Aromatase Inhibitors from *Broussonetia papyrifera*

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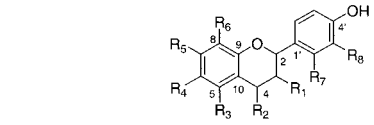
Bioassay-guided fractionation of an ethyl acetate-soluble extract from the whole plants of *Broussonetia papyrifera*, using an in vitro aromatase inhibition assay, led to the isolation of five new active compounds, 5,7,2′,4′-tetrahydroxy-3-geranylflavone (**1**), isogemichalcone C (**8**), 3′-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2′,4′-tetrahydroxychalcone 11′-*O*-coumarate (**9**), demethylmoracin I (**10**), and (2*S*)-2′,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone (**11**), and 10 known (**12**−**21**) compounds which were also found to be active. Of these compounds, the most potent were **9** (IC_{50} 0.5 μM), **11** (IC_{50} 0.1 μM), isolicoflavonol (**12**, IC_{50} 0.1 μM), and (2*S*)-abyssinone II (**13**, IC_{50} 0.4 μM). Additionally, six new compounds, 5,7,3′,4′-tetrahydroxy-6-geranylflavonol (**2**), 5,7,3′,4′-tetrahydroxy-3-methoxy-6-geranylflavone (**3**), (2*S*)-7,4′-dihydroxy-3′-prenylflavan (**4**), 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)propane (**5**), 1-(2,4-dihydroxy-3-prenylphenyl)-3-(4-hydroxyphenyl)propane (**6**), and 1-(4-hydroxy-2-methoxyphenyl)-3-(4-hydroxy-3-prenylphenyl)propane (**7**), were isolated and characterized, but proved to be inactive as aromatase inhibitors, as were an additional 21 known compounds. The structures of the new compounds (**1**−**11**) were elucidated by spectroscopic methods. Structure−activity relationships in the aromatase assay were determined for the benzofurans, biphenylpropanoids, coumarins, and various types of flavonoids (chalcones, flavans, flavanones, and flavones) obtained among a total of 42 constituents of *B. papyrifera*.

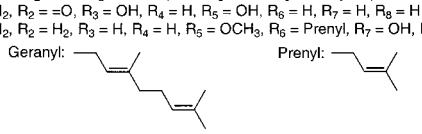
Epidemiological and experimental evidence strongly support a role for estrogens in the development and growth of breast cancer.[1,2] Similarly, the participation of estrogens in prostate neoplasia has been postulated.[3,4] Therefore, one chemotherapeutic or chemopreventive strategy for breast and prostate cancer control is to decrease estrogen production.[5] Accordingly, inhibition of aromatase, an enzyme that catalyzes the final, rate-limiting step in estrogen biosynthesis,[6] is being explored as a target germane to the treatment or prevention of breast and prostate cancers.[5] Aminoglutethimide and its analogues may be considered prototype aromatase inhibitors, and based on the same mechanism of action, substrate androstenedione derivatives, imidazoles, and triazoles have been developed over the past 20 years.[5,7]

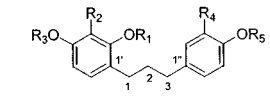
Broussonetia papyrifera (L.) L'Hér. ex Vent. (Moraceae) is a deciduous tree, and its fruits have been used for impotency and to treat ophthalmic disorders in the People's Republic of China.[8,9] Extracts of *B. papyrifera* have shown antifungal,[10] antihepatotoxic,[11] antioxidant,[12] and lens aldose reductase inhibitory activities.[9] Also, several flavonoid constituents of this plant have been shown to inhibit lipid peroxidation[13] and to exhibit antiplatelet effects.[14] Previous phytochemical work on this plant has resulted in the isolation of coumarins,[15] triterpenoids,[16] and various types of flavonoids.[15,17−24]

As part of our continuing search for cancer chemopreventive agents of natural origin, an ethyl acetate-soluble extract of *B. papyrifera* was found to significantly inhibit aromatase activity in an in vitro assay (74% inhibition at 80 μg/mL). Bioassay-guided fractionation of the ethyl acetate-soluble extract of *B. papyrifera* using this assay led to the isolation of five new (**1**, **8**−**11**) and 10 known (**12**−**21**) compounds that were found to be active. Additionally, six new compounds (**2**−**7**) and 21 known compounds were isolated and characterized as inactive when evaluated with this in vitro aromatase assay.[25,26] We currently report the isolation and identification of active and/or new compounds using the aromatase inhibition assay to guide chromatographic purification and describe structure−

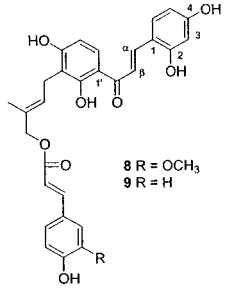


1 R_1 = Geranyl, R_2 = =O, R_3 = OH, R_4 = H, R_5 = OH, R_6 = H, R_7 = OH, R_8 = H, $\Delta^{2(3)}$
2 R_1 = OH, R_2 = =O, R_3 = OH, R_4 = Geranyl, R_5 = OH, R_6 = H, R_7 = H, R_8 = OH, $\Delta^{2(3)}$
3 R_1 = OCH_3, R_2 = =O, R_3 = OH, R_4 = Geranyl, R_5 = OH, R_6 = H, R_7 = H, R_8 = OH, $\Delta^{2(3)}$
4 R_1 = H_2, R_2 = H_2, R_3 = H, R_4 = H, R_5 = OH, R_6 = H, R_7 = H, R_8 = Prenyl
12 R_1 = OH, R_2 = =O, R_3 = OH, R_4 = H, R_5 = OH, R_6 = H, R_7 = H, R_8 = Prenyl, $\Delta^{2(3)}$
13 R_1 = H_2, R_2 = =O, R_3 = H, R_4 = H, R_5 = OH, R_6 = H, R_7 = H, R_8 = Prenyl
14 R_1 = H_2, R_2 = =O, R_3 = OH, R_4 = H, R_5 = OH, R_6 = H, R_7 = OH, R_8 = H
15 R_1 = H_2, R_2 = =O, R_3 = H, R_4 = H, R_5 = OH, R_6 = Prenyl, R_7 = OH, R_8 = H
16 R_1 = OH, R_2 = =O, R_3 = OH, R_4 = H, R_5 = OH, R_6 = Prenyl, R_7 = H, R_8 = Prenyl, $\Delta^{2(3)}$
17 R_1 = H_2, R_2 = =O, R_3 = OH, R_4 = H, R_5 = OH, R_6 = H, R_7 = H, R_8 = H
22 R_1 = H_2, R_2 = H_2, R_3 = H, R_4 = H, R_5 = OCH_3, R_6 = Prenyl, R_7 = OH, R_8 = H





5 R_1 = H, R_2 = H, R_3 = H, R_4 = H, R_5 = H
6 R_1 = H, R_2 = Prenyl, R_3 = H, R_4 = H, R_5 = H
7 R_1 = CH_3, R_2 = H, R_3 = H, R_4 = Prenyl, R_5 = H
18 R_1 = H, R_2 = H, R_3 = CH_3, R_4 = H, R_5 = H



8 R = OCH_3
9 R = H

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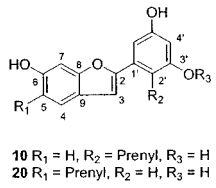
Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**3** in Acetone-d_6[a]

