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(+)-AFZELECHIN 3-RHAMNOSIDE FROM *CASSIPOUREA GERRARDII*

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Key Word Index—*Cassipourea gerrardii*; Rhizophoraceae; bark; (+)-afzelechin 3-*O*-α-L-rhamnopyranoside.

Abstract—A new flavanol glycoside has been isolated from the bark of *Cassipourea gerrardii*. Its structure has been established from spectroscopic and hydrolytic studies as (+)-afzelechin 3-*O*-α-L-rhamnopyranoside. Delayed homonuclear COSY studies show up interesting long-range couplings between specific protons.

INTRODUCTION

Cassipourea gerrardii (Rhizophoraceae, Cunningham 2167b, Natal University), commonly referred to as the Bastard Onion wood, on account of its smell, is a canopy tree growing in forests from the Eastern Cape, through Natal and Swaziland into Transvaal [1]. It is used by Zulu and Asian women in the form of a 'face pack' made from the bark and is extensively sold in herbal medicine shops. Earlier work on the extracts of *Cassipourea* species by Warren and his associates [2, 3] revealed the presence of the novel sulphur-containing alkaloids, cassipourine and gerrardine. Presumably the smell associated with the wood of the tree emanates from these alkaloids and possibly other sulphur-containing compounds.

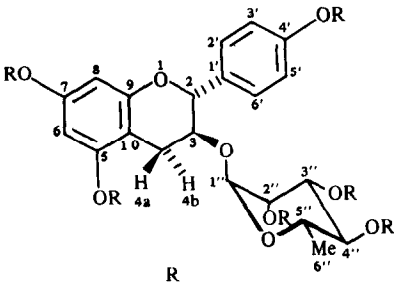
RESULTS AND DISCUSSION

Following our interest in the preservation of endangered indigenous plant species through a better knowledge of the active constituents of these plants, we have examined the bark of *Cassipourea gerrardii*. Our initial studies showed that the ethanolic extract consisted of a number of phenolic components which were extremely intractable. Using a combination of silica gel chromatography (column and centrifugal), Sephadex LH-20 separation, and further separation by silica gel chromatography, a small quantity of semi-crystalline material (0.0007%) identified as (+)-afzelechin-3-rhamnopyranoside (**1a**) was isolated.

Flavan-3-ol glycosides are rare in nature and to date only six of these, substituted at the 3-position, have been characterized [4–7]. All these compounds, apart from being 3-glycosides, are based on catechin and this is also the case for the two dimeric procyanidin glycosides belonging to this unique collection [7]. In contrast to naturally occurring catechin derivatives, afzelechin derivatives are relatively rare and occur in a restricted number of plant species. The present isolation of the first afzelechin flavan-3-ol glycoside with the sugar at C-3 thus represents a significant finding. It is of interest to note that dimeric afzelechin compounds have been known since 1982 [8, 9].

The structure of **1a**, and its acetate (**1b**), was readily determined by examination of the ^1H and ^{13}C NMR spectra at 200 MHz. The 5,7,4′-trihydroxy substitution pattern was clearly discernible and comparison with relevant data [7] leaves no doubt that the rhamnoside moiety is situated at position 3. The typical anomeric proton resonance at δ4.32 (*d*, $J=1.56$ Hz), the methyl resonance at δ1.26 (*d*, $J=6.2$ Hz) and the characteristic chemical shift positions of the remaining carbons on the sugar moiety served to confirm the L-rhamnose residue. The α-configuration was assigned to the glycosidic linkage based on the chemical shift of C-3 and C-5 of rhamnose, in which the signals due to the α-anomer (δ72.5 and 69.4, respectively) resonate at a higher field than those in the β-anomer (δ75.4 and 73.5) [10, 11]. The stereochemistry of substituents on ring C is *trans* as shown by the signal from H-2 appearing as a doublet ($J=7.85$ Hz) at δ 4.74. The hexaacetate (**1b**) was also fully characterized and serves to confirm the structure of the title compound as (+)-afzelechin and 3-*O*-α-L-rhamnopyranoside. The free phenolic form was extremely hygroscopic and no accurate elemental analysis was obtained.

We wish to draw attention to certain interesting observations which could be made from a study of the delayed homonuclear COSY spectrum of **1a** using a coupling constant of 5 Hz: (i) Weak, but quite distinct coupling between the phenolic proton signals on C-5 and C-7 while that from C-4′ shows no coupling. This allows accurate 'labelling' of the position of the phenolic protons. (ii) Distinct coupling between the signals from the C-4 hydroxyl group and the signals from 2′,6′ (but not 3′,5′)



1a H [2*R*,3*S*]
1b Ac

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protons and between C-7 hydroxyl and H-6 and H-8. (iii) Very strong couplings between the singals from H-2′,6′ and H-2 and slightly weaker couplings between H-3′,5′ and H-2. (iv) Strong coupling between the signals from H-8 to H-4a and H-4b and the corresponding coupling between H-6 and H-4a and H-4b. We believe that these long range couplings serve to confirm the proposed structure and that they provide a generally applicable method for establishing the relationship between protons separated by more than one bond.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50.1 MHz unless otherwise stated. Plant material was collected in northern Natal.

