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EPIAFZELECHIN-(4β→8, 2β→0→7)-*ENT*-AFZELECHIN FROM *CASSIPOUREA GERRARDII*

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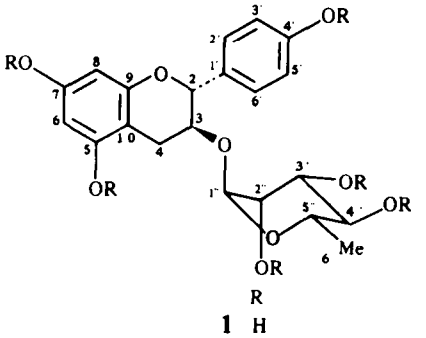
Key Word Index—*Cassipourea gerrardii*; Rhizophoraceae; A-type proanthocyanidin; flavan-3-ol.

Abstract—A new A-type proanthocyanidin has been isolated from the bark of *Cassipourea gerrardii*. Its structure has been established from spectroscopic studies as epiafzelechin-(4β→8, 2β→0→7)-*ent*-afzelechin. ^{1}H NMR analysis of *R*-(+)- and *S*-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of the title compound permits assessment of the absolute stereochemistry at both C-3(C) and C-3(F).

INTRODUCTION

Cassipourea gerrardii is a small tree growing in forests from the Eastern Cape, through Natal and Swaziland into Transvaal [1]. The bark is sold commercially in herbal and medicine shops. In a previous communication [2] we reported on the isolation of the novel flavan-3-ol glycoside afzelechin-3-*O*-α-L-rhamnopyranoside (**1**) from the bark of *C. gerrardii*. From the same source we have now isolated a new proanthocyanidin dimer. It is the major constituent in the EtOAc soluble portion of the bark but proved extremely difficult to purify.



RESULTS AND DISCUSSION

Our initial studies showed that the ethanolic extract from the stem bark of *C. gerrardii* consisted of a number of phenolic components which were extremely intractable. Using a combination of silica gel chromatography, Sephadex LH-20 separation and further separations with Toyopearl HW-40F, an amorphous powder (0.09%) identified as epiafzelechin-(4β→8, 2β→0→7)-*ent*-afzelechin (**2a**) was isolated.

Since the first isolation [3] and structural determination [4, 5] of proanthocyanidin A-2 [epicatechin-(4β→8, 2β→0→7)-epicatechin] a variety of analogues possessing the doubly linked unit of either (2β,4β) or (2α,4α) configuration have been reported [6–10]. However, in contrast to the wide distribution of singly linked proanthocyanidins (B-type), doubly linked proanthocyanidins (A-type) are relatively rare and occur in a restricted number of plant species.

The ^{13}C and ^{1}H NMR spectra of **2a** were similar to those of mahuannin B (**3**) previously isolated by Hikino *et al.* [6], the significant differences being only the chemical shifts and ^{1}H coupling constants of the F-ring protons and carbons. The presence of a 1H doublet (δ 4.83, J = 7.95 Hz) in the ^{1}H NMR spectrum of **2a** attributed to H-2(F) clearly indicates a 2,3-*trans* configuration of the lower F-ring substituents. Furthermore the presence of two 1H doublets (J = 3.56 Hz) at δ4.12 and δ4.13 together with the ^{13}C NMR resonances at δ29.15 and δ100.37 indicate that the flavan-3-ol units are joined via a C-4(C) carbon–carbon linkage and a C-2(C) ether linkage.

Methylation of (**2a**) with ethereal diazomethane yielded the tetramethyl ether (**2b**) and not the pentamethyl ether (**2c**) as expected. The ^{1}H NMR spectrum of **2b** revealed the presence of a sharp 1H singlet at δ6.67. Based on the HETCOR spectrum of **2b** it was concluded that the proton was phenolic. The DELAYED HETCOR (7 Hz) spectrum displayed strong coupling between the phenolic proton and the adjacent C-5(A) and slightly weaker coupling to C-6(A) and C-10(A) carbons. These results establish that the phenolic hydroxyl is located at position 5(A). The unusual high field shift and uncharacteristic sharpness of this phenolic proton resonance, together with the definite non-reactivity towards diazomethane, suggests a through-space interaction with the lower structural unit. Of the three possible linkage isomers *viz.* (4→6, 2→0→7), (4→6, 2→0→5) and (4→8, 2→0→7), stereomodels suggest that it is only in the latter mode of linkage