	δ_H			δ_C		
carbon	**1**	**2**	**3**	**1**	**2**	**3**
2				162.4	146.6	156.6
3				121.8	136.6	139.5
4				183.0	176.5	180.2
5				163.4	158.9	160.0
6	6.25, brs			99.2	111.7	112.3
7				164.7	162.7	162.3
8	6.33, brs	6.59, brs	6.57, s	94.2	93.8	94.0
9				159.3	155.6	155.3
10				105.3	104.0	105.7
1′				113.0	123.8	123.1
2′		7.81, brs	7.69, d (1.8)	157.2	115.6	116.5
3′	6.57, brs			103.8	145.7	146.1
4′				161.4	148.2	149.1
5′	6.51, brd (8.3)	6.99, d (8.6)	6.99, d (8.4)	108.0	116.2	116.4
6′	7.19, d (8.3)	7.68, d (7.9)	7.56, dd (2.0, 8.3)	132.3	121.3	122.2
1″	3.12, d (6.9)	3.37, d (7.1)	3.37, d (7.1)	24.4	21.9	21.9
2″	5.14, m	5.29, brt (6.8)	5.30, m	122.6	123.1	123.3
3″				135.8	135.3	135.7
4″	1.89, m	1.96, m	1.95, m	40.4	40.5	40.6
5″	1.43, s	1.79, s	1.80, s	16.0	16.2	16.2
6″	2.00, m	2.05, m	2.05, m	27.3	27.3	27.5
7″	5.04, m	5.07, m	5.08, m	125.1	125.1	125.4
8″				131.6	131.6	131.7
9″	1.61, s	1.54, s	1.56, s	25.8	17.6	17.7
10″	1.55, s	1.59, s	1.61, s	17.7	28.8	25.9
−OCH_3			3.86, s			60.2

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses.

activity relationships among the various compound classes obtained.



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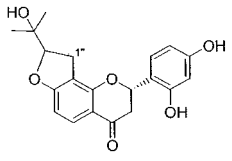

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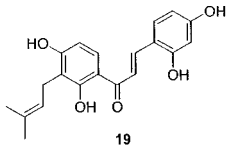

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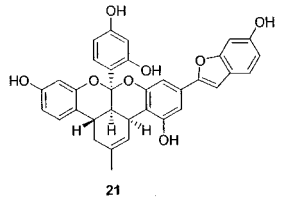

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Results and Discussion

Compound **1** gave a molecular ion $[M]^+$ at *m/z* 422.1719 by HREIMS, consistent with an elemental formula of $C_{25}H_{26}O_6$. In its ^{1}H NMR spectrum (Table 1), characteristic proton signals for a geranyl unit [δ_H 3.12 (2H, J = 6.9 Hz, H-1″), δ_H 5.14 (1H, multiplet, H-2″), δ_H 1.89 (2H, multiplet, H-4″), δ_H 1.43 (3H, singlet, H-5″), δ_H 2.00 (2H, multiplet, H-6″), δ_H 5.04 (1H, multiplet, H-7″), δ_H 1.61 (3H, singlet, H-9″), and δ_H 1.55 (3H, singlet, H-10″)], a set of *meta*-coupled proton signals [δ_H 6.25 (1H, broad singlet, H-6) and δ_H 6.33 (1H, broad singlet, H-8)], and proton signals of an ABX system [δ_H 6.57 (1H, broad singlet, H-3′), δ_H 6.51 (1H, J = 8.3 Hz, H-5′), and δ_H 7.19 (1H, J = 8.3 Hz, H-6′)] were observed. These data suggested that **1** has a flavone skeleton[27] with four hydroxyl groups and one geranyl substituent, and these inferences were confirmed using the APT, COSY, and HMQC NMR techniques. The positions of the substituents were deduced as occurring at C-5, C-7, C-2′, and C-4′ (four hydroxyls) and C-3 (geranyl) using the HMBC NMR technique (see Experimental Section). Additionally, NOE correlations between H-6′ and H-1″, and H-2″ and H-4″, confirmed the position of attachment and the *E* stereochemistry of the geranyl group. Thus, the structure of the new compound **1** was elucidated as 5,7,2′,4′-tetrahydroxy-3-geranylflavone.

The molecular formula of compound **2** was determined as $C_{25}H_{26}O_7$ by HREIMS (*m/z* 438.1683). The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were closely comparable to those of compound **1** except there was evidence of one less aromatic proton. Careful APT, HMQC, and HMBC NMR spectral data interpretation suggested that **2** has a flavonol skeleton with a geranyl group at the C-6 position.[27] The positions of two hydroxyl groups in ring B were concluded to be at C-3′ and C-4′ due to observed HMBC correlations (H-2′/C-2, H-6′/C-2) and the lower field shift of the H-2′ proton signal at δ_H 7.81.[22] Also, the *E* stereochemistry of the geranyl group was confirmed by a NOE correlation between H-2″ and H-4″. Therefore, the new compound **2** was assigned as 5,7,3′,4′-tetrahydroxy-6-geranylflavonol.

Compound **3** showed almost the same ^{1}H and ^{13}C NMR data (Table 1) as those of **2** except for the presence of a methoxyl group [δ_H 3.86 (3H, singlet); δ_C 60.2]. The molecular formula, $C_{26}H_{28}O_7$ (HREIMS, *m/z* 452.1833), was also consistent with an additional methoxyl group in **3** compared with **2**. The position of the methoxyl group was determined as C-3 from the HMBC correlation between the methoxyl signal and C-3. NOE correlations between the methoxyl signal and H-2′ (H-6′), and H-2″ and H-4″, confirmed the position of the methoxyl group and the *E* stereochemistry of the geranyl group, respectively. Thus, the structure of the new compound **3** was deduced as 5,7,3′,4′-tetrahydroxy-3-methoxy-6-geranylflavone.

Compound **4** was obtained as an amorphous brown powder and its molecular formula established as $C_{20}H_{22}O_3$

Table 2. ^{1}H and ^{13}C NMR Data of Compounds **4**, **11**, and **22**[a]

	δ_H			δ_C		
carbon	**4**[b]	**11**[c]	**22**[b]	**4**[b]	**11**[c,d]	**22**[b]
2	4.83, dd (2.2, 9.6)	5.75, m	5.23, dd (1.7, 9.8)	79.1	76.1	74.3
3	1.93, m	2.70, m	1.84, m	31.2	44.1	29.9
	2.04, m	3.03, m	2.14, m			
4	2.62, m		2.62, m	25.4	191.1	25.9
	2.80, m		2.83, m			
5	6.83, d (8.2)	7.70, d (8.4)	6.80, d (8.4)	131.0	129.4	127.8
6	6.30, dd (2.4, 8.2)	6.48 (overlap)	6.41, d (8.4)	109.0	104.8	104.1
7				157.5	167.9	157.6
8	6.24, d (2.3)			104.0	115.0	118.3
9				157.2	160.0	154.8
10				114.3	e	116.0
1′				134.0	117.9	121.4
2′	7.06, d (1.8)			128.5	156.2	156.1
3′		6.48 (overlap)	6.34, d (2.1)	129.1	103.5	103.3
4′				155.8	159.6	158.5
5′	6.73, d (8.1)	6.44, brd (8.4)	6.31, dd (2.0, 8.3)	115.6	108.0	107.4
6′	7.01, dd (2.0, 8.2)	7.36, d (8.4)	7.15, d (8.3)	125.6	129.0	128.3
1″	3.28, d (7.3)	3.09, m	3.23, brd	29.8	28.0	23.1
2″	5.30, m	4.78, dt (2.2, 8.1)	5.14, brt (7.0)	123.9	92.0	124.6
3″				133.0	71.4	131.1
4″	1.68, s	1.28, s	1.64, s	17.8	25.7	18.0
5″	1.71, s	1.21, s	1.61, s	26.0	26.1	26.0
−OCH_3			3.70, s			56.1

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses. [b] MeOH-d_4. [c] Acetone-d_6. [d] Signals derived from HMBC experiment. [e] No signal detected.

