Acutifolins A−F, a New Flavan-Derived Constituent and Five New Flavans from *Brosimum acutifolium*

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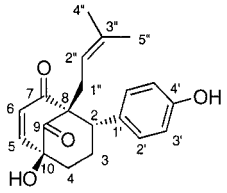
Acutifolin A (**1**), a novel constituent with a bicyclo[3.3.1]non-3-ene-2,9-dione ring, acutifolins B−F (**2**−**6**), five new flavans, and three known flavans were isolated from the bark of *Brosimum acutifolium*, a Brazilian folk medicine ("Mururé"). Their structures were elucidated by spectroscopic methods, including 2D NMR.

In the course of our research into the active compounds of Brazilian medicinal plants,[1] we investigated the constituents of the bark of *Brosimum acutifolium* Huber (Moraceae), commonly known as "Mururé".[2] This plant has been used in Brazilian folk medicine as an antiinflammatory and antirheumatic agent. Previous phytochemical investigations of this species have resulted in the isolation of four new flavans and 10 known compounds.[3,4] In this paper, we report the isolation and structure elucidation of six new constituents, acutifolins A−F (**1**−**6**), from the bark of *B. acutifolium*.



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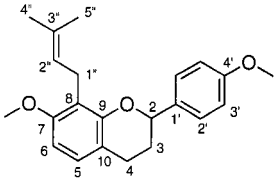

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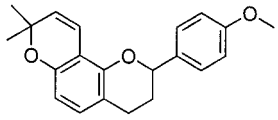

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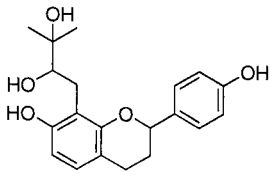

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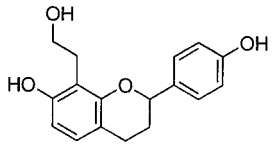

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Results and Discussion

The MeOH extracts of the bark of *B. acutifolium* were partitioned with EtOAc and H_2O. The EtOAc-soluble portions were chromatographed over an ODS column and further purified using reversed-phase HPLC to afford acutifolins A−F (**1**−**6**), together with three known flavans, 4′-hydroxy-7,8-(2″,2″-dimethylpyran)flavan,[3] 7,4′-dihydroxyflavan,[5] and 7,3′-dihydroxy-4′-methoxyflavan.[6]

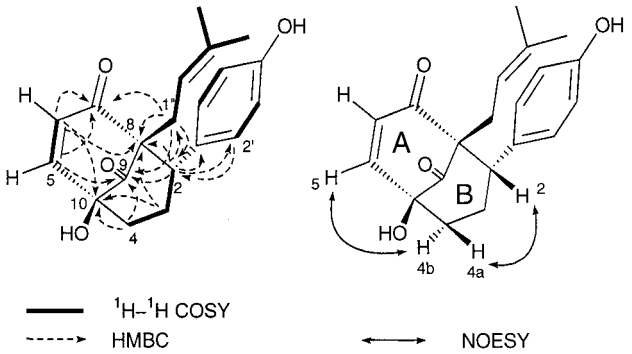

Figure 1. Selected 2D NMR correlations of **1**.

The molecular formula, $C_{20}H_{22}O_4$, of acutifolin A (**1**) was determined by positive-ion HRSIMS [*m*/*z* 327.1573 $(M + H)^+$, Δ −2.3 mmu]. The IR spectrum indicated the presence of the saturated ketone carbonyl group (1734 cm^{-1}), an α,β-unsaturated ketone carbonyl group (1667 cm^{-1}), and hydroxyl (3200−3600 cm^{-1}) groups. The ^{1}H NMR,^{13}C NMR, and HMQC experiments of **1** indicated the presence of two ketone carbonyl, three other sp^2 quarternary, seven sp^2 methine, two sp^3 quaternary, one sp^3 methine, three methylene, and two methyl carbons. Because seven of 10 unsaturations were thus accounted for, it was concluded that **1** contained three rings. The ^{1}H−^{1}H COSY spectrum of **1** suggested the presence of a 1,4-disubstituted benzene ring (H-2′ and H-3′), a 1,1,3-trisubstitued propane (H-2, H-3, and H-4), a disubstituted olefin (H-5 and H-6), and a 3-methyl-2-butenyl group (H-1″, H-2″, H-4″, and H-5″) (Figure 1). The HMBC correlations of H-6 to C-7, C-8, and C-10 and of H-5 to C-7 and C-9 revealed the presence of ring A (Figure 1). On the other hand, the HMBC correlations of H-2 to C-8 and C-9, of H-3 to C-8 and C-10, and of H-4 to C-9 and C-10 revealed the presence of another ring, B. These results indicated that ring A and ring B made a bicyclo[3.3.1]non-3-ene-2,9-dione ring. The HMBC correlations of H-2′ to C-2 and of H-2 to C-1′ and C-2′ showed the presence of a 4-hydroxyphenyl group on C-2. The HMBC correlations of H-1″ to C-2, C-7, C-8, and C-9 and of H-2 to C-1″ showed that the 3-methyl-2-butenyl group attached to C-8. Thus, the assignments of ^{1}H and ^{13}C signals of **1** were made by the combination of ^{1}H−^{1}H COSY, HMQC,

