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BRACKENIN, A DIMERIC DIHYDROCHALCONE FROM *BRACKENRIDGEA ZANGUEBARICA*

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Key Word Index—*Brackenridgea zanguebarica*; Ochnaceae; bark; dimeric dihydrochalcone; dihydroisoliquiritigenin.

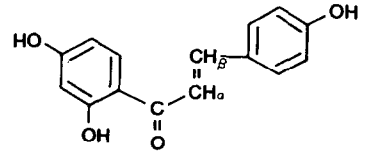
Abstract—Brackenin, a new dimeric dihydrochalcone isolated from *Brackenridgea zanguebarica*, has been shown to consist of two dihydroisoliquiritigenin units joined by a carbon-to-carbon link between the two α-carbon atoms.

INTRODUCTION

Chalcones, of which isoliquiritigenin (**1**) is an example, have long been regarded as key compounds in the biosynthesis of flavonoids [1–3]. More recently, Roux and Ferreira [4] have drawn attention to the central position occupied by a variation of the chalcone skeleton, the α-hydroxychalcones. They have postulated that simple chemical transfomations of these precursors can lead to dihydroflavonols on the one hand, or to 2-hydroxy-2-benzylcoumarones on the other. Isolation of α-hydroxychalcones from a variety of natural sources have strengthened Roux's view regarding the importance of these compounds in the biosynthesis of 3-hydroxyflavonoids.

The dihydrochalcones, another simple variation of the chalcone skeleton, are not abundant in nature and are of sporadic occurrence [5]. We now wish to report the isolation of a dimeric dihydrochalcone (**2**) for which we propose the trivial name brackenin. It appears to possess the symmetrical structure of a meso compound and is composed of two dihydroisoliquiritigenin units linked by a carbon-to-carbon bond between the two α-carbon atoms. It represents the first biflavonoid in this class. Biogenetically, it is feasible to speculate that brackenin forms by head-to-tail union of three acetate units followed by subsequent coupling to a *p*-coumaryl fragment—the common pathway for all chalcones [2]. Following enzymic hydrogenation, two dihydroisoliquiritigenin units could dimerize by a radical [6] or an ionic mechanism. While biflavonoids incorporating two chalcone or two dihydrochalcone units do not appear to exist, there are examples in which one of the two components is a chalcone. In this category fall, amongst others, GB-1a heptamethyl ether [7] (transformation product) and occidentoside [8] recently obtained from *Anacardium occidentale*. In the latter compound chalcone and flavanone units are linked by an ether bridge between aromatic rings.

Brackenridgea zanguebarica is a small tree found in the northern Transvaal and also in Zimbabwe. Amongst the indigenous Venda people it is known as 'mutavhasindi'. The tree is believed to have magical powers in warding off evil spirits and in protecting from lightning strikes [9]. The roots are used as a type of cure-all for many diseases including the treatment of wounds. Our work on the tree has so far been restricted to an investigation of the constituents of the corky bark of the tree. On the outside the bark is greyish, but below the surface it is bright



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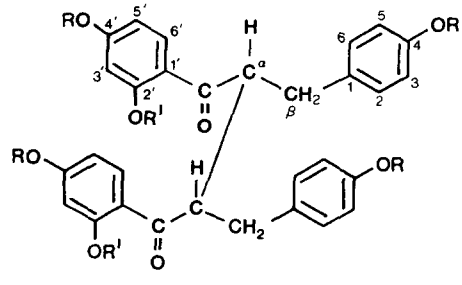
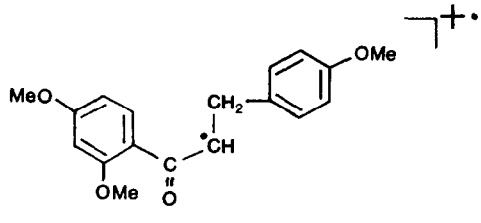


2 R = H, R' = H
3 R = Ac, R' = Ac
4 R = Me, R' = Me
5 R = Me, R' = H



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yellow. Separation of the constituents of the bark has proved to be a formidable task since a complex mixture is present. Brackenin is the first component to be obtained in a pure form. Earlier, Bombardelli *et al.* [10] obtained vitexin, vitexin 2″-*O*-acetyl-7-*O*-methyl ether, iso-orientin and sequojaflavone from the leaves of this tree. The only other biflavonoids from the Ochnaceae are recorded by Okigawa *et al.* [11, 12], who obtained a series of 3′,4′-linked biflavone ethers from *Ochna squarrosa*.