Table 3. ^{1}H and ^{13}C NMR Data of Compounds **5**−**7** in MeOH-d_4[a]

	δ_H			δ_C		
carbon	**5**	**6**	**7**	**5**	**6**	**7**
1	2.51, m	2.52, m	2.53, m	30.3	30.8	29.2
2	1.79, m	1.80, m	1.81, m	33.6	33.1	31.8
3	2.51, m	2.52, m	2.53, m	35.9	35.9	34.8
1′				121.3	121.8	123.1
2′				157.0	154.3	158.2
3′	6.26, d (2.4)		6.35, brd	103.4	117.2	98.7
4′				157.2	155.1	154.8
5′	6.20, dd (2.4, 8.1)	6.27, d (8.2)	6.31, dd (2.4, 8.1)	107.2	108.1	106.3
6′	6.81, d (8.1)	6.68, d (overlap)	6.93, d (8.1)	131.4	128.0	129.9
1″				135.0	135.0	134.9
2″	6.98, d (8.6)	6.98, d (8.4)	6.89, brs	130.3	130.3	130.1
3″	6.67, d (8.6)	6.67, d (overlap)		115.9	116.0	126.6
4″				156.1	156.3	152.0
5″	6.67, d (8.6)	6.67, d (overlap)	6.69, d (7.8)	115.9	116.0	115.5
6″	6.98, d (8.6)	6.98, d (8.4)	6.91 (overlap)	130.3	130.3	127.2
1‴		3.33, brd (9.6)	3.30, d (7.0)		23.6	30.0
2‴		5.21, m	5.29, m		124.7	122.0
3‴					131.7	134.5
4‴		1.77, s	1.76, s		18.0	17.9
5‴		1.66, s	1.74, s		26.0	25.8
−OCH_3			3.76, s			55.3

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses.

by HREIMS (*m*/*z* 310.1564). In its ^{1}H NMR spectrum (Table 2), an ABX proton system at δ_H 6.83 (1H, J = 8.2 Hz, H-5), δ_H 6.30 (1H, J = 2.4 and 8.2 Hz, H-6), and δ_H 6.24 (1H, J = 2.3 Hz, H-8) and a second ABX proton system at δ_H 7.06 (1H, J = 1.8 Hz, H-2′), δ_H 6.73 (1H, J = 8.1 Hz, H-5′), and δ_H 7.01 (1H, J = 2.0 and 8.2 Hz, H-6′) were observed. The signals at δ_H 4.83 (1H, J = 2.2 and 9.6 Hz, H-2), δ_H 1.93 (1H, multiplet, H-3), δ_H 2.04 (1H, multiplet, H-3), δ_H 2.62 (1H, multiplet, H-4), and δ_H 2.80 (1H, multiplet, H-4) were coupled to each other. Also, characteristic prenyl proton signals were observed at δ_H 3.28 (2H, J = 7.3 Hz, H-1″), δ_H 5.30 (1H, multiplet, H-2″), δ_H 1.68 (3H, singlet, H-4″), and δ_H 1.71 (3H, singlet, H-5″). The results obtained from the APT and HMQC NMR spectra indicated that **4** has a flavan skeleton with two hydroxyl groups and one prenyl substituent.[19] The positions of these functional groups were determined unambiguously as C-7 and C-4′ (two hydroxyls) and C-3′ (prenyl), respectively, using the HMBC NMR technique. The absolute configuration at C-2 was confirmed as *S* by CD data comparison with literature values for a group of flavans.[28] Accordingly, the structure of the new compound **4** was assigned as (2*S*)-7,4′-dihydroxy-3′-prenylflavan.

Compound **5** was obtained as an amorphous brown powder, and the ^{1}H and ^{13}C NMR data of **5** (Table 3) were almost superimposable to those of broussonins A (**18**) and B except for the absence of one methoxyl signal, consistent with the molecular formula ($C_{15}H_{16}O_3$; HREIMS, *m*/*z* 244.1098) obtained. These observations suggested that **5** contains a 1,3-diphenyl-substituted propane unit with three hydroxyl substituents.[18] The positions of three hydroxyl groups present were confirmed as C-2′, C-4′, and C-4″ using the COSY and HMBC NMR techniques. Thus, the struc-

ture of the new compound **5** was assigned as 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)propane.

Compound **6** was obtained as an amorphous brown powder with the molecular formula $C_{20}H_{24}O_3$ (HREIMS *m/z* 312.1725). In the ^{1}H NMR spectrum of **6** (Table 3), characteristic signals were observed for a prenyl group at δ_H 3.33 (2H, J = 9.6 Hz, H-1‴), δ_H 5.21 (1H, multiplet, H-2‴), δ_H 1.77 (3H, singlet, H-4‴), and δ_H 1.66 (3H, singlet, H-5‴) and two sets of proton signals coupled to each other at δ_H 6.27 (1H, J = 8.2 Hz, H-5′) and δ_H 6.68 (overlapped, H-6′), and δ_H 6.98 (2H, J = 8.4 Hz, H-2″) and δ_H 6.67 (overlapped, H-3″). In the aliphatic region, the signals coupled to each other at δ_H 1.80 (2H, multiplet, H-2) and δ_H 2.52 (4H, multiplet, H-1 and H-3) suggested the presence of a 1,3-diphenyl-substituted propane unit bearing one prenyl and three hydroxyl groups, which was substantiated using the APT, HMQC, and HMBC NMR techniques.[18] Also, the positions of the functional groups were determined unambiguously as C-2′, C-4′, and C-4″ (three hydroxyls) and C-3′ (prenyl) using 2D NMR techniques (COSY and HMBC). Thus, the structure of the new compound **6** was elucidated as 1-(2,4-dihydroxy-3-prenylphenyl)-3-(4-hydroxyphenyl)propane.

The ^{1}H NMR spectrum of compound **7** ($C_{21}H_{26}O_3$; HREIMS *m/z* 326.1877) showed the same profile in the upfield region as that of **6** except for one methoxyl signal at δ_H 3.76 (3H, singlet) (Table 3). However, in the downfield region, the proton signals for an ABX system at δ_H 6.35 (1H, broad doublet, H-3′), δ_H 6.31 (1H, J = 2.4 and 8.1 Hz, H-5′), and δ_H 6.93 (1H, J = 8.1 Hz, H-6′) and for proton signals of a second ABX system at δ_H 6.89 (1H, broad singlet, H-2″), δ_H 6.69 (1H, J = 7.8 Hz, H-5″), and δ_H 6.91 (overlapped, H-6″) were observed. Thus, the carbon skeleton of **7** was determined as being the same as that of **6**. The various functional groups were placed at C-4′ and C-4″ (two hydroxyls), C-2′ (methoxyl), and C-3″ (prenyl) with the aid of the HMBC NMR technique. Accordingly, the structure of the new compound **7** was assigned as 1-(4-hydroxy-2-methoxyphenyl)-3-(4-hydroxy-3-prenylphenyl)-propane.

