Rhuschalcones II−VI, Five New Bichalcones from the Root Bark of *Rhus pyroides*

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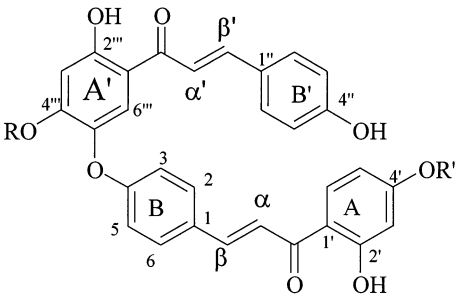
Biflavonoids detected in trace amounts in an earlier investigation of the twigs of *Rhus pyroides* have now been found in the root bark of this species. These new flavonoids belong to a rare bichalcone class and have been identified as 2′,4′,4″,2‴,4‴-pentahydroxy-4-*O*-5‴-bichalcone (rhuschalcone II, **2**), 2′,4′,4″,2‴-tetrahydroxy-4‴-methoxy-4-*O*-5‴-bichalcone (rhuschalcone III, **3**), 4,2′,4″,2‴-tetrahydroxy-4‴-methoxy-4′-*O*-5‴-bichalcone (rhuschalcone IV, **4**), 4,2′,4′,4″,2‴,4‴-hexahydroxy-3,5‴-dihydrochalcone-chalcone (rhuschalcone V, **5**), and 4,2′,4′,4″,2‴,4‴-hexahydroxy-3,5‴-bichalcone (rhuschalcone VI, **6**), repectively. Also obtained was the known compound rhuschalcone I (**1**). Their structures were determined by spectroscopic and chemical methods, and for **1**−**3** by total synthesis. All the bichalcones (**1**−**6**) tested exhibited selective cytotoxic activity against the HT29 and HCT-116 colon tumor cell lines.

The genus *Rhus* consists of ca. 200 species[1] and is rich in biflavonoids.[2] Biflavonoids such as agathisflavone, amentoflavone, hinokiflavone, rhusflavanone, and succedaneaflavone have been sourced from *Rhus* species and evaluated for activity against a range of pathologically significant viruses.[3] In another study, hinokiflavone was found as the most active among 65 natural flavonoids to inhibit the pro-coagulant activity of adherent human monocytes stimulated by endotoxin and interleukin-1-β in vitro.[4] Other *Rhus* biflavonoids have also shown cytotoxic[5] and antimalarial[6] activities.

Rhus pyroides Burch. (Anacardiaceae) is a shrub to a medium-sized tree widely distributed in the eastern part of Botswana. In an earlier investigation of the twigs of this plant we isolated and characterized a new bichalcone, which was given the name rhuschalcone I.[7] In the present paper we report the isolation and characterization of five new bichalcones (**2**−**6**) from the root bark of *R. pyroides*. These compounds were evaluated in the 60-cell line panel of the U.S. National Cancer Institute.[8]

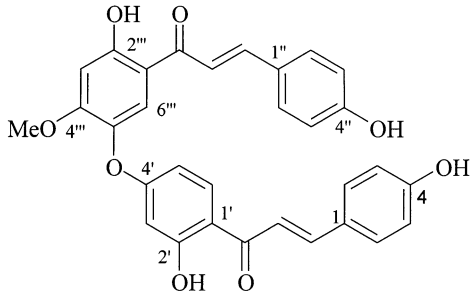


1 R = R' =Me

2 R=R' = H

3 R = Me, R'=H

7 R = H, R' = Me



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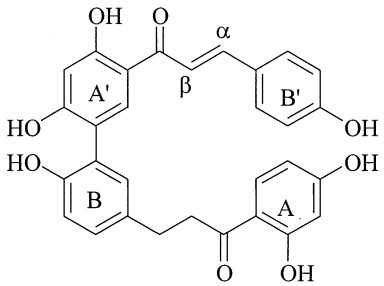

5

6 = $\triangle^{\alpha\beta}$

Results and Discussion

The crude organic extract of the root bark of the plant was freed of most of the tannin material, and the dichloromethane-soluble residue was subjected to flash column chromatography on silica gel followed by purification on Sephadex LH-20 to give rhuschalcone II (**2**) as a yellow solid with mp 160−163 °C. HREIMS indicated an ion at *m*/*z* 510.1308 (M^+, calcd 510.1314), consistent with a molecular formula of $C_{30}H_{22}O_8$. The IR spectrum showed absorption bands at 3419 (OH), 1634 (hydrogen bonded and conjugated carbonyl), and 1505 cm^{-1} (Ar ring). The UV spectrum displayed bands at 373 and 330 nm, which were assignable to a chalcone system. The band at 373 nm was shifted by +46 nm upon addition of NaOMe and by +31 nm when NaOAc was added, indicative of a free hydroxyl group at the C-4′ position. The ^{13}C NMR spectrum of **2** displayed signals for 30 carbons, which were edited by DEPT into 17 methines and 13 nonprotonated carbons consisting of two carbonyls and seven oxygenated carbons.