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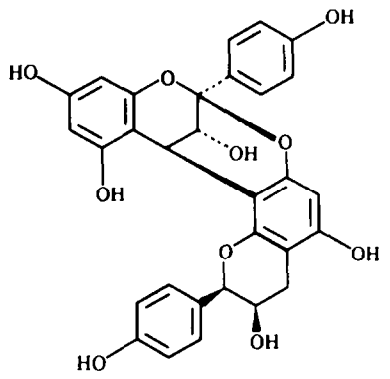

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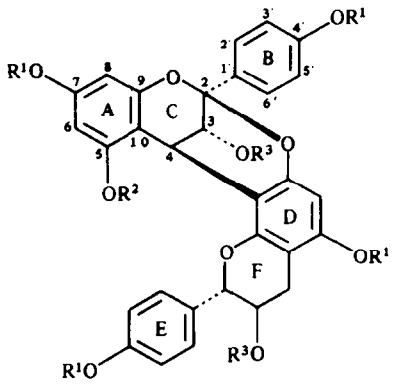

	R^1	R^2	R^3
2a	H	H	H
2b	Me	H	H
2c	Me	Me	H
2d	Me	OC(OMe)(CF$_3$)Ph	(R)
2e	Me	OC(OMe)(Ph)CF$_3$	(S)
2f	Me	Me	Me

that such steric interactions could exist. Confirmation of the mode of linkage was established by applying the powerful ^{1}H NOE technique to the tetramethyl ether (**2b**). Besides the stereochemically insignificant but structurally important NOE association of 5-OH(A) (δ6.67) with H-4(C) (δ4.31, 19.1%) and H-6(A) (δ6.01, 3.4%) this proton exhibited selective associations with both H-2′,6′(E) and H-2(F) (δ7.34, *d*, J = 6.47 Hz; δ4.83, *d*, J = 7.95 Hz; 8.0% and 0.73% resp.). These selective NOE associations thus establish the (4→8, 2→0→7) linkages represented by (**2a**).

The absolute stereochemistry at C-4(C) for (**2a**) was established from the sign of the Cotton effect near 230 nm [11]. Circular dichroism measurements in methanol revealed a negative Cotton effect at 258 nm ($[\theta] = -5.8 \times 10^3$) and a positive Cotton effect at 232 nm ($[\theta] = 1.7 \times 10^3$). This indicates a 4*R* configuration. The configuration at C-2(C) is thus automatically assigned.

It has been established that ^{1}H NMR analysis of *R*-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of different sets of enantiomeric flavan-3-ols and 4-arylflavan-3-ols, respectively, permits assignment of the absolute configuration at C-3 in these compounds [12]. By comparing ^{1}H NMR data in both the *R*-(+)- and *S*-(−)-MTPA esters, this methodology has been extended to assess the absolute configuration in these compounds when only one isomer is available (Mouton, C. H. L., Steenkamp, J. A. and Ferreira, D., unpublished results). A structural appraisal of the A-type proanthocyanidins revealed that this method would be well suited for assigning the absolute stereochemistry at C-3(C) and C-3(F) in these compounds.

The *R*-(+)-and *S*-(−) MTPA esters **2d** and **2e**, respectively, were prepared from the tetramethyl ether (**2b**) via standard procedures [13]. ^{1}H NOE association of H-3(C) and H-3(F), H-2(F) with H-2′,6′(B) and H-2′,6′(E), respectively, permitted accurate 'labelling' of these aromatic protons in both the *R*-(+)- and *S*-(−)-MTPA esters. ^{1}H NMR spectra of the *R*-(+)- and *S*-(−)-MTPA esters revealed that shielding, and therefore an upfield shift of both H-2′,6′(B) and H-2′,6′(E) (δ −0.17 and δ −0.18, respectively, occurs in the *S*-(−)-MTPA ester relative to the *R*-(+)-MTPA ester. Thus the *R*-configuration may be assigned to both C-3(C) and C-3(F). Finally, since the relative stereochemistry between H-2(F) and H-3(F) is known, C-2(F) may be assigned the *S*-configuration. The heptamethyl ether derivative (**2f**) was also fully characterized and serves to confirm the structure of the title compound as epiafzelechin-(4β→8, 2β→0→7)-*ent*-afzelechin.