RESULTS ND DISCUSSION

Proof of structure for brackenin (**2**) is based largely on spectroscopic evidence. The colourless needles gave an elemental analysis corresponding with $(C_{15}H_{13}O_4)_n$. Mass spectral analysis indicated three intense peaks at *m/z* 257 (52%), 137 (base peak) and 107 (55%) (see below). The ^{1}H NMR spectrum showed an ABX and AA′BB′ pattern of substitution on two aromatic rings together with a hydrogen-bonded phenolic hydroxyl group (far downfield at δ 13.05) and two sets of multiplets for the aliphatic protons. Since the signals from the aliphatic protons were unclear at 80 MHz, the spectrum was subsequently recorded at 500 MHz. The two-proton 'multiplet' centred at δ 2.82 was then seen as two sets of peaks, centred at δ 2.76 and 2.89, and each consisting of a doublet of doublets. The one-proton 'multiplet' centred at δ 4.18 was not so well resolved and resembled a doublet of doublets. This appeared to indicate non-equivalence of the two protons on the methylene group leading to geminal coupling (J = 13.4 Hz). Each of these protons was in turn coupled with the methine proton (J = 9.6 and 3.3 Hz) thus giving rise to the observed splitting pattern.

Acetylation of brackenin gave a derivative having an $[M]^+$ *m/z* 748 corresponding to $C_{42}H_{36}O_{13}$. This, we believed, was derived from the hexa-acetate of $(C_{15}H_{13}O_4)_2$ following loss of water from the $[M]^+$. Other prominent peaks in the mass spectrum of the hexa-acetate occurred at *m/z* 706, 664, 622, 580 and 538 (small), consistent with the los of successive ketene units. At the lower end of the scale, significant peaks were present at *m/z* 383, 341, 300, 257, 137 (base peak) and 107. These fragments derived from the dihydroisoliquiritigenin dimer after fission of the intermolecular bond and loss of the acetyl groups. A critical step in the process was enolization of one of the carbonyl groups followed by intramolecular dehydration to give the $[M]^+$ at *m/z* 748. The pattern of substitution deduced from the ^{1}H NMR spectrum was substantiated by the characteristic peaks at *m/z* 383, 257 (accurately determined as $C_{15}H_{13}O_4$), 137 ($C_7H_5O_3$) and 107.

The hexamethyl ether of brackenin (methylation using dimethyl sulphate–potassium carbonate) showed an intense peak at *m/z* 580 and a small $[M]^+$ at *m/z* 598 indicative of water loss analogous to the hexa-acetate. The base peak in the spectrum occurred at *m/z* 299 for which the ion **6** is suggested. Methylation of brackenin with diazomethane afforded a crystalline tetramethyl ether with the first major peak at *m/z* 285. This fragment was analogous to **6** but with a free phenolic group at the C-2′ position. Formation of the tetramethyl ether with diazomethane is not unexpected and reflects the hydrogen bonding of the C-2′ hydroxyl group with the adjacent carbonyl [13].

Evidence from fully decoupled ^{13}C NMR spectra and from single frequency off-resonance decoupled spectra (SFORD) of brackenin provided further evidence for the proposed structure. Brackenin itself exhibited thirteen carbons, as anticipated. In the SFORD spectrum, the α- and β-carbon stood out as a doublet and triplet, respectively, thus confirming the position of the intermolecular link. The ^{13}C NMR spectra of brackenin and two of its derivatives are shown in Table 1. The chemical shift positions are in good agreement with those found in davidigenin [5] (a dihydrochalcone with the same pattern of substitution) and in other related compounds [14, 15].