The ^1H NMR spectrum (600 MHz, acetone-d_6) showed resonances due to two chelated hydroxyls at δ 13.6 and 13.5 and two pairs of AA′BB′-type signals, one pair at δ 7.85

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Table 1. NMR Data for Compound **2**

position	δ_H (mult., J_{Hz}) acetone-d_6	δ_H (mult., J_{Hz}) CD_3CN-d_3	δ_C acetone-d_6	COSY	HMBC H → C
1			129.3		
2	7.85 (d, 8.8)	7.76 (d, 8.8)	130.9	H-3	*β*, 1, 3/5, 4
3	7.00 (d, 8.8)	7.00 (d, 8.8)	116.2	H-2	1, 2/6, 4,
4			161.5		
5	7.00 (d, 8.8)	7.00 (d, 8.8)	116.2	H-6	1, 2/6, 4,
6	7.85 (d, 8.8)	7.76 (d, 8.8)	130.9	H-5	*β*, 1, 3/5, 4
1′			113.9		
2′			165.2		
3′	6.39 (d, 2.3)	6.37 (d, 2.4)	103.2	H-5′	1′, 2′, 4′, 5′
4′			166.7		
5′	6.46 (dd, 8.9, 2.3)	6.46 (dd, 8.9, 2.4)	108.3	H-3′, H-6′	1′, 3′
6′	8.13 (d, 8.9)	8.02 (d, 8.9)	132.9	H-5′	2′, 4′, C=O
C=O			192.2		
α	7.80−7.90 m	7.51 (d, 15.4)	119.2	H-*β*	1, *β*, C=O
β	7.80−7.90 m	7.85 (d, 15.4)	143.9	H-α	1, α, 2, 6, C=O
1″			129.3		
2″	7.74 (d, 8.6)	7.65 (d, 8.6)	131.5	H-3″	*β*′, 1″, 3″/5″,4″
3″	6.90 (d, 8.6)	6.87 (d, 8.7)	116.2	H-2″	1″, 2″/6″,4″
4″			160.6		
5″	6.90 (d, 8.6)	6.87 (d, 8.7)	116.2	H-6″	1″, 2″/6″,4″
6″	7.74 (d, 8.6)	7.65 (d, 8.6)	131.5	H-5″	*β*′, 1″, 3″/5″,4″
1‴			113.5		
2‴			163.9		
3‴	6.61 (s)	6.58 (s)	104.9	H-6‴	1‴, 2‴ 4‴, 5‴
4‴			157.7		
5‴			134.9		
6‴	8.10 (s)	7.93 (s)	124.6	H-*β*′	2‴, 4‴, 5‴, C=O
C=O			192.3		
α′	7.80−7.90 m	7.67 (d, 15.3)	117.7	H-α′	1″, *β*′, C=O′
β′	7.80−7.90 m	7.86 (d, 15.3)	145.4		2″, 6″, α′, C=O′

and 7.00 and another pair at *δ* 7.74 and 6.90 of two *para*-substituted phenolic derivatives. An ABX proton spin system was observed at *δ* 6.39, 6.46, and 8.13, characteristic of a 1,3,4-trisubstituted benzene unit. In addition, two singlet signals integrating for one proton each were observed at *δ* 8.10 and 6.61. The above data strongly suggested **2** to be a bichalcone. Signals of *trans*-alkene protons of the bichalcone system were not clearly observed due to signal overlap in acetone-d_6, but became quite clear when the spectrum was recorded in acetonitrile-d_3 (Table 1). One set was observed at *δ* 7.51 and 7.85 (each 1H, J = 15.4 Hz) and the second set at *δ* 7.67 and 7.86 (each 1H, J = 15.3 Hz). Detailed analysis of the 1D and 2D NMR data revealed that the first set of AA′BB′ doublets belonged to the protons at the 2,6 and 3,5 positions of one chalcone moiety and the other set to the 2″,6″ and 3″,5″ protons of a second chalcone moiety, respectively (Table 1). The two singlets at *δ* 6.61 and 8.10 were assigned to the 3‴ and 6‴ protons, respectively, of the 2‴,4‴,5‴-trisubstituted chalcone ring A′. Detailed analysis of the HMBC and HMQC data enabled the full assignment of all protons and carbons of this compound (Table 1). The structure of rhuschalcone II, a new compound, was therefore deduced to be 2′,4′,4″,2‴,4‴-pentahydroxy-4-*O*-5‴-bichalcone (**2**).

Rhuschalcone III (**3**) was isolated as a yellow solid, mp 159−161 °C. HREIMS showed a molecular ion at *m*/*z* 524.1472 (M^+, calcd 524.1471), indicating the molecular formula $C_{31}H_{24}O_8$. The spectroscopic properties of this compound were very similar to those of rhuschalcone II (**2**), and it was assumed to be a methylated derivative (Experimental Section). The upfield portion of the ^1H NMR spectrum showed one methoxy resonance at *δ* 3.87. The location of this methoxy group was deduced to be at C-4‴ from the following observations (Figure 1).

Irradiation of the methoxy signal resulted in the NOE enhancement of the singlet signal (H-3‴, 1.7%) at *δ* 6.70. The HMBC spectrum showed a ^3J correlation of the

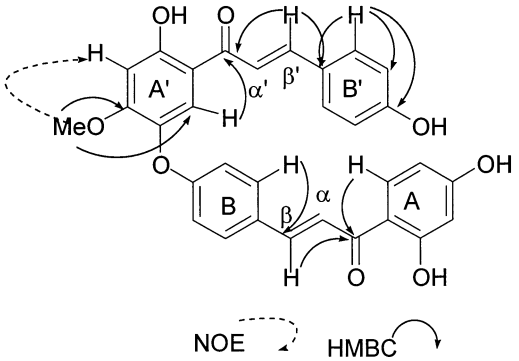

Figure 1. Important HMBC (single arrows) and NOE (dashed arrow) correlations for compound **3**.

methoxy proton signals to the oxygenated Ar−C signal at *δ* 159.4, which was in turn correlated through a ^3J interaction to the two singlet signals of H-3‴ and H-6‴, respectively, at *δ* 6.70 and 8.08. The chemical shifts of the carbonyl groups were assigned on the basis of the HMBC data, which showed ^3J interactions of the H-6‴ singlet at *δ* 8.08 and the H-*β*′ signal at *δ* 7.89 with the upfield C=O′ signal at *δ* 192.4. Likewise the signal of the second H-*β* was correlated to the other C=O resonance at *δ* 191.7. Full analysis of the HMBC and HMQC data enabled the complete assignment of the proton and carbon signals of **3** (Experimental Section), and the structure of rhuschalcone III, a new compound, was identified as 2′,4′,4″,2‴-tetrahydroxy-4‴-methoxy-4-*O*-5‴-bichalcone (**3**).