Compound **8** was obtained as an orange powder and was shown to possess a molecular formula of $C_{30}H_{28}O_9$ by positive HRFABMS (*m/z* [M + Na]$^+$, 555.1577). The ^{1}H and ^{13}C NMR spectra of **8** exhibited characteristic chalcone signals at δ_H 7.80 (1H, J = 15.4 Hz, H-α), δ_H 8.22 (1H, J = 15.4 Hz, H-*β*), δ_C 117.5 (C-α), δ_C 140.9 (C-*β*), and δ_C 193.4 (CO) and signals for a ferulate group at δ_H 7.34 (1H, J = 1.6 Hz, H-2″), δ_H 6.85 (1H, J = 8.1 Hz, H-5″), δ_H 7.12 (1H, J = 1.7 and 8.2 Hz, H-6″), δ_H 7.57 (1H, J = 16.0 Hz, H-7″), δ_H 6.40 (1H, J = 15.9 Hz, H-8″), δ_H 3.91 (3H, singlet, OCH_3), δ_C 145.6 (C-7″), δ_C 115.8 (C-8″), and δ_C 167.3 (C-9″).[29] On the basis of these observations and by comparison of its spectral data with those of gemichalcone C,[29] compound **8** was concluded to be a regioisomer of gemichalcone C. This was confirmed using a NOESY NMR experiment. Thus, the NOE correlations between H-7′ and H-10′, and H-8′ and H-11′, clearly indicated *E* stereochemistry of the prenyl group. Moreover, the chemical shift differences at positions C-10′ and C-11′ of the *E* and *Z* isomers supported the stereochemistry proposed.[29,30] Therefore, the new compound **8** was assigned as 3′-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2′,4′-tetrahydroxychalcone 11′-*O*-ferulate and has been accorded the trivial name isogemichalcone C.

Compound **9** was also obtained as an orange powder and was deduced as having a molecular formula of $C_{29}H_{26}O_8$ by positive HRFABMS (*m/z* [M + Na]$^+$, 525.1484). The ^{1}H and ^{13}C NMR spectra of **9** were almost superimposable with those of **8** except for the ferulate moiety of the latter compound. The presence of AA′XX′-type proton signals at δ_H 7.54 (2H, J = 8.6 Hz, H-2″ and H-6″) and δ_H 6.87 (2H, J = 8.5 Hz, H-3″ and H-5″) and the absence of AMX-type proton signals and any methoxy signal indicated that **9** has a coumarate moiety rather than a ferulate unit as in **8**.[30] The *E* stereochemistry was deduced in the same manner as described for **8**. Accordingly, the structure of the new compound **9** was determined as 3′-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2′,4′-tetrahydroxychalcone 11′-*O*-coumarate.

The ^{1}H and ^{13}C NMR data of compound **10** were almost the same as those of moracin I[31] except for the absence of one methoxyl signal. This was consistent with the molecular formula ($C_{19}H_{18}O_4$; HREIMS, *m/z* 310.1208) obtained. The ^{1}H NMR data of **10** clearly indicated the presence of a benzofuran moiety [δ_H 6.66 (1H, singlet, H-3), δ_H 7.33 (1H, J = 8.4 Hz, H-4), δ_H 6.72 (1H, J = 2.2 and 8.4 Hz, H-5), and δ_H 6.87 (1H, J = 2.1 Hz, H-7)], a prenyl group [δ_H 3.42 (2H, J = 6.3 Hz, H-1″), δ_H 5.13 (1H, multiplet, H-2″), and δ_H 1.64 (6H, s, H-4″ and H-5″)], and *meta*-coupled protons [δ_H 6.61 (1H, J = 2.5 Hz, H-2′) and δ_H 6.33 (1H, J = 2.5 Hz, H-4′)]. Thus, the structure of the new compound **10** was proposed as demethylmoracin I and confirmed using 2D NMR techniques.

Compound **11**, a minor component, was obtained as an amorphous yellow powder and its molecular formula established as $C_{20}H_{20}O_6$ by positive HRFABMS (*m/z* [M + H]$^+$, 357.1327). The ^{1}H NMR spectrum of **11** (Table 2) revealed an ABX system of proton signals at δ_H 6.48 (overlapped, H-3′), δ_H 6.44 (1H, J = 8.4 Hz, H-5′), and δ_H 7.36 (1H, J = 8.4 Hz, H-6′) and a set of protons coupled to each other at δ_H 7.70 (1H, J = 8.4 Hz, H-5) and δ_H 6.48 (overlapped, H-6). Additionally, three proton signals at δ_H 5.75 (1H, multiplet, H-2), δ_H 2.70 (1H, multiplet, H-3), and δ_H 3.03 (1H, multiplet, H-3) and four proton signals at δ_H 3.09 (2H, multiplet, H-1″), δ_H 4.78 (1H, doublet of triplets, H-2″), δ_H 1.28 (3H, singlet, H-4″), and δ_H 1.21 (3H, singlet, H-5″) indicated that **11** is based on a flavanone skeleton with a 1-hydroxy-1-methylethyldihydrofuran group.[32] The locations of each functional group were confirmed using 2D NMR techniques as C-2′ and C-4′ (two hydroxyls) and [2,3-*h*] (dihydrofuran ring). The absolute configuration at C-2 was confirmed by a negative Cotton effect in the $\pi \rightarrow \pi^*$ transition region (~290 nm) in the CD spectrum, which is characteristic for the 2*S* configuration of flavanones.[33] Thus, the structure of the new compound **11** was elucidated as (2*S*)-2′,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone.

Additionally, 10 active compounds of previously known structures were identified as isolicoflavonol (**12**),[34] (2*S*)-abyssinone II (**13**),[35] (2*S*)-5,7,2′,4′-tetrahydroxyflavanone (**14**),[36] (2*S*)-euchrenone a7 (**15**),[37] broussoflavonol F (**16**),[16] (2*S*)-naringenin (**17**),[38] broussonin A (**18**),[18] 2,4,2′,4′-tetrahydroxy-3′-prenylchalcone (**19**),[39] moracin N (**20**),[40] and albanol A (**21**),[41] by spectral data interpretation and comparison with literature values. Furthermore, 21 known compounds, (2*S*)-2′,4′-dihydroxy-7-methoxy-8-prenylflavan (**22**) (for ^{1}H and ^{13}C NMR data, see Table 2),[42] (2*S*)-7,4′-dihydroxyflavan,[20] (2*R*,3*R*)-lespedezaflavanone C,[43] bavachin,[44] (2*R*,3*R*)-katuranin,[45] gancaonin P,[46] (2*R*,3*R*)-5,7,2′,4′-tetrahydroxyflavanonol,[47] broussonins B,[18] E,[20] and F,[20] broussochalcones A[22] and B,[22] isobavachalcone,[48] 2,4,2′,4′-tetrahydroxychalcone,[39] moracins D,[49] I,[31] and M,[31] (3*S*,5*R*)-loliolide,[50] marmesin,[18] *trans*-resveratrol,[51] and 5,7-dihydrocoumarin,[52] were identified in turn by comparison

Table 4. Aromatase Inhibitory Activity of Compounds **1**, **8**−**21**, and Aminoglutethimide[a]

compound	IC_{50} (μM)
1	24.0
8	7.1
9	0.5
10	31.1
11	0.1
12	0.1
13	0.4
14	2.2
15	3.4
16	9.7
17	17.0
18	30.0
19	4.6
20	31.1
21	7.5
aminoglutethimide	6.4

[a] Compounds **2**−**7**, **22**, (2*S*)-7,4′-dihydroxyflavan, (2*R*,3*R*)-lespedezaflavanone C, bavachin, (2*R*,3*R*)-katuranin, gancaonin P, (2*R*,3*R*)-5,7,2′,4′-tetrahydroxyflavanonol, broussonins B, E, and F, broussochalcones A and B, isobavachalcone, 2,4,2′,4′-tetrahydroxychalcone, moracins D, I, and M, (3*S*,5*R*)-loliolide, marmesin, *trans*-resveratrol, and 5,7-dihydrocoumarin were evaluated and found to be inactive as inhibitors of aromatase (IC_{50} > 40 μg/mL).

with published physical and spectral data. All of these 21 known compounds were inactive in the aromatase inhibition assay at the dose levels used (IC_{50} > 40 μg/mL).