Brackenin showed no optical rotation. Since the α-C is a chiral atom and since the available evidence points to a highly symmetrical molecule, the mode of link between the two C_{15}-units has been shown in such a way as to reflect a meso compound. In this orientation the two hydrogens on the α-C are always *cis* to one another and the configuration of upper unit is *R* and the lower *S*. However, at this stage the possibility of a racemic mixture cannot be excluded.

EXPERIMENTAL

All mps are uncorr. NMR spectra were recorded at 80 and 500 MHz; unless otherwise stated spectra were recorded in Me_2CO-d_6.

Bark from stem and roots of *B. zanguebarica* (Oliver), collected in the Thengwe area of Venda (authentic sample lodged in Herbarium, University of Natal), was freed of the outer grey covering. The yellow, corky residue (400 g) was ground in a pestle and mortar and subsequently extracted in a Soxhlet apparatus with petrol (60–80°), followed by C_6H_6 and CH_2Cl_2. This procedure removed a small quantity of white, waxy material. Finally the bark was extracted for 36 hr with EtOH to give a golden yellow powder (181 g).

Extraction and separation. The crude extract was subjected to a preliminary purification by CC on silica gel 60 (230–400 mesh) with C_6H_6–EtOH (4:1). The orange-yellow powder was subsequently separated using gel permeation chromatography. Orange powder (8 g), dissolved in a minimum vol. of EtOH, was chromatographed on a Sephadex LH-20 column (100 g, 135 cm × 4 cm) using EtOH–H_2O (47:53) as eluant. The flow rate was adjusted to 2.8 ml/min using a peristaltic pump and 12 ml fractions were collected. Column eluant was monitored by continuous recording of the absorption at 280 nm. By this technique a colourless fraction followed by three coloured fractions could be collected. Typically the colourless material resided in tubes 200–350. The combined contents from these tubes were concd *in vacuo* and then freeze-dried. The pale yellow residue (1.9 g) was again chromatographed on silica gel using C_6H_6–EtOH (9:1) as eluant and this yielded pale cream-coloured rosettes of brackenin, 1.3 g, mp 255° (C_6H_6–Me_2CO) (Found: C, 69.8; H, 5.38. $C_{30}H_{26}O_8$ requires: C, 70.0; H, 5.05%). IR ν_{max}^{nujol} cm^{-1}: 3500, 3410 (OH), 1680 (sh) and 1610 (CO). MS *m/z* (rel. int.): 292 (0.6), 257 (49.7), 240 (11.6), 163 (8.1), 152 (7.3), 147 (10.3) 137 (100), 120 (24.6), 107 (52). ^{1}H NMR: δ H 2.87–3.03 (*m*, 2H, β-CH_2). 4.20 (*m*, 1H, α-CH), 6.33 (*d*, 1H, J = 2.3 Hz, H-3′), 6.28, 6.41 (*dd*, 1H, J = 2.3 Hz, H-5′), 6.65, 6.98 (*dd*, 4H, J = 8.5 Hz, H-3,5, H-2,6), 7.56 (*d*, 1H, J = 9.5 Hz, H-6′), 13.07 (*s*, 1H, OH on C-2′). ^{13}C NMR: see Table 1.