Rhuschalcone IV (**4**) was obtained as a yellow solid, mp 140−142 °C. HREIMS (M^+, *m*/*z* 524.1461, calcd 524.1471) indicated the molecular formula to be $C_{31}H_{24}O_8$. The ^{13}C and DEPT spectra of this compound suggested the presence of a methoxy group (δ_C 53.6), 13 nonprotonated carbons (including two carbonyls at δ_C 189.8 and 192.5 and seven oxygenated carbons), and 17 methine carbons. Examination

of the ^{1}H NMR spectrum revealed that this compound is also composed of four aromatic rings (of which two contain one AA′BB′ system each, one ABX-substituted and one tetrasubstituted) and four *trans*-alkene protons. This compound is isomeric with compound **3** and is also a bichalcone containing the same number and type of functional groups. The notable difference in the ^{1}H NMR spectrum of this compound from that of **3** was the downfield shift of signals assigned to H-3‴ and H-6‴ by 0.35 and 0.25 ppm, respectively. It was noted that this compound was slightly more polar than **3** (R_f 0.63 and 0.77, respectively, $CHCl_3$−EtOAc, 1:1). Although there were only slight differences in the major UV absorption bands between **3** and **4**, a much bigger shift (+64 nm compared to +29) was observed for the former upon addition of NaOMe. Initially the possibility was considered that **4** differed from **3** only in the location of the methoxy group, but this was ruled out when NOE irradiation of this group resulted in the enhancement of the H-3‴ as was observed for **3**. However, the same NOE irradiation yielded a piece of crucial information in establishing the nature of the inter-chalcone linkage, since signals belonging to H-3′ and H-5′ of ring A of one of the chalcone moieties were also enhanced. It became clear at this stage that this bichalcone differs from **3** in the manner in which the two moieties are linked. Detailed analysis, employing HMBC, HMQC, and selective NOESY experiments, allowed the full assignment of the proton and carbon signals as shown (Experimental Section). Thus, the new compound, **4**, was identified as 4,2′,4″,2‴-tetrahydroxy-4‴-methoxy-4′-*O*-5‴-bichalcone.

Rhuschalcone V (**5**) was isolated as a yellow solid, mp 125−127 °C, $[\alpha]_D^{25}$ −6.5° (*c* 0.03, MeOH). The molecular formula $C_{30}H_{24}O_8$ was determined from HREIMS ([M$^+$] *m*/*z* 512.1468, calcd 512.1471). The UV spectrum had absorptions at 381 and 279 nm. The IR spectrum showed the presence of hydroxyl (3714 cm^{-1}), carbonyl (1635 cm^{-1}), and aromatic (1541 and 1511 cm^{-1}) groups. The ^{1}H NMR spectrum (Experimental Section) indicated the presence of signals corresponding to two chelated hydroxyl groups at δ 13.61 and 12.83 and one *para*-substituted aromatic ring forming an AA′BB′-type system at δ 7.76 (2H, J = 8.6 Hz) and 6.92 (2H, J = 8.6 Hz), typical for ring B of a flavonoid. Signals due to two trisubstituted aromatic rings were observed at δ 6.34 (1H, J = 2.3 Hz), 6.44 (1H, J = 7.8, 2.3 Hz), and 7.87 (1H, J = 7.8 Hz) and at δ 6.91 (1H, J = 8.6 Hz), 7.19 (1H, J = 8.3, 2.2 Hz), and 7.26 (1H, J = 2.1 Hz). Two singlet signals of a tetrasubstituted aromatic ring were also observed at δ 6.51 and 8.10. One set of the protons of a *trans*-alkene at δ 7.88 (1H, J = 14.8 Hz) and 7.85 (1H, J = 14.8 Hz) and two sets of triplets at δ 3.14 (2H, J = 7.5 Hz) and 3.01 (2H, J = 7.6 Hz) showed the presence of a chalcone moiety and a dihydrochalcone moiety, respectively. The ^{13}C NMR and DEPT experiments also gave results that were consistent with the above by indicating the presence of 14 methines, two methylenes, and 14 nonprotonated carbons (including two carbonyls at δ 205.6 and 194.1 and six oxygenated carbons). The above data indicated the structure of **5** as a bichalcone formed from a chalcone unit and a dihydrochalcone unit. The chalcone−dihydrochalcone interlinkage sites were established from HMBC spectral data (Figure 2). The presence of cross-peaks between C-3 (δ 125.1) and H-6‴ (δ 8.10) and between C-5‴ (δ 119.3) and H-2 (δ 7.26) indicated that rings A′ and B were connected via a C-3−C-5‴ bond. Full assignments of the proton and carbon NMR signals were made by use of HMQC and HMBC spectra. Therefore, the structure of the new compound rhuschalcone V was determined as 4,2′,4′,4″,2‴,4‴-hexahydroxy-3.5‴-dihydrochalcone/chalcone (**5**).