Out of a series of 42 compounds from *B. papyrifera*, comprising benzofurans, biphenylpropanoids, coumarins, and various types of flavonoids (chalcones, flavans, flavanones, and flavones), only certain representatives of the latter class of compounds showed potent aromatase inhibition activity. The IC_{50} values of compounds **1** and **8**−**21** are summarized in Table 4. Flavanone **11** (IC_{50} 0.1 μM) and flavone **12**[34] (IC_{50} 0.1 μM) were the most potent flavonoids obtained, exhibiting potency that was approximately 60-fold greater than aminoglutethimide, the positive control used for this assay. The functionalized chalcone **9** (IC_{50} 0.5 μM) and the flavanone **13**[35] (IC_{50} 0.4 μM) were approximately 10 times more active than aminoglutethimide. Interestingly, the various benzofurans [demethylmoracin I (**10**), moracins D,[49] I,[31] M,[31] and N (**20**)[40]], biphenylpropanoids [**5**−**7**, broussonins A (**18**),[18] B,[18] E,[20] and F[20]], flavanonols [(2*R*,3*R*)-lespedezaflavanone C,[43] (2*R*,3*R*)-katuranin,[45] and (2*R*,3*R*)-5,7,2′,4′-tetrahydroxyflavanonol[47]], and flavans [**4**, **22**, and (2*S*)-7,4′-dihydroxyflavan[20]] tested, which are quite closely related structurally to the active compounds, did not show potent anti-aromatase activity. It was noted that a carbonyl group in compounds of the chalcone, flavone, and flavanone classes is required for the exhibition of potent aromatase inhibition activity. However, the presence of a C-5 hydroxyl group among the flavanones decreased activity significantly (**14**,[36] IC_{50} 2.2 μM, and **17**,[38] IC_{50} 17.0 μM), and flavones or flavanones with a prenyl or geranyl unit at C-6 (**2**, **3**, bavachin,[44] and gancaonin P[46]) were not active. Presumably such a bulky substituent at C-6 prevents these compounds from interacting with the enzyme.

It has been reported that some flavonoids (flavones, flavanones, and isoflavones) inhibit aromatase activity.[25,26,53,54] In the present study, inhibition was achieved at physiologically relevant concentrations (100−1000 nM) of dietary flavonoids. Moreover, the fruits of *B. papyrifera* have been consumed by individuals in the People's Republic of China, albeit for the treatment of various medical disorders, rather than as an edible plant.[8,9] Accordingly, these compounds show significant potential for development as cancer chemopreventive agents. To the best of our knowledge, this paper reports the most potent aromatase inhibitors (**11**, IC_{50} 0.1 μM, and **12**,[34] IC_{50} 0.1 μM) derived from a natural source thus far.[55]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. CD measurements were performed using a JASCO 600 CD spectrometer. IR spectra were collected on a JASCO 410 FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and Bruker DRX-500 MHz spectrometers using a 5 mm or a 2.5 mm sample tube. MS and HRMS were recorded on a Finnigan MAT 90 instrument operating at 70 eV and a HPLC-ESMS system (Hewlett-Packard 5989B mass spectrometer, 5998A electrospray interface). MALDI-TOF-MS data were obtained on a Bruker Reflex III TOF mass spectrometer. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography. HPLC was performed using a Hitachi system with a L-7100 pump and a L-7100 UV detector and a Waters system with a 515 pump and a 2487 UV detector.

Plant Material. Whole plants of *Broussonetia papyrifera* (L.) L'Hér. ex Vent. were collected at Shawnee National Forest, Harrisburg, IL, in September 1998 and dried. A voucher specimen (accession number 2208806) has been deposited at the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The dried plant material (4.8 kg) was ground and extracted with MeOH (3 × 10 L) by maceration. The extracts were combined and concentrated in vacuo at 40 °C. The concentrated extract was suspended in 90% MeOH and then partitioned with petroleum ether (3 × 3 L) to afford a petroleum ether-soluble syrup (D001, 43.5 g) on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (2 L) and partitioned again with EtOAc (3 × 2 L) to give an EtOAc-soluble extract (D002, 64.8 g) and an aqueous residue (D003, 170.0 g). The EtOAc-soluble extract significantly inhibited aromatase activity (D002, 74% inhibition at 80 μg/mL; D001, 27% inhibition; D003, 14% inhibition).

Fractionation of the EtOAc-soluble extract (D002) was initiated by vacuum-liquid chromatography over Si gel as stationary phase using a $CHCl_3$−MeOH gradient as mobile phase to afford 13 pooled fractions (F001−F013). Of these, F005−F007 showed the most potent aromatase inhibitory activity (94−95% inhibition at 80 μg/mL) and were worked up separately. Thus, F005 [eluted with $CHCl_3$−MeOH (40:1); 94% inhibition at 80 μg/mL] was eluted on Si gel with gradient mixtures of $CHCl_3$−MeOH to afford fractions F014−F021. Of these, F018 [eluted with $CHCl_3$−MeOH (30:1); 50% inhibition at 8 μg/mL] was chromatographed over Si gel with petroleum ether−EtOAc (20:1 → 2:1), resulting in the isolation of broussoflavonol F (**16**, 30 mg, 0.00063%)[16] and marmesin (12 mg, 0.00025%).[18] Additional chromatographic separation of a fraction eluted by petroleum ether−EtOAc (10:1) over MCI-gel CHP 20P (Supleco, Bellefonte, PA) using a H_2O−MeOH gradient yielded broussochalcone B (2 mg, 0.000042%)[22] and isobavachalcone (2.5 mg, 0.000052%).[48] Further separation of an impure fraction eluted by petroleum ether−EtOAc (9:1) by HPLC [YMC ODS-AQ Pack (YMC, Wilmington, NC), 250 × 20 mm i.d., 85% MeOH in H_2O, flow rate 7 mL/min] resulted in the purification of 1-(4-hydroxy-2-methoxyphenyl)-3-(4-hydroxy-3-prenylphenyl)propane (**7**, t_R 16 min, 2.5 mg, 0.000052%). F019 [eluted with $CHCl_3$−MeOH (20:1); 51% inhibition at 8 μg/mL] was chromatographed on a Si gel column developed with petroleum ether−EtOAc (15:1 → 2:1) to afford fractions F022−F031. (3*S*,5*R*)-Loliolide (7 mg, 0.00015%)[50] was crystallized from F031 (petroleum ether−EtOAc, 1:1). F028 [eluted with petroleum ether−EtOAc (10:1); 77% inhibition at 8 μg/mL] was passed over a column containing Sephadex LH-20 (Sigma, St. Louis, MO) using MeOH for elution, resulting in two separate fractions. From the latter fraction, broussonin