Hexa-acetate (**3**). Brackenin (87 mg) was acetylated using Ac_2O–pyridine. The solid material was recovered from H_2O, then recrystallized from MeOH as fine white needles (90 mg), mp 155–156° (Found: C, 65.43; H, 4.69. $C_{42}H_{38}O_{14}$ requires: C, 65.77; H, 4.99%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1675, 1609 (CO). MS *m/z* (rel. int.): 748.21553 ($C_{42}H_{36}O_{13}$: requires 748.21014), 706 (14.0), 664 (16.1), 622 (13.3), 580 (10.0), 538 (3.8), 383 (30.0), 341 (68.0), 300 (43.7), 299 (32.0), 282 (14.9), 257 (21.7), 240 (14.7), 221 (10.8),

Table 1. ^{13}C NMR shifts of two derivatives of brackenin and davidigenin [5]

Carbon No.	Brackenin (2)	Brackenin hexa-acetate (3)	Brackenin tetramethyl ether (5)	Davidigenin [5] (dihydroiso-liquiritigenin)
1	129.53 (*s*)	135.69	130.26	132.1
2	130.06 (*d*)	129.60	130.03	129.6
3	115.27 (*d*)	121.19	113.82	115.3
4	156.03 (*s*)	153.53	158.30	155.8
5	115.27 (*d*)	121.14	113.82	115.3
6	130.06 (*d*)	129.60	130.03	129.6
1′	114.97 (*s*)	128.28	115.32	113.3
2′	165.39 (*s*)	149.08	165.91	165.6
3′	102.87 (*d*)	117.27	100.91	103.0
4′	166.03 (*s*)	149.49	166.54	164.7
5′	108.28 (*d*)	118.67	107.79	108.1
6′	133.17 (*d*)	130.58	132.26	133.0
CO	206.43 (*s*)	200.33	206.62	200.7
α-C	49.76 (*d*)	52.31	49.66	39.7
β-C	36.67 (*t*)	35.40	37.43	29.5
OMe			55.64; 55.22	
OAc		168.49; 168.76 167.84		
OAc		20.39 20.39 20.26		

179 (34.6), 163 (10.8), 137 (100), 120 (16.0), 107 (75.7). ^1H NMR: δ H 2.22 (*s*, 6H, acetate), 2.27 (*s*, 12H, acetate), 2.89 (*m*, 2H, β-CH_2), 4.23 (*br s*, 1H, α-CH), 6.80–7.29 (*m*, 14H, aromatic H). ^{13}C NMR: see Table 1.

Hexamethyl ether (**4**). To brackenin (50 mg), dry Me_2CO (5 ml) and dry K_2CO_3 (158 mg) were added, followed by a one molar excess of Me_2SO_4 (0.11 ml). The mixture was refluxed with stirring for 15 hr. After reaction, the soln was carefully basified with conc NH_4OH and poured onto ice. The white solid (48 mg) was recrystallized from MeOH, mp 86°. MS *m/z* (rel. int.): $[M]^+$ 598 (0.2), 580 (95.0), 299 (100), 165 (95), 134 (40), 121 (90).

Tetramethyl ether (**5**). To brackenin (100 mg), dissolved in MeOH (10 ml), a three molar excess of CH_2N_2–Et_2O was added at −10°. After 48 hr the solvent was reduced *in vacuo* and the oily residue examined by TLC. It consisted of one major and three minor components. These were separated by chromatography on silica gel using C_6H_6–EtOAc (19:1) as solvent. This afforded 45 mg of colourless rosettes, mp 157° (Found: C, 71.4; H, 5.72. $C_{34}H_{34}O_8$ requires: C, 71.6; H, 5.96%). IR $\nu_{max}^{CD_2Cl_2}$ cm^{-1}: 3200 and 1620 (CO). MS *m/z* (rel. int.): 285, 151 (10), 135 (3.4), 121 (11.0). ^1H NMR: δ 2.75–2.86 (*m*, 2H, β-CH_2), 3.64 (*s*, 6H, 2 × OMe), 3.76 (*s*, 6H, 2 × OMe), 4.08–4.22 (*m*, 1H, α-CH), 6.18–6.35 (*m*, 2H, aromatic H), 6.59, 6.88 (*dd*, 4H, J = 8.4 Hz, H-3,5, H-2,6), 7.37 (*d*, 1H, J = 8.7 Hz, H-6′), 13.07 (*s*, 1H, OH on C-2′). ^{13}C NMR: see Table 1.

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