An interesting observation may be made concerning the ^{1}H NMR spectra of **2a** and **3**. Besides the obvious difference in the signals arising from the F-ring protons, the H-2′,6′(E) proton signals of (**2a**) are upfield (δ −0.23) relative to the same signals in (**3**). This relative upfield shift suggests greater steric interaction between these protons and the A-ring. This observation is confirmed by Dreiding models and may serve to explain the difference in reactivity at 5-OH(A) in **2a** and **3**.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50.1 MHz unless otherwise stated. IR spectra were recorded on a Shimadzu FTIR-4300. Plant material was collected in Northern Natal.

Extraction and isolation. Air-dried and powdered stem bark of *C. gerrardii* (2.48 kg) was exhaustively extracted (Soxhlet) with EtOH (95%) and the combined extracts concentrated to dryness under reduced pressure. The residue was suspended in water and successively extracted with hexane, $CHCl_3$ and EtOAc. Evaporation of the EtOAc fraction afforded a residue (10.0 g) which was fractionated on silica gel by column chromatography using $CHCl_3$–acetone (2:3) as eluent. This solvent removed less polar, 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 fractions. Compound (**2a**) [R_f 0.31 in CH_2Cl_2–acetone (1:1)] was present in tubes 60–84. Further separations of this fraction by centrifugal TLC (Chromatotron, Model 7924T) using CH_2Cl_2–acetone (1:1) and column chromatography over Toyopearl HW-4OF eluting with EtOH (80%) afforded pure (**2a**) (2.1 g).

Epiafzelechin-(4β→8, 2β→0→7)-ent-*afzelechin* (**2a**). This is an amorphous, hygroscopic powder, mp 238–245°, $[\alpha]_D^{25}$ + 64.29° (MeOH; *c* 0.126); $[\alpha]_{365}^{25}$ + 199.21° (MeOH; *c* 0.126); UV λ_{max}^{MeOH}

Table 1. ^{1}H NMR (200 MHz) spectral data for compounds **2a**, **2b** and **2f**

H	**2a** (in CD_3OD)	**2b** (in $CDCl_3$)	**2f** (in $CDCl_3$)
3(C)	4.12 *d* (3.56)	4.12 *d* (3.65)	3.79 *d* (3.59)
4(C)	4.31 *d* (3.56)	4.27 *d* (3.65)	4.99 *d* (3.59)
6(A)	6.01 *d* (2.22)	6.13 *d* (2.47)	5.94 *d* (2.32)
8(A)	6.13 *d* (2.22)	6.27 *d* (2.47)	6.24 *d* (2.32)
2',6'(B)	7.55 *dd* (2.10, 6.79)	7.64 *dd* (2.12, 6.86)	7.61 *dd* (6.87, 2.04)
3',5'(B)	6.85 *dd* (2.10, 6.79)	6.96 *dd* (2.12, 6.86)	6.90 *dd* (6.87, 2.04)
2(F)	4.83 *d* (7.95)	4.73 *d* (8.63)	5.21 *d* (7.23)
3(F)	4.21 *ddd* (8.39, 5.60, 7.95)	4.13 *ddd* (9.02, 5.94, 8.63)	3.69 *m*
4(F) eq.	2.63 *dd* (8.39, 16.41)	2.57 *dd* (16.58, 9.02)	2.44 *dd* (16.95, 4.53)
4(F) ax.	2.99 *dd* (5.60, 16.41)	3.06 *dd* (16.58, 5.94)	2.73 *dd* (16.95, 3.85)
2',6'(E)	7.34 *dd* (2.1, 6.47)	7.38 *dd* (2.10, 6.60)	7.10 *dd* (6.76, 1.96)
3',5'(E)	6.88 *dd* (2.1, 6.47)	6.98 *dd* (2.10, 6.60)	6.74 *dd* (6.76, 1.96)
6(D)	6.15 *s*	6.25 *s*	6.13 *s*
3(C)–OH		2.00 *s*	
3(F)–OH		2.03 *s*	
5(A)–OH		6.671 *s*	
7(A)–OMe		3.70 *s*	3.71 *s**
4'(B)–OMe		3.81 *s*	3.71 *s**
5(D)–OMe		3.74 *s*	3.65 *s*
4'(E)–OMe		3.81 *s*	3.77 *s**
5(A)–OMe			3.24 *s*
3(C)–OMe			2.97 *s*
3(F)–OMe			3.35 *s*

*May be interchanged.