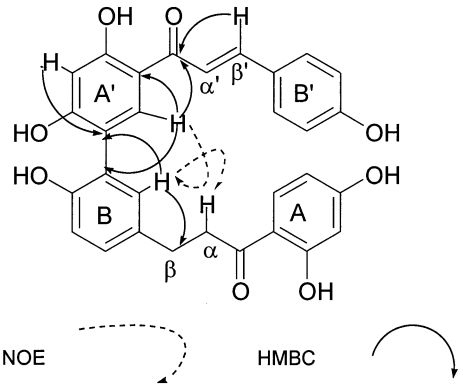

Figure 2. Important HMBC (single arrows) and NOE (dashed arrows) correlations for compound **5**.

Rhuschalcone VI (**6**) was isolated as yellow solid, mp 119−121 °C, $[\alpha]_D^{25}$ −6.3° (*c* 0.03, MeOH). The molecular formula $C_{30}H_{22}O_8$ was deduced from EIMS and ^{13}C NMR data. HREIMS: [M$^+$] *m*/*z* 510.1315, calcd 510.1321. The spectral data obtained for this compound suggested that it was a dehydro derivative of **5**. The UV spectrum had absorptions at 372 and 306 nm, indicating additional unsaturation compared to **5**. The most striking difference in the ^{1}H NMR spectrum of **6** from that of **5** was the absence of the two triplets that were assigned to the α and β methylene protons in **5**. Instead there was a second set of *trans*-alkene protons which overlapped with other signals in the δ 7.8−7.9 region. Although the spectra of these two compounds were quite similar in the δ 6.3−7.0 region, the complex overlapping signals of protons in the lower field region (δ 7.8−7.9) were clarified only through extensive selective 1D TOCSY experiments. The points of interlinkage of the two chalcone moieties was deduced from the HMBC spectra, which showed cross-peaks between C-3 (δ 126.2) and H-6‴ (δ 8.18) and between C-5‴ (δ 116.2) and H-2 (δ 7.84), indicating that rings A′ and B were connected via a C-3−C-5 bond as in **5**. Rhuschalcone VI was thus deduced to be a new compound having the structure 4,2′,4′,4″,2‴,4‴-hexahydroxy-3,5‴-bichalcone (**6**).

Compound **1** was isolated as yellow needles from methanol (mp 230−232 °C). This compound was found to be identical (mp, mmp, IR, UV, ^{1}H and ^{13}C NMR) to rhuschalcone I, previously isolated by Masesane et al. from the twigs and leaves of the same plant.[7]

As part of the proof of the identity of the foregoing chalcones, their total syntheses were undertaken. This resulted in the successful total synthesis of three (**1**−**3**) of them. The key step in the synthesis was the Ulmann coupling of the trimethylated phenolic chalcone **8** and the bromochalcone **9** (Scheme 1).[9] Microwave irradiation of a mixture of the two solids in the presence of potassium carbonate and copper(I) chloride gave a 19% yield of the bichalcone **10**. Treatment of **10** with boron tribromide led, after chromatographic separation, to four bichalcones (**1**, **2**, and **3**, and a fourth compound which was deduced to be **7**, principally from MS and ^{1}H NMR data) (Experimental Section). The synthetic bichalcones (**1**−**3**) were identical in all respects to the natural products.

Bichalcones **2**−**6** are new compounds and are reported here for the first time. Compound **1** was reported before from the twigs of the same plant.[4] Simple bichalcones are very rare in nature, and the few representatives known include 3′,3‴-bis(2′,4′,6′-trihydroxy-4-methoxydihydro-

Scheme 1

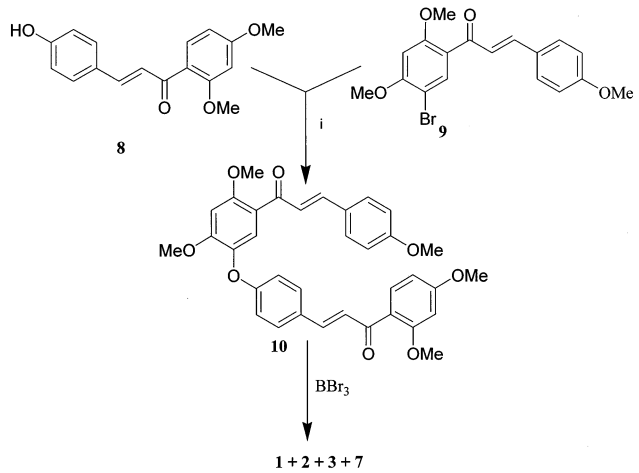

Table 2. Cytotoxicity of Compounds **1**− **6** (ED_{50} Values Given in μg/mL)

cell type	**1**	**2**	**3**	**4**	**5**	**6**
Colo 205	11	15	4	23	*a*	24
HCT 116	5	15	5	*a*	25	5
HCT 15	51	*a*	4	*a*	*a*	*a*
HT 29	3	14	3	*a*	25	10
KM 12	17	19	4	*a*	*a*	*a*
SW 620	42	*a*	3	*a*	*a*	*a*

[a] Not reached at the concentration tested.

chalcone), isolated from *Iryanthera sagotiana*,[10] and a tetrahydrobichalcone, brackenin, isolated from *Brackenridgea zanguebarica*.[11] Compounds **5** and **6** appear to be the first examples of this class of compounds having a biaryl linkage between two chalcone units and a dihydrochalcone and chalcone unit, respectively. Both **5** and **6** are optically active, and this is attributed to the restricted rotation along the biaryl axis leading to configurationally stable unsymmetrical molecules.