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Table 1. ^{1}H NMR Spectral Data of Acutifolins A−F (δ, ppm, in CD_3OD, except as noted; J in Hz, in parentheses)

position	**1**	**2**[a]	**3**[a]	**4**	**5**	**6**
2	2.86 dd (13.4, 4.0)	5.02 dd (10.1, 2.4)	5.02 dd (10.2, 1.5)	4.9[b]	4.93 dd (10.2, 1.9)	4.92 dd (10.1, 1.6)
3a	2.0	2.1	2.1	2.1	2.1	2.1
3b	1.8	2.0	2.0	1.9	1.9	2.0
4a	2.1	2.9	2.9	2.9	2.9	2.9
4b	1.9	2.7	2.7	2.7	2.7	2.7
5	7.03 d (9.9)	6.87 d (8.4)	6.82 d (8.3)	6.75 d (8.2)	6.77 d (8.2)	6.73 d (8.3)
6	6.43 d (9.9)	6.46 d (8.4)	6.36 d (8.3)	6.38 d (8.2)	6.38 d (8.2)	6.33 d (8.3)
MeO-7		3.80 s, 3H				
2′	6.82 d, 2H (8.4)	7.34 d, 2H (8.5)	7.35 d, 2H (8.8)	7.24 d, 2H (8.6)	7.25 d, 2H (8.5)	7.24 d, 2H (8.6)
3′	6.68 d, 2H (8.4)	6.90 d, 2H (8.5)	6.92 d, 2H (8.8)	6.78 d, 2H (8.6)	6.77 d, 2H (8.5)	6.78 d, 2H (8.6)
MeO-4′		3.82 s, 3H	3.83 s, 3H			
1″a	2.26 dd (14.7, 6.8)	3.34 d, 2H (7.6)	6.67 d (10.1)	3.06 dd (13.8, 9.9)	3.05 dd (13.8, 10.0)	2.99, 2H
1″b	2.1			2.61 dd (13.8, 2.3)	2.61 dd (13.8, 2.2)	
2″	4.83 brt (6.8)	5.23 t (7.6)	5.52 d (10.1)	3.52 dd (9.9, 2.3)	3.56 dd (10.0, 2.2)	3.65, 2H
4″	1.54 s, 3H	1.64 s, 3H	1.42 s, 3H	1.15 s, 3H	1.15 s, 3H	
5″	1.46 s, 3H	1.64 s, 3H	1.42 s, 3H	1.13 s, 3H	1.15 s, 3H	

[a] In $CDCl_3$. [b] Overlapped with CD_3OD.

Table 2. ^{13}C NMR Spectral Data of Acutifolins A−F (δ, ppm, in CD_3OD, except as noted)