B (8 mg, 0.00017%)[18] was obtained. Further purification of the first fraction was carried out by HPLC (YMC ODS-AQ Pack, 250 × 20 mm i.d., 80% MeCN in H_2O, flow rate 7 mL/min) to afford (2*S*)-naringenin (**17**, t_R 11 min, 1.6 mg, 0.000033%),[38] (2*S*)-abyssinone II (**13**, t_R 20 min, 0.5 mg, 0.00001%),[35] and bavachin (t_R 22 min, 0.3 mg, 0.0000063%).[44] F029 [eluted with petroleum ether−EtOAc (8:1); 83% inhibition at 8 μg/mL] was further chromatographed on TSK-gel Toyopearl HW 40F (Supleco, Bellefonte, PA) using a H_2O−MeOH gradient, resulting in the isolation of broussonin A (**18**, 3 mg, 0.000063%),[18] (2*R*,3*R*)-lespedezaflavanone C (1.3 mg, 0.000027%),[43] and moracins D (1.3 mg, 0.000027%)[49] and I (5.5 mg, 0.00015).[31] The impure fraction eluted with 70% MeOH in H_2O was subjected to preparative TLC using $CHCl_3$−MeOH (20:1) to afford (2*S*)-7,4′-dihydroxyflavan (1.1 mg, 0.000023%)[20] and broussonins E (2 mg, 0.000042)[20] and F (1 mg, 0.000021%).[20] F030 [eluted with petroleum ether−EtOAc (5:1); 59% inhibition at 8 μg/mL] was subjected to passage over C_{18} reversed-phase Si gel (Sigma, St. Louis, MO) using 70% MeOH in H_2O, resulting in the purification of (2*S*)-7,4′-dihydroxy-3′-prenylflavan (**4**, 10 mg, 0.00021%), (2*S*)-2′,4′-dihydroxy-7-methoxy-8-prenylflavan (**22**, 5 mg, 0.0001%),[42] and 1-(2,4-dihydroxy-3-prenylphenyl)-3-(4-hydroxyphenyl)propane (**6**, 3.5 mg, 0.000073%).

Fraction F006 [eluted with $CHCl_3$−MeOH (30:1); 95% inhibition at 80 μg/mL] was chromatographed on Si gel with gradient mixtures of $CHCl_3$−MeOH, resulting in the preparation of fractions F032−F041. Then, F037 [eluted with $CHCl_3$−MeOH (30:1); 66% inhibition at 8 μg/mL] was further chromatographed on TSK-gel Toyopearl HW 40F using MeOH, producing subfractions F042−F049. F043, F044, F047, and F048 were purified on C_{18} reversed-phase Si gel using a H_2O−MeOH gradient, leading to the isolation of 5,7-dihydroxycoumarin (3 mg, 0.000063%),[52] (2*R*,3*R*)-katuranin (6.5 mg, 0.00014%),[45] moracin N (**20**, 4 mg, 0.000083%),[40] and 2,4,2′,4′-tetrahydroxy-3′-prenylchalcone (**19**, 8 mg, 0.00017%),[39] respectively. F045 and F046 were purified using HPLC (YMC ODS-AQ Pack, 250 × 20 mm i.d., 60% MeCN in H_2O, flow rate 7 mL/min), resulting in the purification of demethylmoracin I (**10**, t_R 15 min, 2.5 mg, 0.000052%), (2*S*)-2′,4′-dihydroxy-2″-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone (**11**, t_R 19 min, 0.5 mg, 0.00001%), 5,7,3′,4′-tetrahydroxy-3-methoxy-6-geranylflavone (**3**, t_R 11 min, 3 mg, 0.000063%), and 5,7,2′,4′-tetrahydroxy-3-geranylflavone (**1**, t_R 12 min, 2 mg, 0.000042%), respectively. F038 [eluted with $CHCl_3$−MeOH (20:1); 61% inhibition at 8 μg/mL] was subjected to passage over Sephadex LH-20 using MeOH, resulting in pooled fractions F050−F059. *trans*-Resveratrol (12 mg, 0.00025%)[51] was crystallized from F051. F052 (62% inhibition at 4 μg/mL) was further purified by C_{18} reversed-phase Si gel using 50% MeOH in H_2O, resulting in the purification of (2*S*)-5,7,2′,4′-tetrahydroxyflavanone (**14**, 5 mg, 0.0001%),[36] 5,7,3′,4′-tetrahydroxy-6-geranylflavonol (**2**, 3.5 mg, 0.000073%), and 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)propane (**5**, 4 mg, 0.000083%). F054 (67% inhibition at 4 μg/mL) was purified by HPLC (YMC ODS-AQ Pack, 250 × 20 mm i.d., 50% MeCN in H_2O, flow rate 5 mL/min), leading to the isolation of euchrenone a7 (**15**, t_R 20 min, 1.1 mg, 0.000023%),[37] gancaonin P (t_R 29 min, 2 mg, 0.000042%),[46] and broussochalcone A (t_R 42 min, 0.9 mg, 0.000019%).[22] F055 (72% inhibition at 4 μg/mL) was chromatographed over C_{18} reversed-phase Si gel using 40% MeOH in H_2O, resulting in pure moracin M (4 mg, 0.000083%)[31] and 2,4,2′,4′-tetrahydroxychalcone (1 mg, 0.000021%).[39]

Fraction F007 [eluted with $CHCl_3$−MeOH (20:1); 94% inhibition at 80 μg/mL] was eluted on Sephadex LH-20 using a H_2O−MeOH gradient producing fractions F060−F064. F062 (eluted with 60% MeOH in H_2O; 75% inhibition at 4 μg/mL) was purified using HPLC (YMC ODS-AQ Pack, 250 × 20 mm i.d., 50% MeCN in H_2O, flow rate 5 mL/min) to afford pure 3′-[γ-hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2′,4′-tetrahydroxychalcone 11′-*O*-coumarate (**9**, t_R 27 min, 2 mg, 0.000042%), isogemichalcone C (**8**, t_R 31 min, 1.5 mg, 0.000031%), and isolicoflavonol (**12**, t_R 38 min, 0.8 mg, 0.000017%).[34] F063 (eluted with 80% MeOH in H_2O; 76% inhibition at 4 μg/mL) was purified using HPLC (YMC ODS-AQ Pack, 250 × 20 mm i.d., 30% MeCN in H_2O, flow rate 5 mL/min), resulting in the purification of (2*R*,3*R*)-5,7,2′,4′-tetrahydroxyflavanonol (t_R 11 min, 3.5 mg, 0.000073%)[47] and albanol A (**21**, t_R 21 min, 3.7 mg, 0.000077%).[41]

5,7,2′,4′-Tetrahydroxy-3-geranylflavone (**1**): brown powder; mp 94−95 °C; UV (MeOH) λ_{max} (log ε) 314 (4.16), 258 (4.40), 207 (4.78) nm; IR (NaCl) ν_{max} 3335, 2922, 1652, 1507, 1163 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HMBC correlations H-6/C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-3′/C-1′, C-2′, C-4′, C-5′; H-5′/C-1′, C-3′; H-6′/C-2, C-2′; H-1″/C-2, C-3, C-4; OH-5/C-5, C-6, C-10; NOESY correlations: H-6′/H-1″; H-2″/H-4″; H-7″/H-9″; EIMS *m/z* 422 (M^+, 45), 353 (100), 311 (31), 299 (51), 297 (51), 153 (38), 149 (25); HREIMS *m/z* 422.1719, calcd for $C_{25}H_{26}O_6$, 422.1729.

5,7,3′,4′-Tetrahydroxy-6-geranylflavonol (**2**): brown powder; mp 158−156 °C; UV (MeOH) λ_{max} (log ε) 376 (4.34), 258 (4.34), 206 (4.66) nm; IR (NaCl) ν_{max} 3365, 2920, 1652, 1540 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HMBC correlations H-2′/C-2, C-4′, C-6′; H-5′/C-1′, C-3′; H-6′/C-2, C-2′, C-4′; H-1″/C-5, C-6, C-7, C-2″, C-3″; OH-5/C-5, C-6, C-10; NOESY correlations H-2″/H-4″; H-7″/H-9″; EIMS *m/z* 438 (M^+, 45), 369 (84), 353 (25), 315 (100), 143 (35); HREIMS *m/z* 438.1683, calcd for $C_{25}H_{26}O_7$, 438.1679.