Samples of all the rhuschalcones (**1**−**6**) were submitted to the U.S. National Cancer Institute for in vitro primary cytotoxic screening using a panel of 60 different human tumor cell lines. All the bichalcones manifested varying degrees of cytotoxic activity on some cell lines, but the bichalcones as a group showed more activity on colon cancer cell lines, especially the HT29 and HCT-116 cell lines (Table 2). Rhuschalcone IV (**4**) is noted as the most potent substance, and it also showed similar level of activity on melanoma cell lines (LC_{50} = 4.8, 4.6, and 4.6 on SK-MEL-5, SK-MEL-28, and UACC-62 cell lines, respectively). The two least active bichalcones were **5** and **6**.

Experimental Section

General Experimental Procedures. Melting points were determined on a Stuart Scientific (SMP1) apparatus and are uncorrected. Optical rotations were taken on a Polartronic D polarimeter (Schmidt & Haensch) (25 °C, 10 cm cell). IR spectra were recorded as KBr pellets on a Perkin-Elmer 2000 FT-IR spectrophotometer. UV spectra were measured on a Shimadzu UV-2101 PC UV−vis scanning spectrophotometer. ^{1}H NMR (600.13 MHz) and ^{13}C NMR (150.9 MHz) spectra were measured on a Bruker DRX 600 instrument using $CDCl_3$ (δ 7.26 and 77.01), acetone-d_6 (δ 2.01), and CD_3CN (δ 7.26 and 77.01) solvents and internal ^{1}H and ^{13}C standards. Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 7 Hz). LRAPCIMS were determined on a Finnigan LC Q DECA. For TLC, precoated Si gel 60 F_{254} plates were used and spots were detected under UV light and further visualized by spraying with vanillin−sulfuric acid. Preparative TLC was run on a 0.5 mm thick Si gel layer coated on 20 × 20 cm glass plates. Column chromatography was carried out on Si gel 60 (60−200 mesh, Merck). Microwave irradiation was performed in a Sharp Carousel convection microwave oven (R-9H10) at a setting of 180 °C.

Plant Material. Root bark from trees of *R. pyroides* was collected from the banks of the Notwane River in the city of Gaborone in December 1999. The plant material was identified by Dr. L. M. Turton (Botswana National Museum, Gaborone), and a voucher specimen (No. M99) has been deposited at the University of Botswana Herbarium.

Extraction and Isolation. The air-dried material (1.8 kg root bark) was ground and exhaustively extracted with 5.4 L of CH_2Cl_2−MeOH (1:1) for 24 h followed by methanol for 1 h. The extracts were combined and freed of solvent to give 130 g of reddish residue. The extract was dissolved in a mixture of MeOH−H_2O (1:1, 600 mL) and extracted with CH_2Cl_2 (4 × 300 mL) to give, after concentration in vacuo, 10 g of a CH_2Cl_2-soluble extract. This residue was subjected to flash chromatography on 120 g of Si gel and eluted with $CHCl_3$ (300 mL) followed with $CHCl_3$ containing increasing amounts of MeOH. Forty-eight fractions (25 mL each) were collected and pooled according to their similarity on analytical TLC plates and dried. Combined fractions 1−19 (0.2 g), which were eluted with $CHCl_3$−MeOH (99:1), were applied on a Sephadex LH-20 column (2.5 × 17 cm), eluted with $CHCl_3$−MeOH (2:1) to yield rhuschalcone I (**1**, 109 mg). Combined fractions 20−25 (0.45 g), which were eluted from a flash column with $CHCl_3$−MeOH (98:2), were subjected to Si gel column chromatography (10 g) and eluted with $CHCl_3$−MeOH (98:2), followed by preparative TLC using the same solvent system, to give **4** (8 mg, R_f = 0.63 in EtOAc−$CHCl_3$, 1:1). Fractions 26−28 (0.7 g), eluted from a flash column with $CHCl_3$−MeOH (97:3), were purified over a short column of Si gel (14 g) (same solvent) to give **3** (57 mg, R_f = 0.77 in EtOAc−$CHCl_3$, 1:1). Fractions 29−31 (0.23 g), obtained from the flash column using $CHCl_3$−MeOH (96:4), were filtered through Sephadex LH-20 ($CHCl_3$−MeOH, 2:1) to yield 54 mg of **2**. Fractions 43−47 (0.34 g), eluted from the flash column using $CHCl_3$−MeOH (9:1), were subjected to successive chromatographic separations on Sephadex LH-20, Si gel, and finally preparative TLC ($CHCl_3$−MeOH, 95:5) to yield **5** (30 mg, R_f = 0.27) and **6** (15 mg, R_f = 0.22). Repeated column chromatography over Sephadex LH-20 using $CHCl_3$−MeOH (1:1) of fraction 48 (0.2 g) (eluted from the flash column using $CHCl_3$−MeOH, 9:1), followed by preparative TLC ($CHCl_3$−MeOH, 4:1), afforded a mixture of catechin and epicatechin (150 mg).

2′,4′,4″,2‴,4‴-Pentahydroxy-4-*O*-5‴-bichalcone (rhuschalcone II, 2): orange solid; mp 160−163 °C; UV (MeOH) λ_{max} (log ϵ) 373 (3.51), 330 (2.38) nm; (MeOH + NaOMe) λ_{max} (log ϵ) 418 (3.57), 278 (2.52), 205 (3.81) nm; (MeOH + $AlCl_3$) λ_{max} (log ϵ) 372 (3.53), 226 (2.69) nm; (MeOH + $AlCl_3$ + HCl) λ_{max} (log ϵ) 368 (3.54), 231 (2.39) nm; (MeOH + NaOAc) λ_{max} (log ϵ) 418 (3.57), 278 (2.52), 205 (3.81) nm; IR (KBr) ν_{max} 3419, 2361, 1634, 1554, 1505, 1366, 1293, 1223 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR, see Table 1; EIMS *m*/*z* 510 [M^+] (100), 391 (63), 363 (55), 348 (40), 255 (94), 228 (54), 202 (32), 163 (48), 151 (47); HREIMS *m*/*z* 510.1308 (calcd for $C_{30}H_{22}O_8$, 510.1314).