Table 2. ^{13}C NMR (50 MHz) spectral data for compounds **2a**, **2b** and **2f**

C	**2a** (in CD_3OD)	**2b** (in $CDCl_3$)	**2f** (in $CDCl_3$)
2(C)	100.37 *s*	99.07 *s*	98.42 *s*
3(C)	67.66 *s*	66.52 *d*	75.67 *d*
4(C)	29.15 *d*	27.40 *d*	24.47 *d*
5(A)	156.69 *s*	155.25 *s*	158.19 *s*
6(A)	98.06 *d*	96.90 *d*	92.31 *d*
7(A)	158.01 *s*	159.94 *s*	159.51 *s**
8(A)	96.48 *d**	94.72 *d*	92.80 *d*
9(A)	154.20 *s*	152.36 *s**	153.72 *s*
10(A)	103.93 *s*	102.51 *s*	104.297 *s*
1'(B)	131.57 *s*	127.74 *s*	131.40 *s*
2'6'(B)	129.46 *d*	128.22 *d*	128.30 *d*
3'5'(B)	115.48 *d*	113.65 *d*	113.15 *d*
4'(B)	158.66 *s*	160.21 *s*	159.80 *s**
2(F)	84.14 *d*	83.37 *d*	78.23 *d*
3(F)	68.04 *d*	67.34 *d*	76.01 *d*
4(F)	29.15 *t*	28.01 *t*	21.46 *t*
5(D)	156.03 *s*	157.30 *s*	157.09 *s*
6(D)	96.53*	93.00 *d*	91.61 *d*
7(D)	152.09 *s*	150.96 *s**	151.40 *s*
8(D)	106.74 *s*	105.61 *s*	105.72 *s*
9(D)	151.40 *s*	149.67 *s*	151.04 *s*
10(D)	103.1 *s*	103.28 *s*	101.29 *s*
1'(E)	129.80 *s*	130.12 *s*	131.37 *s*
2'6'(E)	130.10 *d*	129.30 *d*	127.38 *d*
3'5'(E)	116.26 *d*	114.56 *d*	113.54 *d*
4'(E)	158.76	160.45 *s*	158.96 *s*
–OMe		55.30–55.64	54.88–57.39

*May be interchanged.

nm 237.0, 237.2; IR ν_{max} cm^{-1}: 3492, 1614, 1516, 1140, 832. ^{1}H and ^{13}C NMR, Tables 1 and 2. CD $[\theta]_{200}$ + 0.73 × 10^3, $[\theta]_{232}$ + 1.7 × 10^3, $[\theta]_{241}$ 0.0, $[\theta]_{258}$ − 5.8 × 10^3, $[\theta]_{285}$ 0.0.

Tetramethyl ether (**2b**). The free phenolic form of the proanthocyanidin (**2a**) (110 mg) was methylated with ethereal diazomethane to afford 70 mg of the tetramethyl ether as an amorphous powder, mp 152–154°, $[\alpha]_D^{25}$+ 40.00 ($CHCl_3$; *c* 0.095), $[\alpha]_{365}^{25}$ + 104.21° ($CHCl_3$; *c* 0.095); IR ν_{max} cm^{-1}: 3445, 1603, 1516, 1252, 1036, 831; ^{1}H and ^{13}C NMR, Tables 1 and 2.

Heptamethyl ether (**2f**). NaH (3 eq.) was added to a stirred solution of (**2b**) (70 mg) in anhydrous THF (30 ml) at 0°. The mixture was stirred at room temperature for 30 min, treated with MeI (3 eq.) and stirred for a further 6 hr. The reaction was quenched with water and extracted with ether. Purification by flash chromatography (CH_2Cl_2) afforded the heptamethyl ether (42 mg) as a pale yellow amorphous powder, mp 134°, $[\alpha]_D^{25}$ − 78.0° ($CHCl_3$; 0.100), $[\alpha]_{365}^{25}$ − 332.0 ($CHCl_3$; *c* 0.100); IR ν_{max} cm^{-1}: 1615, 1518, 1250, 1115, 1040; MS *m/z* (rel. int.): 642.2397 $[M]^+$ (30) (calc. for $C_{37}H_{38}O_{10}$, 642.2465), 611 (52), 447 (41), 164 (64), 149 (100), 121 (57), 91 (69). ^{1}H and ^{13}C NMR, Tables 1 and 2.

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