position	**1**	**2**[a]	**3**[a]	**4**	**5**	**6**
2	56.0	78.2	78.2	80.8	80.9	79.0
3	27.9	30.1	30.0	31.0	31.4	31.4
4	39.0	24.8	24.7	25.9	26.0	25.9
5	152.3	126.6	128.9	128.5	128.5	128.4
6	132.8	103.4	108.6	109.2	109.3	108.7
7	199.2	153.2	151.9	155.8	155.7	155.7
8	72.9	114.5	110.0	115.2	115.3	113.6
9	209.0	153.2	150.4	154.8	154.7	155.2
10	78.9	117.7	113.8	114.6	114.7	114.2
1′	130.2	134.5	134.1	134.2	134.4	134.6
2′	131.3	127.1	127.2	128.3	128.3	128.3
3′	115.6	113.7	113.8	116.1	116.1	116.1
4′	158.0	159.0	159.4	157.9	158.0	158.0
5′	115.6	113.7	113.8	116.1	116.1	116.1
6′	131.3	127.1	127.2	128.3	128.3	128.3
1″	29.7	22.3	117.0	26.7	26.7	27.7
2″	121.1	123.0	128.7	79.0	79.1	62.7
3″	134.2	130.8	75.6	74.0	74.1	
4″	18.1	17.8	27.7	24.8	24.7	
5″	26.0	25.8	27.7	25.8	26.0	
MeO-7		55.9				
MeO-4′		55.3	55.3			

[a] In $CDCl_3$.

and HMBC data (Tables 1 and 2). The NOESY correlations of H-2 to H-4a and of H-4b to H-5 indicated α-orientation of 8-prenyl and 10-hydroxyl groups and β-orientation of the 2-(4′-hydroxyphenyl) group (Figure 1). Therefore, the relative stereochemistry of acutifolin A was determined as **1**. The establishment of the absolute stereochemistry by the chemical modification of **1** failed due to the unstable nature of **1**.

The molecular formula, $C_{22}H_{26}O_3$, of acutifolin B (**2**) was determined by positive-ion HRSIMS [m/z 339.1965 $(M + H)^+$, Δ +0.5 mmu]. The ^{1}H and ^{13}C NMR spectra of **2** indicated the presence of seven substituted sp^2 (three of these were bearing oxygen atoms), seven sp^2 methine, one sp^3 methine (bearing an oxygen atom), three methylene, and four methyl (two of these were methoxy) carbons. Because seven of 10 unsaturations were thus accounted for, it was concluded that **2** also contained three rings. The ^{1}H−^{1}H COSY spectrum revealed the prsence of a 1,4-disubstituted benzene ring (H-2′ and H-3′), a 1,1,3-trisubstitued propane (H-2, H-3, and H-4), a disubstituted olefin (H-5 and H-6), and a 3-methyl-2-butenyl group (H-1″, H-2″, H-4″, and H-5″) (Figure 2), which were similar to those of acutifolin A (**1**). NOESY correlations of **2** were shown in Figure 2, and the chemical shifts of ^{1}H and ^{13}C NMR were shown in Tables 1 and 2, respectively. These data revealed that **2** was a flavan substituted with a prenyl group at C-8 and with two methoxy groups at C-7 and C-4′. Thus, the structure of acutifolin B was elucidated as **2**.

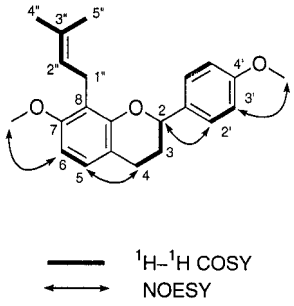

Figure 2. Selected ^{1}H−^{1}H COSY and NOESY correlations of **2**.

The molecular formula, $C_{21}H_{22}O_3$, of acutifolin C (**3**) was determined by positive-ion HRSIMS [m/z 323.1635 $(M + H)^+$, Δ −1.2 mmu]. The ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were similar to those of acutifolin B (**2**) except for the absence of one of two methoxy groups and a prenyl group and instead the presence of another isoprene unit. These NMR data indicated that the molecule possessed a 4′,7,8-trisubstituted flavan ring, a 1,2-disubstituted olefin, one oxygenated sp^3 quarternary carbon, one methoxy, and two methyl groups. The results of NOESY and HMBC experiments of **3** (Figure 3) revealed that a methoxy group was attached to C-4′. Since 10 (a flavan ring which has nine unsaturations and one olefin) of 11 unsaturations were accounted for, it was concluded that **3** contained one more ring (ring D) in the molecule. HMBC correlations (Figure 3) of H-4″ to C-2″, C-3″, and C-5″, of H-2″ to C-3″, C-4″, and C-8, and of H-1″ to C-3″, C-7, C-8, and C-9 revealed the presence of a 2,2-dimethylpyran ring