5,7,3′,4′-Tetrahydroxy-3-methoxy-6-geranylflavone (**3**): brown powder; mp 98−99 °C; UV (MeOH) λ_{max} (log ε) 351 (4.11), 270 (4.11), 260.5 (4.13), 205 (4.50) nm; IR (NaCl) ν_{max} 3362, 2925, 1646, 1472 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HMBC correlations H-8/C-6, C-7, C-9, C-10; H-2′/C-2, C-4′, C-6′; H-5′/C-1′, C-3′; H-6′/C-2, C-2′, C-4′; H-1″/C-5, C-6, C-7, C-2″, C-3″; OCH_3/C-3; OH-5/C-5, C-6, C-10; NOESY correlations OCH_3/H-2′; H-1″/H-5″; H-2″/H-4″; H-7″/H-9″; EIMS *m/z* 452 (M^+, 46), 409 (7), 383 (99), 329 (100), 137 (16); HREIMS *m/z* 452.1833, calcd for $C_{26}H_{28}O_7$, 452.1835.

(2*S*)-7,4′-Dihydroxy-3′-prenylflavan (**4**): brown powder; mp 116−117 °C; $[\alpha]_D^{20}$ −4.9° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 305 (3.10), 283 (3.40), 207 (4.28) nm; CD (MeOH) nm $\Delta\epsilon_{282}$ −10.9; IR (NaCl) ν_{max} 3364, 2920, 1617, 1507 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HMBC correlations H-2/C-3, C-4, C-9, C-1′, C-2′, C-6′; H-3/C-2, C-4, C-1′; H-4/C-2, C-3, C-5, C-9; H-5/C-4, C-7, C-9; H-6/C-7, C-8, C-10; H-8/C-6, C-9, C-10; H-2′/C-2, C-4′, C-6′, C-1″; H-5′/C-1′, C-3′, C-4′, C-6′; H-6′/C-2, C-2′, C-4′; H-1″/C-3′, C-2″, C-3″; H-2″/C-4″, C-5″; EIMS *m/z* 310 (M^+, 100), 188 (80), 175 (37), 133 (50); HREIMS *m/z* 310.1564, calcd for $C_{20}H_{22}O_3$, 310.1568.

1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)propane (**5**): brown powder; mp 92−93 °C; UV (MeOH) λ_{max} (log ε) 280 (3.59), 224 (4.07), 205.5 (4.28) nm; IR (NaCl) ν_{max} 3335, 2929, 1615, 1511 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HMBC correlations: H-1/C-2, C-1′, C-2′; H-2/C-1, C-3, C-1′, C-1″; H-3/C-2, C-1″, C-2″; H-3′/C-1′, C-2′, C-5′; H-5′/C-1′, C-3′, C-4′; H-6′/C-1, C-2′; H-2″/C-3, C-3″, C-4″; H-3″/C-1″, C-4″; EIMS *m/z* 244 (M^+, 68), 134 (23), 123 (100), 107 (32); HREIMS *m/z* 244.1098, calcd for $C_{15}H_{16}O_3$, 244.1099.

1-(2,4-Dihydroxy-3-prenylphenyl)-3-(4-hydroxyphenyl)-propane (**6**): brown powder; mp 115−116 °C; UV (MeOH) λ_{max} (log ε) 279 (3.28), 232 (3.55) nm; IR (NaCl) ν_{max} 3421, 2909, 1652, 1515 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HMBC correlations H-1/C-1′, C-2′, C-6′; H-2/C-1′, C-1″; H-3/C-1″, C-2″; H-5′/C-1′, C-3′, C-4′; H-6′/C-1, C-2′, C-4′; H-2″/C-3, C-1″, C-4″; H-3″/C-1″, C-4″; H-1‴/C-2′, C-4′; H-4‴ and H-5‴/C-2‴, C-3‴; EIMS *m/z* 312 (M^+, 67), 257 (12), 191 (100), 135 (74); HREIMS *m/z* 312.1725, calcd for $C_{20}H_{24}O_3$, 312.1725.

1-(4-Hydroxy-2-methoxyphenyl)-3-(4-hydroxy-3-prenylphenyl)propane (**7**): brown powder; mp 85−86 °C; UV (MeOH) λ_{max} (log ε) 281 (3.59), 228 (3.97) nm; IR (NaCl) ν_{max} 3420, 2925, 1651, 1507 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HMBC correlations H-1/C-2, C-1′, C-2′; H-2/C-1, C-3, C-1′, C-1″; H-3/C-2, C-1″, C-6″; H-3′/C-1′, C-2′, C-4′, C-5′; H-5′/C-1′, C-3′, C-4′; H-6′/C-1, C-2′; H-2″/C-1″, C-4″, C-1‴; H-5″/C-4″; H-6″/C-3, C-4″; H-1‴/C-2″, C-3″, C-4″, C-2‴; H-2‴/C-1‴, C-4‴, C-5‴; OCH_3/C-2′; EIMS *m/z* 326 (M^+, 66), 175 (41), 137 (100); HREIMS *m/z* 326.1877, calcd for $C_{21}H_{26}O_3$, 326.1881.

Isogemichalcone C (**8**): orange powder; UV (MeOH) λ_{max} (log ε) 386 (4.40), 321 (4.39), 206 (4.65) nm; IR (NaCl) ν_{max} 3267, 2922, 1676, 1599, 1492, 1368, 1242, 1176 cm^{-1}; ^1H NMR

(CD_3COCD_3, 500 MHz) δ 1.88 (3H, s, H-10′), 3.46 (2H, d, J = 7.4 Hz, H-7′), 3.91 (3H, s, OCH_3), 4.54 (2H, s, H-11′), 5.69 (1H, brt, J = 8.0 Hz, H-8′), 6.40 (1H, d, J = 15.9 Hz, H-8″), 6.45 (1H, brd, J = 8.5 Hz, H-5), 6.51 (1H, brs, H-3), 6.53 (1H, d, J = 8.9 Hz, H-5′), 6.85 (1H, d, J = 8.1 Hz, H-5″), 7.12 (1H, dd, J = 1.7 and 8.2 Hz, H-6″), 7.34 (1H, d, J = 1.6 Hz, H-2″), 7.57 (1H, d, J = 16.0 Hz, H-7″), 7.68 (1H, d, J = 8.5 Hz, H-6), 7.80 (1H, d, J = 15.4 Hz, H-α), 7.91 (1H, d, J = 8.8 Hz, H-6′), 8.22 (1H, d, J = 15.4 Hz, H-β); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 14.2 (C-10′), 22.0 (C-7′), 56.3 (OCH_3), 70.2 (C-11′), 103.6 (C-3), 107.9 (C-5′), 109.1 (C-5), 111.2 (C-2″), 114.5 (C-1′), 115.0 (C-3′), 115.2 (C-1), 115.8 (C-8″), 116.0 (C-5″), 117.5 (C-α), 124.0 (C-6″), 127.5 (C-1″), 127.7 (C-8′), 130.2 (C-6′), 131.2 (C-9′), 131.7 (C-6), 140.9 (C-β), 145.6 (C-7″), 148.7 (C-3″), 150.0 (C-4″), 159.9 (C-2), 162.3 (C-4), 162.5 (C-4′), 165.1 (C-2′), 167.3 (C-9″), 193.4 (CO); HMBC correlations H-6/C-β; H-α/CO; H-β/CO; H-6′/CO; H-7′/C-2′, C-3′, C-4′; H-11′/C-9″; NOESY correlations H-7′/H-10′; H-8′/H-11′; H-2″/OCH_3; FABMS m/z 555 $[M + Na]^+$, 479 (25), 329 (100), 307 (22), 284 (15), 198 (50); HRFABMS m/z 555.1577, calcd for $C_{30}H_{28}O_9Na$, 555.1623.