2′,4′,4″,2‴-Tetrahydroxy-4‴-methoxy-4-*O*-5‴-bichalcone (rhuschalcone III, 3): yellow sold; mp 159−161 °C; UV (MeOH) λ_{max} (log ϵ) 398 (3.63), 320 (3.18), 209 (4.10) nm; (MeOH + $AlCl_3$) λ_{max} (log ϵ) 428 (3.61), 386 (3.49), 331 (2.99) nm; (MeOH + $AlCl_3$ + HCl) λ_{max} (log ϵ) 427 (3.60), 377 (3.49), 272 (2.77), 236 (3.09) nm; (MeOH + NaOAc) λ_{max} (log ϵ) 392 (3.67), 277 (3.20), 222 (4.22) nm; IR (KBr) ν_{max} 3450, 1637, 1564, 1510, 1366, 1222 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.08 (1H, s, H-6‴), 8.05 (1H, d, J = 8.9 Hz, H-6′), 7.89 (1H, d, J = 15.2 Hz, H-β′), 7.84 (1H, d, J = 14.6 Hz, H-β), 7.81 (2H, d, J = 8.9 Hz, H-2, H-6), 7.80 (1H, d, J = 14.6, H-α) 7.79 (1H, d, J = 15.2, H-α′), 7.73 (2H, d, J = 8.5 Hz, H-2″, H-6″), 6.94 (2H, d, J = 8.7 Hz, H-3, H-5), 6.89 (2H, d, J = 8.4 Hz, H-3″, H-5″), 6.70 (1H, s, H-3‴), 6.46 (1H, dd, J = 8.6, 1.6 Hz, H-5′),

6.36 (1H, d, J = 1.6 Hz, H-3′), 3.87 (3H, s, OMe-4‴); ^{13}C NMR (acetone-d_6, 150 MHz) δ 192.4 (s, C=O′), 191.7 (s, C=O), 167.3 (s, C-2′), 167.2 (s, C-4′), 164.6 (s, C-2‴), 161.5 (s, C-4), 161.4 (s, C-4″), 159.4 (s, C-4‴), 145.8 (d, C-β′), 143.4 (d, C-β), 135.5 (s, C-5‴), 132.7 (d, C-6′), 131.7 (d, C-2″, C-6″), 130.9 (d, C-2, C-6), 129.4 (s, C-1), 126.6 (s, C-1″), 124.1 (d, C-6‴), 119.5 (d, C-α), 117.3 (d, α′), 116.4 (d, C-3″, C-5″), 116.0 (d, C-3, C-5), 113.4 (s, C-1′), 113.3 (s, C-1‴), 109.2 (d, C-5′), 103.5 (d, C-3′), 101.8 (d, C-3‴), 56.2 (q, OMe-4‴); EIMS m/z 524 [M$^+$] (100), 405 (21), 378 (41), 268 (19), 242 (27), 229 (19), 137 (33), 94 (24); HREIMS m/z 524.1472 (calcd for $C_{31}H_{24}O_8$, 524.1471).

4,2′,4″,2‴-Tetrahydroxy-4‴-methoxy-4′-*O*-5‴-bichalcone (rhuschalcone IV, 4): yellow solid; mp 140−142 °C; UV (MeOH) λ_{max} (log ϵ) 371 (3.25) nm; (MeOH + NaOMe) λ_{max} (log ϵ) 435 (3.26), 349 (2.88), 209 (3.98) nm; (MeOH + $AlCl_3$) λ_{max} (log ϵ) 429 (3.29) nm; (MeOH + $AlCl_3$ + HCl) λ_{max} (log ϵ) 427 (3.29), 378 (3.15) nm; (MeOH + NaOAc) λ_{max} (log ϵ) 378 (3.32), 285 (2.88), 223 (4.18) nm; IR (KBr) ν_{max} 3406, 1635, 1503, 1361, 1222 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.17 (1H, d, J = 9.0, H-6′), 7.88 (1H, d, J = 15.3 Hz, H-β′), 7.83 (1H, s, H-6‴), 7.82 (1H, d, J = 15.3 Hz, H-α), 7.78 (2H, d, J = 8.7 Hz, H-2, H-6), 7.67 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.76 (1H, d, J = 15.5, H-β′), 7.70 (1H, d, J = 15.5, H-α′), 6.96 (2H, d, J = 8.6 Hz, H-3, H-5), 6.86 (2H, d, J = 8.7 Hz, H-3″, H-5″), 6.54 (1H, dd, J = 8.9, 2.5 Hz, H-5′), 6.45 (1H, d, J = 2.5 Hz, H-3′), 6.35 (1H, s, H-3‴), 3.89 (3H, s, OMe-4‴); ^{13}C NMR (acetone-d_6, 150 MHz) δ 192.5 (s, C=O), 189.8 (s, C=O′), 166.6 (s, C-2′), 166.6 (s, C-4′), 166.6 (s, C-2‴), 166.6 (s, C-4‴), 162.4 (s, C-4), 160.4 (s, C-4″), 144.7 (d, C-β), 143.0 (d, C-β′), 137.2 (s, C-5‴), 132.2 (d, C-6′), 131.0 (d, C-2″, C-6″), 130.9 (d, C-2, C-6), 128.4 (s, C-1), 127.1 (s, C-1″), 123.2 (d, C-6‴), 118.5 (d, C-α′), 118.3 (d, C-α), 116.3 (d, C-3″, C-5″), 116.2 (d, C-3, C-5), 114.5 (s, C-1′), 109.9 (s, C-1‴), 107.5 (d, C-5′), 105.4 (d, C-3‴), 101.2 (d, C-3′), 53.6 (q, OMe-4‴); EIMS m/z 524 [M$^+$] (100), 419 (22), 405 (52), 379 (63); HREIMS m/z 524.1461 (calcd for $C_{31}H_{24}O_8$, 524.1471).

