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A bichalcone from the twigs of *Rhus pyroides*

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Abstract

The twigs of *Rhus pyroides* yielded a novel bichalcone 2′,4″,2‴-trihydroxy-4′,4‴-dimethoxy-4-*O*-5‴-bichalcone. It was identified on the basis of spectroscopic data including 1D and 2D NMR spectroscopy. The name rhuschalcone-1 is proposed.

Keywords: *Rhus pyroides*; Anacardiaceae; Biflavonoid; Bichalcone; Rhuschalcone-1

1. Introduction

The genus *Rhus* consists of ca 200 species and is known to be rich in biflavonoids. Interest in biflavonoids has increased in recent times due to realization of a variety of biological activities manifested by them. Biflavonoids agathisflavone, robustaflavone and hinokiflavone from *Rhus succedanea* have been shown to have HIV-1 reverse transcriptase activity (Lin et al., 1997). Hinokiflavone was identified as one of 65 natural flavonoids to inhibit the interleukin-1ß-induced procoagulant activity of adherent human monocytes (Lale, Herbert, Augereau, Billon, Leconte & Gleye, 1996).

Rhus pyroides is a shrub growing to a medium sized tree found widely distributed in the Eastern part of Botswana. It has been observed that this plant is avoided by the corn cricket (*Heterodes popus* L) (Private communication between Dr. J. Wollard and local farmers) which often invades agricultural farms and devours a wide range of crops and plants. We were intrigued by this observation and decided to investigate the plant in an effort to identify possible anti-feedants from the plant. In this paper we report the isolation and characterization of a novel bichalcone which has week anti-feedant properties. The name rhuschalcone-1 is proposed for this novel compound.

2. Results and discussion

Rhuschalcone-1 was isolated as yellow needles from methanol (m.p. 232–234°C). The molecular formula $C_{32}H_{26}O_8$ was deduced from the HR-EIMS (M^+/z 538.1620). The IR spectrum showed absorption bands at 3451 (*br*, –OH), 1635 (chelated –C═O), and bands attributable to aromatic rings at 1565 and 1504 cm^{-1}. The UV spectrum displayed bands assignable to a chalcone system at 371, 240 and 201 nm. The use of UV-shift reagents (Section 3) led to the suggestion that the compound contained 2′ and/or 4′–OH (bathochromic shifts with NaOH, NaOAc) and no *o*–diOH functionality ($AlCl_3$/HCl). The biflavonoid nature of the compound was deduced from the molecular formula and the ^1H NMR spectrum which displayed two chelated –OH resonances and 17 signals of aromatic/vinylic methine hydrogens. The two pairs of doublets, one at δ7.85 (1H, $J = 15.5$ Hz) and 7.58 (1H, $J = 15.5$

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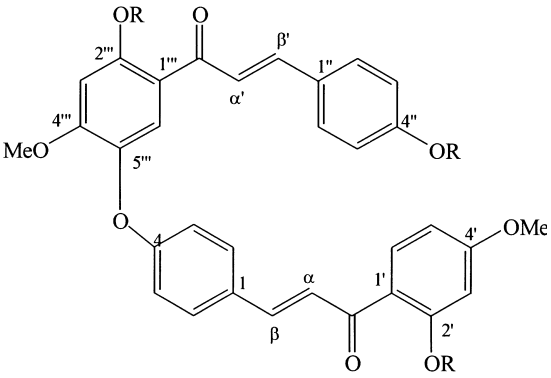
Hz), and the other at δ7.83 (1H, $J = 15.4$ Hz) and 7.71 (1H, $J = 15.4$ Hz), characteristic of *trans* protons of double bonds revealed the bichalcone nature of the compound under investigation. The spectrum also showed two sets of A_2B_2-type doublets, one set at δ7.70 and 6.90, and the other one at δ7.61 and 6.80 (see Table 1). From detailed analysis of the 1D and 2D NMR data it was possible to conclude that the first set belonged to the 2,6 and the 3,5 protons of one chalcone moiety and the other set to the 2″,6″ and the 3″,5″ protons of the other chalcone moiety, respectively. The signals at δ6.45, 6.53, and 8.04 correspond to the 3′, 5′, 6′ ABX set of protons of a 2′, 4′-disubstituted ring-A of a chalcone moiety, respectively. The remaining two singlets at δ6.69 and 7.93 corresponded to 3‴ and 6‴ protons, respectively, of a 2‴, 4‴, 5‴-trisubstituted ring-A of another chalcone moiety. The upfield portion of the ^{1}H NMR spectrum showed two methoxy resonances at δ3.82 and 3.85. The locations of the methoxy groups at C4′ and C4‴ were established from the NOESY data. The signal at δ3.82 was assigned to the methoxy group at C4‴ since it displayed a cross peak to the C-3‴ proton resonance at δ6.69. Similarly the signal at δ3.85 showed cross peaks to the signals of the C-3′ and C-5′ protons at δ6.45 and 6.53, respectively, establishing the location of the second methoxy group at C-4′. The HMBC spectrum also showed ^3J correlation of the methoxy proton signal at δ3.82 to the oxygenated Ar–C signal C-4‴ (δ158.8). The latter signal also was correlated through a ^3J interaction to the two singlets at δ6.69 and 7.93 (H6‴ and H3‴, respectively).