3′-[γ-Hydroxymethyl-(*E*)-γ-methylallyl]-2,4,2′,4′-tetrahydroxychalcone 11′-*O*-coumarate (**9**): orange powder; UV (MeOH) λ_{max} (log ϵ) 387 (4.28), 312 (4.39), 207 (4.56) nm; IR (NaCl) ν_{max} 3160, 2923, 1674, 1602, 1444, 1368, 1240, 1168, 1109 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 1.88 (3H, s, H-10′), 3.46 (2H, d, J = 7.1 Hz, H-7′), 4.55 (2H, s, H-11′), 5.68 (1H, brt, J = 7.0 Hz, H-9′), 6.35 (1H, d, J = 16.0 Hz, H-8″), 6.45 (1H, brd, J = 8.4 Hz, H-5), 6.51 (1H, brs, H-3), 6.53 (1H, d, J = 8.8 Hz, H-5′), 6.87 (2H, d, J = 8.5 Hz, H-3″ and H-5″), 7.54 (2H, d, J = 8.6 Hz, H-2″ and H-6″), 7.59 (1H, d, J = 16.0 Hz, H-7″), 7.69 (1H, d, J = 8.5 Hz, H-6), 7.79 (1H, d, J = 15.4 Hz, H-α), 7.90 (1H, d, J = 8.9 Hz, H-6′), 8.21 (1H, d, J = 15.4 Hz, H-β); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 14.2 (C-10′), 22.0 (C-7′), 70.2 (C-11′), 103.6 (C-3), 107.8 (C-5′), 109.1 (C-5), 114.5 (C-1′), 115.0 (C-3′), 115.2 (C-1), 115.6 (C-8″), 116.6 (C-3″ and C-5″), 117.4 (C-α), 127.0 (C-1″), 127.7 (C-8′), 130.2 (C-6′), 130.9 (C-2″ and C-6″), 131.2 (C-9′), 131.7 (C-6), 140.9 (C-β), 145.3 (C-7″), 159.9 (C-2), 160.5 (C-4″), 162.3 (C-4′), 162.5 (C-4), 165.1 (C-2′), 167.3 (C-9″), 193.4 (CO); HMBC correlations H-6/C-β; H-α/CO; H-β/CO; H-6′/CO; H-7′/C-2′, C-3′, C-4′; H-11′/C-9″; FABMS m/z 525 $[M + Na]^+$, 460 (35), 307 (100), 289 (95), 273 (43), 242 (30); HRFABMS m/z 525.1484, calcd for $C_{29}H_{26}O_8Na$, 525.1518.

Demethylmoracin I (**10**): brown powder; mp 82−83 °C; UV (MeOH) λ_{max} (log ϵ) 310 (4.30), 214 (4.47) nm; IR (NaCl) ν_{max} 3364, 2924, 1621, 1488, 1145 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 1.64 (6H, s, H-4″ and H-5″), 3.42 (2H, d, J = 6.3 Hz, H-1″), 5.13 (1H, m, H-2″), 6.33 (1H, d, J = 2.5 Hz, H-4′), 6.61 (1H, d, J = 2.5 Hz, H-2′), 6.66 (1H, s, H-3), 6.72 (1H, dd, J = 2.2 and 8.4 Hz, H-5), 6.87 (1H, d, J = 2.1 Hz, H-7), 7.33 (1H, d, J = 8.4 Hz, H-4); ^{13}C NMR (CD_3OD, 125 MHz) δ 18.1 (C-4″), 25.9 (C-5″), 26.0 (C-1″), 98.4 (C-7), 103.8 (C-4′), 105.5 (C-3), 113.0 (C-5 and C-2′), 119.3 (C-1′), 121.9 (C-4), 123.0 (C-9), 125.7 (C-2″), 131.4 (C-3″), 133.0 (C-6′), 156.2 (C-3′), 156.6 (C-2), 156.9 (C-8), 157.0 (C-6), 157.9 (C-5′); HMBC correlations H-3/C-2, C-9; H-2′/C-2, C-1′, C-4′; H-1″/C-1′, C-5′, C-6′; EIMS m/z 310 (M^+, 100), 295 (37), 267 (55), 188 (67), 123 (26); HREIMS m/z 310.1208, calcd for $C_{19}H_{18}O_4$, 310.1205.

2*S*-2′,4′-Dihydroxy-2″-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-*h*]flavanone (**11**): yellow powder; UV (MeOH) λ_{max} (log ϵ) 387 (3.23), 297 (3.30), 284.5 (3.38), 219 (3.82) nm; CD nm (MeOH) $\Delta\epsilon_{294}$ −7.2; IR (NaCl) ν_{max} 3228, 2923, 1683 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HMBC correlations H-2/C-2′, C-6′; H-3/C-2, C-4; H-5/C-4, C-7, C-9; H-6/C-7, C-8; H-3′/C-2′, C-4′, C-5′; H-5′/C-1′, C-3′; H-6′/C-2, C-2′, C-4′; H-1″/C-7, C-8, C-9, C-2″, C-3″; H-2″/C-7, C-4″, C-5″; FABMS m/z 357 $[M + H]^+$, 307 (40), 253 (15), 176 (80), 154 (100), 119 (95), 90 (85); HRFABMS m/z 357.1327, calcd for $C_{20}H_{21}O_6$, 357.1332.

Assay for Inhibition of Aromatase Activity. Microsomes were prepared from freshly delivered human term placentas using 0.05 M potassium phosphate buffer, pH 7.4, and stored frozen in plastic tubes at −70 °C. Reaction mixtures were prepared in glass tubes containing 4 μL of placental microsomes (5 mg/mL), 0.3 μL of [1,2-^{3}H]androstenedione (42.0 Ci/mmol, 1.0 mCi/mL) (NEN Life Science Products, Boston, MA), 5 μL of unlabeled androstenedione (0.875 μM), 5 μL of NADPH (0.48 mM), 10 μL of test sample (dissolved in DMSO), and 0.05 M potassium phosphate buffer, pH 7.4 (500 μL, final volume). After a 4 min incubation at 37 °C, the reaction was terminated by adding 3 mL of chloroform. The tubes were centrifuged at 2000*g* for 10 min, and then 300 μL of the aqueous phases were transferred to tubes containing 300 μL of charcoal/dextrin solution (5%). Following another 10 min centrifugation at 2000*g*, supernatant fractions (500 μL) were used for the determination of radioactivity. Inhibition of aromatase activity was calculated using the following equation:

$$\%\ \text{inhibition} = \left(1 - \frac{\text{sample(DPM)} - \text{blank(DPM)}}{\text{DMSO(DPM)} - \text{blank(DPM)}}\right) \times 100$$

Samples were tested in duplicate, and the mean values were used to prepare dose−response curves. Results were typically expressed as IC_{50} values. Aminoglutethimide (Sigma, St. Louis, MO) was used as a positive control.[25,26,56,57]

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Supporting Information Available: UV, CD, IR, ^{1}H NMR, ^{13}C NMR, and MS data of the known compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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