4,2′,4′,4″,2‴,4‴-Hexahydroxy-3,5‴-dihydrochalcone-chalcone (rhuschalcone V, 5): yellow solid ($CHCl_3$); mp 125−127 °C; $[\alpha]^{25}_D$ −6.5° (c 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 438 (3.47), 331 (3.45) nm; (MeOH + $AlCl_3$) λ_{max} (log ϵ) 435 (3.28), 388 (3.24), 305 (3.16), 218 (3.39) nm; (MeOH + $AlCl_3$ + HCl) λ_{max} (log ϵ) 431 (3.25), 376 (3.27), 300 (3.18), 319 (3.36) nm; (MeOH + NaOAc) λ_{max} (log ϵ) 414 (3.55), 331 (3.68), 222 (4.24); IR (KBr) ν_{max} 3714, 2961, 1635, 1541, 1511, 1366, 1208 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.10 (1H, s, H-6‴), 7.88 (1H, d, J = 14.8 Hz, H-β′), 7.87 (1H, d, J = 7.8, H-6′), 7.85 (1H, d, J = 14.8 Hz, H-α′), 7.76 (2H, d, J = 8.6 Hz, H-2″, H-6″), 7.26 (1H, d, J = 2.1 Hz, H-2), 7.19 (1H, dd, J = 8.3, 2.2 Hz, H-6), 6.92 (2H, d, J = 8.6 Hz, H-3″, H-5″), 6.91 (1H, d, J = 8.6, H-5), 6.51 (1H, s, H-3‴), 6.44 (1H, dd, J = 7.8, 2.3 Hz, H-5′), 6.34 (1H, d, J = 2.3 Hz, H-3′), 3.14 (2H, t, J = 7.5, H-α), 3.01 (2H, t, J = 7.6 Hz, H-β); ^{13}C NMR (acetone-d_6, 150 MHz) δ 205.6 (s, C=O), 194.1 (s, C=O′), 166.6 (s, C-4′), 165.7 (s, C-2‴), 165.0 (s, C-2′), 162.5 (s, C-4‴), 160.5 (s, C-4″), 153.2 (s, C-4), 144.8 (d, C-β′), 134.3 (d, C-6‴), 133.2 (d, C-6′), 132.9 (s, C-1), 132.3 (d, C-2), 131.4 (d, C-2″, C-6″), 129.1 (d, C-6), 127.0 (s, C-1″), 125.1 (s, C-3), 119.3 (s, C-5‴), 118.7 (d, C-α′), 116.4 (d, C-5), 116.2 (d, C-3″, C-5″), 114.7 (s, C-1‴), 113.8 (s, C-1′), 108.3 (d, C-5′), 103.8 (d, C-3‴), 103.0 (d, C-3′), 39.9 (t, C-α), 29.9 (t, C-β); EIMS m/z 512 [M$^+$] (52), 375 (50), 288 (43), 275 (26), 255 (30), 233 (34), 168 (34), 153 (100), 137 (90), 107 (28); HREIMS m/z 512.1468 (calcd for $C_{30}H_{24}O_8$, 512.1471).

4,2′,4′,4″,2‴,4‴-Hexahydroxy-3,5‴-bichalcone (rhuschalcone VI, 6): yellow solid ($CHCl_3$); mp 119−121 °C; $[\alpha]^{25}_D$ −6.3° (c 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 372 (3.32), 306 (3.06), 226 (3.07) nm; (MeOH + $AlCl_3$) λ_{max} (log ϵ) 428 (3.36), 333 (2.89) nm; (MeOH + $AlCl_3$ + HCl) λ_{max} (log ϵ) 424 (3.35) nm; (MeOH + NaOAc) λ_{max} (log ϵ) 399 (3.40), 306 (3.15), 222 (3.77); IR (KBr) ν_{max} 3417, 2928, 1634, 1619, 1558, 1511, 1365, 1211 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) δ 8.18 (1H, s, H-6‴), 8.16 (1H, d, J = 8.9 Hz, H-6′), 7.92 (1H, d, J = 15.4, H-β), 7.91 (1H, d, J = 15.4 Hz, H-β′), 7.88 (1H, d, J = 15.6 Hz, H-α), 7.87 (1H, d, J = 15.3 Hz, H-α′), 7.84 (1H, d, J = 2.6 Hz, H-2), 7.75 (1H, dd, J = 8.6, 2.6 Hz, H-6), 7.75 (2H, d, J = 8.6, H-2″, H-6″), 7.06 (1H, d, J = 8.4 Hz, H-5), 6.89 (2H, d, J = 8.6 Hz, H-3″, H-5″), 6.53 (1H, s, H-3‴), 6.45 (1H, dd, J = 8.9, 2.3 Hz, H-5′), 6.37 (1H, d, J = 2.3 Hz, H-3′); ^{13}C NMR (acetone-d_6, 150 MHz) δ 192.6 (s, C=O′), 192.4 (s, C=O), 167.1 (s, C-2′), 166.4 (s, C-2‴), 165.1 (s, C-4′), 163.1 (s, C-4‴), 160.6 (s, C-4″), 158.4 (s, C-4), 144.9 (d, C-β′), 144.7 (d, C-β), 134.3 (d, C-6‴), 132.9 (d, C-6′), 132.3 (d, C-2), 131.4 (d, C-2″, C-6″), 130.7 (d, C-6), 127.2 (s, C-1), 127.0 (s, C-1″), 126.2 (s, C-3), 118.1 (d, C-α), 117.9 (d, C-α′), 116.9 (d, C-5), 116.2 (s, C-5‴), 116.2 (d, C-3″, C-5″), 114.2 (s, C-1‴), 114.0 (s, C-1′), 108.2 (d, C-5′), 103.7 (d, C-3‴), 103.2 (d, C-3′); EIMS m/z 510 [M$^+$] (52), 472 (12), 430 (12), 415 (32), 396 (100), 381 (17), 329 (18), 314 (16), 275 (22), 265 (36), 255 (43), 221 (28), 213 (29), 181 (25), 173 (30), 165 (53); HREIMS ([M$^+$] m/z 510.1315, calcd 510.1321).