Table 1
^{1}H NMR data for **1** ($CDCl_3$, 600 MHz) and **2** ($CDCl_3$ 300 MHz) multiplicities J (Hz) values given in parentheses

H	1	2
2,6	7.70 *d* (8.8)	7.60[a] *d* (8.7)
3,5	6.90 *d* (8.8)	6.96[b] *d* (8.7)
3′	6.45 *d* (2.4)	6.67 *d* (2.4)
5′	6.53 *dd* (9.0, 2.5)	6.86 *dd* (8.7, 2.4)
6′	8.04 *d* (9.1)	7.76 *d* (8.4)
2″,6″	7.61 *d* (8.6)	7.57[a′] *d* (8.4)
3″,5″	6.80 *d* (8.7)	7.14[b′] *s* (8.4)
3‴	6.69 *s*	6.80 *s*
6‴	7.93 *s*	7.60 *s*
α	7.71 *d* (15.4)	7.55[c] *d* (15.9)
β	7.83 *d* (15.4)	7.61[d] *d* (15.9
α′	7.58 *d* (15.5)	7.16[c′] *d* (15.6)
β′	7.85 *d* (15.5)	7.53[d/e],
4′-OMe	3.85 *s*	3.87 *s*
4‴-OMe	3.82 *s*	3.86 *s*

[a] May be interchanged with a′.
[b] May be interchanged with b′.
[c] May be interchanged with c′.
[d] May be interchanged with d′.
[e] Signal buried under other multiplets.

Acetylation by refluxing in acetic anhydride led to a triacetate derivative. This led to the conclusion that the compound contains one more –OH group in addition to the two chelated –OH groups. Rhuschalcone-1 has 8 oxygen atoms, seven of which are now accounted for in three OH, two methoxy and two carbonyl groups. The remaining oxygen must, therefore, be involved in the interflavonoid linkage. On the basis of the above structure **1** is assigned to rhuschalcone-1. The chemical shifts of the carbonyl carbons were assigned by making use of HMBC data (see Fig. 1) which linked the singlet at δ7.93 (H-6‴) and the α′-H (7.58) to the upfield C=O signal at δ191.5. Also the signal of the second α-H was cross-linked to the other C=O resonance at δ191.8. Full analysis of the HMBC (Fig. 1) and HMQC data enabled the complete assignment of all protons and carbons. This bichalcone may be regarded as an unsymmetrical dimer of the chalcone isoliquiritigenin. The ^{13}C data recorded and assigned to the lower moiety of this bichalcone was found to be very close to those reported for 4′- geranylisoliquiriti-



1 R= H
2. R = Ac

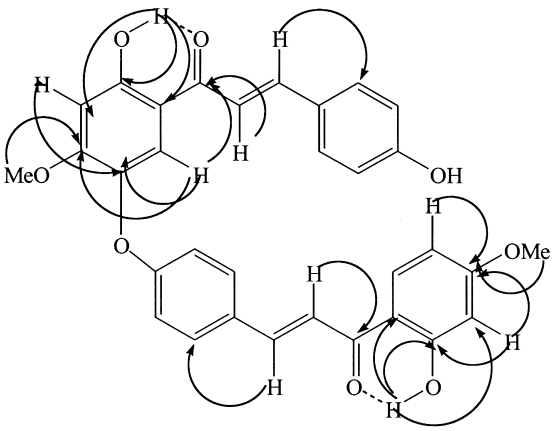


Fig. 1. Relevant ^2J and ^3J-correlations observed in the HMBC spectrum of rhuschalcone-1.

genin (Dagne, Bekele, Noguchi, Shibuya & Sankawa, 1990).