Extraction and isolation. Air-dried and powdered stem bark of *Cassipourea gerrardii* (2.48 kg) was exhaustively extracted (Soxhlet) with EtOH (95%) and the combined extracts concd to dryness under red. pres. The residue was suspended in H_2O and successively extracted with hexane, $CHCl_3$ and EtOAc.

Evapn of the EtOAc fr. afforded a residue (10.0 g) which was fractionated on silica gel by CC using $CHCl_3$–Me_2CO (2:3) as eluent. This solvent removed non-phenolic contaminants. The residue (8.0 g) was then applied to a Sephadex LH-20 column and eluted with EtOH (95%), collecting 6 ml frs. Compound **1a** [R_f 0.23 in CH_2Cl_2–Me_2CO (2·3)] was present in tubes 30–48. This fr. was finally purified by centrifugal TLC (Chromatotron, Model 7924T) using MeOH–CH_2Cl_2–Me_2CO (3:47:50) as eluent to give pure (**1a**) (170 mg).

(+)-*Afzelechin* 3-O-α-L-*rhamnopyranoside* (**1a**). This is a semi-crystalline hygroscopic powder, mp 110–115°, $[\alpha]_D^{28}$ −14.95° (Me_2CO; *c* 0.107). $[\alpha]_{365}^{28}$ −37.38° (Me_2CO; *c* 0.107); UV λ_{max}^{MeOH} nm: 213, 224, 279; IR ν_{max} cm^{-1}: 3384, 1614, 1317, 1141. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Hexaacetate. Amorphous powder, mp 69°, $[\alpha]_D^{28}$ +23.89° ($CHCl_3$; *c* 0.180), $[\alpha]_{365}^{28}$ +90.0° ($CHCl_3$; *c* 0.180); UV λ_{max}^{MeOH} nm: 210; IR ν_{max} cm^{-1}: 3430, 1753, 1620, 1130; ^{1}H and ^{13}C NMR Tables 1 and 2. (Found: C, 58.52; H, 5.62, $C_{33}H_{36}O_{15}$ requires: C, 58.92; H, 5.40%.)

Table 2. ^{13}C NMR (50 MHz) spectral data from flavanols **1a** and **1b** (in [$(CD_3)_2CO$] and $CDCl_3$, respectively)

C	1a	1b
2	80.45 *d*	79.48 *d*
3	74.82 *d*	74.37 *d*
4	27.89 *t*	27.54 *t*
5	156.91 *s*[a]	150.86 *s*[a]
6	95.52 *d*	108.73 *d*
7	157.38 *s*[a]	149.29 *s*[a]
8	96.41 *d*	107.82 *d*
9	158.04 *s*[a]	155.03 *s*
10	100.35 *s*	111.03 *s*
1′	131.09 *s*	134.97 *s*
2′,6′	129.38 *d*	127.84 *d*
3′,5′	115.99 *d*	122.02 *d*
4′	158.32 *s*[a]	149.80 *s*[a]
1″	101.49 *d*	97.98 *d*
2″	71.51	68.84 *d*
3″	72.29 *d*	68.84 *d*
4″	73.66 *d*	70.82 *d*
5″	69.67 *d*	67.09 *d*
6″-Me	17.94 *q*	17.28 *q*
6 × Me		20.67, 20.79, 21.03
6 × CO		169.99, 169.85, 169.58
		169.02, 168.94, 168.34

[a]May be interchanged.

Enzymatic hydrolysis of compound **3a**. The glycoside (45 mg) was dissolved in an acetate buffer (0.02 M, 10 ml, pH 3.8) and incubated (40°) with hesperidinase (20 mg) for 12 hr. Extraction with EtOAc afforded the crude aglycone, which, after separation on the chromatotron (CH_2Cl_2–Me_2CO, 1:1) gave (+)-afzelechin $[\alpha]_D^{28}$ +15° (Me_2CO; *c* 0.100). The sugar residue was confirmed to be rhamnose by co-TLC with an authentic sample.