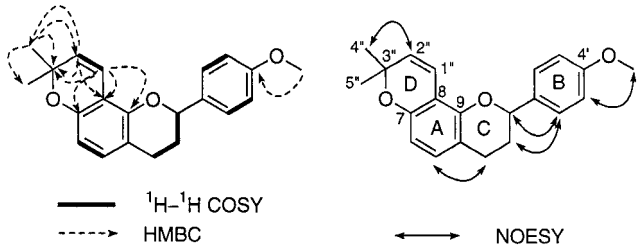

Figure 3. Selected 2D NMR correlations of **3**.

fused to ring A at the C-7 and C-8 positions. Thus, the structure of acutifolin C was elucidated as **3**.

The molecular formula, $C_{20}H_{24}O_5$, of acutifolin D (**4**) was determined by positive-ion HRSIMS [*m*/*z* 345.1709 (M + H)$^+$, Δ +0.8 mmu]. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were also similar to those of acutifolin B (**2**) except for the absence of two methoxy groups and a prenyl group and the presence of another isoprene unit instead. These NMR data indicated that the molecule possessed a 4′,7,8-trisubstituted flavan ring, one oxygenated sp^3 quarternary carbon, one oxygenated sp^3 methine, one sp^3 methylene, and two methyl groups. Since its flavan ring accounted for all of nine unsaturations, it was concluded that **4** did not contain any more rings in the molecule. The ^{1}H−^{1}H COSY spectrum revealed connectivities of C-1″ and C-2″. These data suggested the presence of a 2,3-dihydroxy-3-methylbutyl group connected to C-8. Thus, the structure of acutifolin D was elucidated as **4**.

The molecular formula, $C_{20}H_{24}O_5$, of acutifolin E (**5**) was determined by positive-ion HRSIMS [*m*/*z* 345.1723 (M + H)$^+$, Δ +2.2 mmu] and was the same as that of acutifolin D (**4**). The ^{1}H and ^{13}C NMR of **5** were also very similar to those of acutifolin D (**4**) (Tables 1 and 2). These data indicated that acutifolin E (**5**) was the diastereomer of acutifolin D (**4**) with asymmetric centers at C-2 and C-2″.

The molecular formula, $C_{17}H_{18}O_4$, of acutifolin F (**6**) was determined by positive-ion HRFABMS [*m*/*z* 287.1289 (M + H)$^+$, Δ +0.6 mmu]. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were also similar to those of acutifolins D (**4**) and E (**5**) except for the absence of a 2,3-dihydroxy-3-methylbutyl group and the presence of a 2-hydroxyethyl group instead. The presence of a 2-hydroxyethyl group attached to C-8 was also elucidated by HMBC correlations of H-1″ to C-7, C-8, and C-9 and of H-2″ to C-1″ and C-8″. Thus, the structure of acutifolin F was elucidated as **6**.

Acutifolin A (**1**) is the first natural product with a bicyclo[3.3.1]non-3-ene-2,9-dione ring. Although a bicyclo[3.3.1]nonane-2,4,9-trione ring of catechinic acid is similar to the ring system of acutifolin A (**1**), catechinic acid is reported as a base rearrangement product of catechin.[7] Biogenetically, acutifolin A (**1**) may be derived from brosimine B (**7**), which was previously reported as a constituent of this plant.[4] The bicyclo[3.3.1]non-3-ene-2,9-dione ring of acutifolin A (**1**) may be generated through oxidative ring cleavage of the C-2−O-1 bond followed by formation of the C-2−C-8 bond (Figure 4).

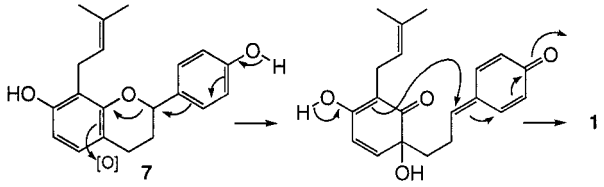

Figure 4. Possible scheme for the formation of acutifolin A (**1**) from bromisine B (**7**).

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AMX500 spectrometer using tetramethylsilane as the internal standard. Mass spectra were obtained on HITACHI M-2000A (SIMS) or JEOL MS-700 (FAB) spectrometers. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV and IR spectra were obtained on Shimadzu UV-260 and JASCO FT/IR-5300 spectrometers, respectively.