Simple bichalcones are in fact rare. The known ones include: a tetrahydrobichalcone, (Drewes & Hudson, 1983; Masaoud, Ripperger, Himmelreich & Adam, 1995) and a dihydrochalcone (Manchanda & Khan, 1985). There are also a few biflavonoids, which are based on chalcones with subsequent modifications in their structures (Drewes, Hudson, Bates & Linz, 1987; Tih, Sondengam, Martin and Bodo, 1989; Messanga, Tih, Sondengam, Martin & Bodo, 1994). To our knowledge this is the first example of a biflavonoid with two intact chalcones moieties. Biflavonoids with interflvonoid (4-*O*-5‴) linkages through oxygen are not uncommon. These include the common biflavone, hinokiflavone, tetrahydrohinokiflavone (Geiger & Harborne, 1986), and lanaroflavone (Dora & Edwards, 1991).

3. Experimental

3.1. General

M.ps. Uncorr.; UV–visible: MeOH solution, EIMS: direct inlet, 70 eV; IR: KBr disk, ^{1}H and ^{13}C NMR ($CDCl_3$) 600 and 300 MHz for proton and 125 and 75 MHz for carbon, residual solvent peaks as internal references.

3.2. Plant material

The twigs of *Rhus pyroides* were collected from a single tree on the banks of the Kolobeng river near Kumakwane village, ca 30 km west of Gaborone, Botswana. It was identified by Dr. L.Turton and a voucher specimen (BMA-301) is kept in the University of Botswana.

3.3. Extraction, isolation and characterization

The air-dried plant material (800 g) was extracted with a 1:1 mixture of CH_2Cl_2–MeOH, MeOH and water. Removal of the solvent from the combined organic extracts gave 40 g of organic extract. The crude extract (35 g) was pre-adsorbed onto 20 g of Si gel and put on to a slurry of 150 g of silica in petrol in a glass column set up for flash chromatography. For elution of the column a petrol stepped ethyl acetate gradient was introduced (9:1, 4:1, 1:1, 100% EtOAc and EtOAc/MeOH 19:1). 65 Frs each of 250 ml were collected. Frs were monitored by TLC. Frs 35 and 36 eluted with petrol/EtOAc (1:1) were combined and concentrated to give 300 mg of residue. This sample was purified by passage through a short column (25 g Si gel) eluting with $CHCl_3$/MeOH (19:1) and subsequently subjected to prep TLC developing with $CHCl_3$ to yield rhuschalcone-1 (35 mg), m.p. 232-234°C. Subsequent fractions yielded trace quantities of biflavonoids, which were not sufficient for complete characterization.

3.3.1. Rhuschalcone-1

Yellow needles from methanol, m.p. 232–234°C; HR-EIMS 538.1620 (calc. $C_{32}H_{26}O_8$: 538.1628); EIMS *M*/*z* (rel. int.): 538 [M^+] (66), 419 (38), 268 (62), 255 (46), 151 (100); IR ν_{max}^{KBr} cm^{-1}: 3451, 1635, 1565, 1504, 1222; UV λ_{max}^{MeOH} nm (log ε): 370 (3.85), 299 (3.53), 205 (3.98); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 433 (3.86), 324 (3.37), 279 (3.21), 206 (3.00);$\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 430 (3.84), 378 (3.70), 280 (3.25), 207 (3.02); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 369 (3.82), 295 (3.56); ^{1}H NMR (600 MHz, $CDCl_3$), see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$) δ:129.4 (C-1), 130.7 (C-2, C-6), 115.8 (C-3, C-5), 158.6 (C-4), 113.9 (C-1′), 166.6 (C-2′), 101.1 (C-3′), 166.1 (C-4′), 107.7 (C-5′), 130.9 (C-6′), 191.5 (C═O), 118.6 (C- α), 143.8 (C-ß), 128.9 (C-1″), 130.3 (C-2″, C-6″), 116.0 (C-3″, C-5″), 160.9 (C-4″), 113.0 (C- 1‴), 164.6 (C-2‴), 101.5 (C-3‴), 158.8 (C-4‴), 135.1 (C-5‴), 122.8 (C-6‴), 191.5 (C═O), 117.4 (c-α′), 144.9 (C-ß′), 56.2 (C-4′-OMe), 55.6 (C-4‴-OMe).

3.3.2. Acetylation of rhuschalcone-1

Fifteen miligram of the chalcone was dissolved in acetic anhydride (1 ml) and heated on an oil bath for 3 h. The excess acetic anhydride was evaporated under reduced pressure. The yellow residue (16 mg), gave a single spot on TLC ($CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$), see Table 1. HR-EIMS: 644.1945 (calc. for $C_{38}H_{32}O_{11}$ 664.1945); EIMS *m*/*z* (rel. int.) 664 [M^+] (4), 580 [M-2 × CH_2═C═O]$^+$ (100%), 268 (29), 43 (40).

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