.287); UV (MeOH) λ_{max} (log ϵ) 201.9 (4.32), 226.2 (4.22), 272.3 (3.04) nm; IR ν_{max} (thin film) 3402, 2928, 1740−1610, 1283, 1074, 1042 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6) (identical to **6** except for 3-*O*-glucuronic ester) δ 4.52 (1H, d, J = 7.4 Hz, H-1), 3.68 (3H, s, C_6OOCH_3) ppm; ^{13}CNMR (150 MHz, DMSO-d_6) 3-*O*-glucuronic ester 103.5 (C-1), 51.7 (C_6OOCH_3) ppm; (−)-LRESMS 1196 ([M + Cl]$^-$), 1159 ([M − H]$^-$); (+)-LRESMS 1183 ([M − H + Na]$^+$), 1184 ([M + Na]$^+$), 697 ([M − (Gal-Xyl-GlcA) + Na]$^+$); (−)-HRESMS 1159.5727 (calcd for $C_{59}H_{85}O_{22}$ 1159.5689).

Acutanguloside F (7) (F80.6.2), 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-21,22-*O*-tigloylbarringtogenol C: amorphous white powder (6.0 mg); $[\alpha]_D^{23.5}$ −14.1° (*c* 0.493, MeOH); UV (MeOH) λ_{max} (log ϵ) 199.5 (4.41) nm; IR ν_{max} (thin film) 3382, 2918, 1730−1640, 1279, 1075, 1041 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6), see Tables 3 and 4; ^{13}CNMR (150 MHz, DMSO-d_6), see Tables 1 and 2; (−)-HRESMS 1147 ([M − 2H + Na]$^-$), 1123 ([M − H]$^-$), 992 ([M − Xyl]$^-$); (+)-HRESMS 1170 ([M − H + 2Na]$^+$), 1148 ([M + Na]$^+$), 963 ([M − Gal + H]$^+$), 699 ([M − (Gal-Xyl-

GlcA) + 2Na]$^+$), 677 ([M − (Gal-Xyl-GlcA) + H + Na]$^+$), 494 ([(Gal-Xyl-GlcA) + H + Na]$^+$); (−)-HRESMS 1123.5657 (calcd for $C_{57}H_{87}O_{22}$ 1123.5689).

Acutanguloside F methyl ester (7a) (F70.4.3.2), 3-*O*-*β*-D-xylopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-methylglucuronopyranosyl-21,22-*O*-tigloylbarringtogenol C: amorphous white powder (0.4 mg); $[\alpha]_D^{23.3}$ −13.7° (*c* 0.187, MeOH); UV (MeOH) λ_{max} (log ϵ) 200.5 (4.43) nm; IR ν_{max} (thin film) 3401, 2925, 1745−1580, 1278, 1075, 1042 cm^{-1}; ^{1}HNMR (600 MHz, DMSO-d_6) (identical to **7** except for 3-*O*-glucuronic ester) δ 4.52 (1H, d, J = 7.5 Hz, H-1), 3.68 (1H, s, C_6OOCH_3) ppm; ^{13}CNMR (150 MHz, DMSO-d_6) 3-*O*-glucuronic ester 103.6 (C-1), 51.7 (C_6OOCH_3) ppm; (−)-HRESMS 1251 ([M + TFA]$^-$), 1173 ([M + Cl]$^-$), 1138 ([M − H]$^-$); (+)-HRESMS 1161 ([M + Na]$^+$), 593 ([M − (Gal-Xyl-GlcA) − tiglate + Na]$^+$); (−)-HRESMS 1137.5825 (calcd for $C_{58}H_{89}O_{22}$ 1137.5846).

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