Plant Material. The bark of *Brosimum acutifolium* was purchased in Sao Paulo, Brazil. The plant was identified by Dr. K. Yoneda (Osaka University), and a voucher specimen has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Isolation. The MeOH extract (52.12 g) of the bark of *B. acutifolium* (1.5 kg) was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (12.56 g) was subjected to reversed-phase column chromatography (MCI Gel ODS IMY, Mitsubishi Chemical Corp., step gradient with H_2O−CH_3CN) to give six fractions. The third fraction (3.51 g) was eluted with H_2O−CH_3CN (60:40) and further chromatographed over ODS (MCI Gel ODS IMY, step gradient with H_2O−MeOH) to give nine fractions. The fifth fraction (600 mg) and the sixth fraction (860 mg), which were eluted with H_2O−MeOH (55:45 and 50:50, respectively), were purified by reversed-phase HPLC (Capcellpack C18 UG 80, Shiseido, H_2O−CH_3CN) to afford acutifolins A (**1**, 15.0 mg, 0.0010%), D (**4**, 446.0 mg, 0.030%), E (**5**, 24.7 mg, 0.0016%), and F (**6**, 2.0 mg, 0.00013%), 7,4′-dihydroxyflavan (2.6 mg, 0.00017%), and 7,3′-dihydroxy-4′-methoxyflavan (4.8 mg, 0.00032%). The sixth fraction (570 mg) of the first chromatography, which was eluted with CH_3CN, was also purified by reversed-phase HPLC to afford acutifolins B (**2**, 1.3 mg, 0.000087%) and C (**3**, 2.8 mg, 0.00019%) and 4′-hydroxy-7,8-(2″,2″-dimethylpyran)flavan (3.4 mg, 0.00023%).

Acutifolin A (1): colorless amorphous solid; $[\alpha]^{22}_D$ +94.7° (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ϵ) 275 (3.66), 280 (sh, 3.63) nm; IR (KBr) ν_{max} 3422 (br), 2930, 1734, 1667, 1612, 1516, 1447, 1373, 1269 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); HRSIMS *m*/*z* 327.1573 [M + H]$^+$ (calcd for $C_{20}H_{23}O_4$, 327.1596).

Acutifolin B (2): colorless amorphous solid; $[\alpha]^{22}_D$ −2.0° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 277 (3.11) nm; IR (KBr) ν_{max} 2926, 1613, 1516, 1491, 1447, 1383, 1248 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); HRSIMS *m*/*z* 339.1965 [M + H]$^+$ (calcd for $C_{22}H_{27}O_3$, 339.1960).

Acutifolin C (3): colorless amorphous solid; $[\alpha]^{22}_D$ −54.3° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (3.93), 290 (sh, 3.87), 310 (sh, 3.68) nm; IR (KBr) ν_{max} 2930, 1612, 1516, 1477, 1462, 1375, 1248 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); HRSIMS *m*/*z* 323.1635 [M + H]$^+$ (calcd for $C_{21}H_{23}O_3$, 323.1647).

Acutifolin D (4): colorless amorphous solid; $[\alpha]^{22}_D$ −100.0° (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (3.64) nm; IR (KBr) ν_{max} 3274 (br), 2924, 1616, 1599, 1520, 1487, 1447, 1370, 1206 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); HRSIMS *m*/*z* 345.1709 [M + H]$^+$ (calcd for $C_{20}H_{25}O_5$, 345.1701).

Acutifolin E (5): colorless amorphous solid; $[\alpha]^{22}_D$ +8.1° (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 279 (3.61) nm; IR (KBr) ν_{max} 3420 (br), 2924, 1614, 1518, 1487, 1454, 1372, 1221 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); HRSIMS *m*/*z* 345.1723 [M + H]$^+$ (calcd for $C_{20}H_{25}O_5$, 345.1701).

Acutifolin F (6): colorless amorphous solid; $[\alpha]^{22}_D$ −42.4° (*c* 0.085, MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (3.62) nm; IR (KBr) ν_{max} 3426 (br), 2926, 1616, 1518, 1456, 1373, 1244 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); HRFABMS *m*/*z* 287.1289 [M + H]$^+$ (calcd for $C_{17}H_{19}O_4$, 287.1283).

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References and Notes

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