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Table 1. ^{1}H NMR (200 MHz) spectral data for flavanols **1a** and **1b** (in [$(CD_3)_2CO$] and $CDCl_3$, respectively)

H	1a	1b
2	4.74 *d* (7.85)	4.87 *d* (8.46)
3	4.02 *ddd* (7.85, 5.67, 8.03)	3.97 *ddd* (8.50 , 6.05, 8.06)
4eq	2.67 *d* (16.27, 8.33)	2.78 *dd* (16.36, 9.09)
4ax	2.92 *dd* (16.27, 5.75)	2.90 *dd* (16.36, 6.13)
6	5.94 *d* (2.29)	6.58 *d* (2.21)
8	6.09 *d* (2.29)	6.63 *d* (2.21)
2′6′	7.28 *d* (8.57)	7.43 *d* (8.52)
3′5′	6.88 *d* (8.57)	7.09 *d* (8.52)
OH-5	8.2	
OH-7	8.5	
OH-4	8.6	
1″	4.33 *d* (1.56)	3.99 *d* (1.58)
2″	3.56 *dd* (3.31, 1.57)	4.95 *dd* (3.44, 1.64)
3″	3.65 *dd* (9.43, 3.39)	5.21 *dd* (10.17, 3.41)
4″	3.42 *dd* (9.44, 9.44)	4.97 *dd* (9.89, 9.89)
5″	3.73 *dq* (9.44, 6.20)	3.85 *dq* (9.82, 6.32)
6″	1.26 *d* (6.20)	1.17 *d* (6.28)
2″,3″,4″ Acetates		1.96, 2.04, 2.05 *s*
5,7,4′ Acetates		2.27, 2.28, 2.33 *s*

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TWO TYROSINASE INHIBITING FLAVONOL GLYCOSIDES FROM *BUDDLEIA CORIACEA*

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Key Word Index—*Buddleia coriacea*; Loganiaceae; flavonol glucoside; buddlenoid A; buddlenoid B; tyrosinase inhibitory activity.

Abstract—Two new flavonol glycosides, buddlenoids A and B, have been isolated and identified as tyrosinase inhibitors from the aerial parts of *Buddleia coriacea*. Their structures were deduced from spectroscopic evidence to be kaempferol 7-(6″-*p*-coumaroylglucoside) and isorhamnetin 7-(6″-*p*-coumaroylglucoside). Both buddlenoids showed high inhibition of mushroom tyrosinase.

INTRODUCTION

The aerial parts of *Buddleia coriacea*, known as 'Quishuara', are used as a remedy for venereal diseases in Bolivia [1]. There has been no previous report on the chemical constituents. In our continuing search for alternative insect control agents, we have isolated tyrosinase inhibitors from several plants. Tyrosinase is one of the most important key enzymes in the insect moulting process [2]. Moreover, tyrosinase inhibitors have also become increasingly important for cosmetic products in relation to hyperpigmentation [3]. Thus, tyrosinase inhibitors may control over production of the dermal melanin pigment since tyrosinase plays an important role in the process of melanin biosynthesis [4, 5]. In our preliminary screening of tyrosinase inhibitory activity, the methanol extract of Quishuara proved to exhibit potent inhibitory activity. Bioassay guided fractionation, after repeated various CC techniques, led to the isolation of two active principles.

RESULTS AND DISCUSSION

The methanol extract of the aerial parts of *B. coriacea* exhibited mushroom tyrosinase inhibitory activity in our preliminary assay. Fractionation guided by the tyrosinase inhibitory activity led to the isolation of two new active principles which were designated as buddlenoids A (**1**) and B (**2**).

Buddlenoid A (**1**) had a molecular formula of $C_{30}H_{26}O_{13}$, which was established by observation of *m/z* 593 $[M-H]^-$ by negative ion FAB mass spectrometry, in conjunction with the ^1H and ^{13}C NMR data. Extensive analysis of the NMR data (Tables 1 and 2) indicated that **1** consists of kaempferol, glucose and *p*-coumaroyl moieties. Also the signals of *meta* coupling between H-6 and H-8 (δ6.70 and 6.71, each *d*, $J = 1$ Hz) and of AA′BB′ type of H-2′, H-6′ and H-3′, H-5′ (δ7.48 and 7.15, each *d*, $J = 5$ Hz) in the ^1H NMR spectrum indicated the characteristic A- and B-ring moieties of kaempferol. The UV spectrum showed absorption maxima at 267 and 317 nm and bathochromic shifts on addition of NaOAc, indicated that the C-5 and C-4′ hydroxyls were unsubstituted [6]. This was also partially supported by the observation of a downfield signal at δ12.45 in the ^1H NMR spectrum indicating the presence of an intramolecular hydrogen bond between the OH-5 and C=O groups of flavonols. In addition, the signals of C-2 and C-3 observed at δ149.5 and 135.1 in the ^{13}C NMR spectrum indicated that the hydroxyl group at