Synthesis of 2′,4′,4″,2‴,4‴-Pentamethoxy-4-*O*-5‴-bichalcone (10). 5′-Bromo-4,2′,4′-trimethoxychalcone (**9**, 412 mg, 1.09 mmol), 4-hydroxy-2′,4′-dimethoxychalcone (**8**, 310 mg, 1.09 mmol), anhydrous potassium carbonate (450 mg, 3.26 mmol), and copper(I) chloride (215 mg, 2.15 mmol) were thoroughly mixed in an agate mortar. The fine yellow powder was heated in a microwave oven at a setting of 180 °C for 2 h. The cooled mixture was sonicated in 50 mL of CH_2Cl_2 and filtered and the organic phase washed with 5% aqueous NaOH (20 mL), dried ($MgSO_4$), and concentrated in vacuo to give 180 mg of crude product. Si gel chromatographic purification using petroleum ether−EtOAc (7:3) yielded 120 mg of **10** (19% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.76 (1H, d, J = 8.6 Hz), 7.68 (1H, d, J = 15.8 Hz), 7.65 (1H, d, J = 15.8 Hz), 7.57 (2H, d J = 8.5 Hz), 7.56 (1H, s), 7.54 (2H, d, J = 8.6 Hz), 7.42 (1H, d, J = 15.8 Hz), 6.94 (2H, d, J = 8.8 Hz), 6.92 (2H, d, J = 8.8 Hz), 6.57 (1H, dd, J = 8.5, 2.3 Hz), 6.51 (1H, d, J = 2.3 Hz), 3.99, 3.91, 3.91, 3.88, 3.86 (each 3H, s); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 191.0 (C=O′), 190.0 (C=O), 164.4 (s, C-4′), 161.8 (s, C-4″) 160.7 (s, C-4), 160.5 (s, C-2′), 157.7 (s, C-4‴), 156.1 (s, C-2‴) 143.1, 142.2, 137.7, 133.2, 130.5 (d, C-2, C-6), 130.3 (d, C-2″, C-6″), 129.9, 128.4, 126.1, 125.1, 124.9, 122.8, 122.2, 116.7 (d, C-3″, C-5″), 114.7 (d, C-3, C-5), 105.5 (d, C-5′), 99.1 (s, C-3′), 97.7 (s, C-3‴), 56.9 (q, OMe C-4‴), 56.6 (q, OMe C-2‴), 56.2 (q, −OMe C-2′), 55.9 (q, −OMe C-4′), 55.8 (q, −OMe C-4″); APCI-MS m/z 581 [M + H]$^+$.

Demethylation of 2′,4′,4″,2‴,4‴-Pentamethoxy-4-*O*-5‴-bichalcone (10). A sample of 2′,4′,4″,2‴,4‴-pentamethoxy-4-*O*-5‴-bichalcone (**7**, 100 mg) was dissolved in dry CH_2Cl_2 (5 mL), 1 M BBr_3 in CH_2Cl_2 (3 mL) was added, and the mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was quenched by addition of MeOH and evaporated in vacuo. The resulting orange solid was partitioned between EtOAc and 1 M NaOH. The organic layer was discarded, and the aqueous layer was extracted with EtOAc (20 mL × 4). After cooling to 0 °C, the aqueous layer was acidified to pH 3 by dropwise addition of 3 M HCl. The EtOAc extract was concentrated in vacuo, and the crude product (70 mg) was purified on preparative TLC using $CHCl_3$−MeOH (95:5) to give **6** (14.5 mg, R_f = 0.22), 2′, 4″,2‴,4‴-tetrahydroxy-4′-methoxy-4-*O*-5‴-bichalcone (5 mg), **2** (9.3 mg), and **1** (10.2 mg).

2′,4″,2‴,4‴-Tetrahydroxy-4′-methoxy-4-*O*-5‴-bichalcone (7): yellow solid; ^{1}H NMR (acetone-d_6, 300 MHz) δ 13.66 (1H, s), 13.63 (1H, s), 8.18 (1H, d, J = 9.0 Hz, H-6′), 8.08 (1H, s, H-6‴), 7.89−7.83 (6H, overlapping multiplet; H-2, H-6, α,α′,β,β′), 7.75 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.00 (2H, d, J = 8.9 Hz, H-3, H-5), 6.89 (2H, d, J = 8.7 Hz, H-3″, H-5″), 6.58 (1H, s, H-3‴), 6.54 (1H, dd, J = 8.7, 2.3 Hz, H-5′), 6.47 (1H, d, J = 2.5 Hz, H-3′), 3.90 (3H, s, −OMe-4′); APCI-MS m/z 525.1 [M + H]$^+$.

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