Phytochemistry, Vol. 26, No. 7, pp. 2049–2051, 1987.
Printed in Great Britain.

0031–9422/87 \$3.00 + 0.00
Pergamon Journals Ltd.

(+)-CATECHIN-GALLOYL ESTERS FROM THE BARK OF *ACACIA GERRARDII*

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(*Received* 11 *November* 1986)

Key Word Index—*Acacia gerrardii*; Leguminosae; Minosoideae; (+)-catechin-3'-gallate; (+)-catechin4'-gallate; (+)-catechin-7-gallate; (+)-catechin-4',7-digallate; (+)-catechin-3',7-digallate.

Abstract—Five (+)-catechin galloyl esters were isolated and characterized from the acetone extract of the bark of *Acacia gerrardii*.

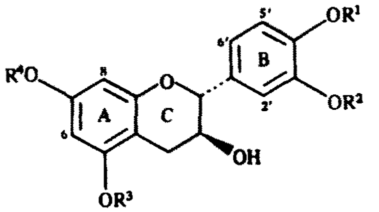
INTRODUCTION

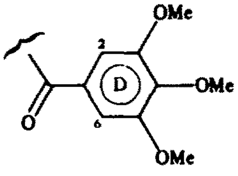
Acacia gerrardii is a small tree up to 8 m in height occurring in woodland and wooded grassland south of the equator in Zambia, Zimbabwe, eastern Botswana, western Maputo, and northern and eastern parts of South Africa. The bark is dark grey to reddish and varies from rough to smooth [1]. Gallic acid [2] and its metabolites [3] are well documented since the former was first isolated from the roots of *Bergenia* species [4].

RESULTS AND DISCUSSION

The milled bark of *Acacia gerrardi* was first extracted with hexane followed by extraction with acetone. The acetone extract on silica gel was eluted from the column with an acetone–benzene mixture. The fractions obtained gave a number of over-lapping spots on TLC with lower R_f values than (+)-catechin (1). Very evident was the high degree of decomposition of the compounds on TLC, this was manifested in an unsuccessful attempt to purify the original compounds. Derivatization with dimethyl sulphate and potassium carbonate yielded only trimethoxygallic acid and penta-*O*-methyl-(+)-catechin. The conventional procedure for acetylation with acetic anhydride and pyridine yielded a tarry product with almost no other derivatives than the acetates of gallic acid and (+)-catechin. When however the procedure was changed and the acetic anhydride was added first, left at about 60° for 30 min to dissolve the material and the pyridine then added, the amount of tarry product was less and quite a few derivatives were seen on TLC but the amount present were still very low. On treatment with ethereal diazomethane the yield of the respective derivatives other than that of gallic acid and (+)-catechin were substantially higher but still very low as reflected in the experimental section. The facile decomposition of the (+)-catechin galloyl esters under slightly alkaline conditions (cf methylation and acetylation) could be the reason why these compounds have not been discovered before.

The ^{1}H NMR patterns (Table 1) of the *O*-methyl-3-hydroxy-(+)-catechin galloyl esters (3–7) are very little





Trimethoxybenzoyl group

	R^1	R^2	R^3	R^4
1	H	H	H	H
2	Me	Me	Me	Me
3	Me	TMB	Me	Me
4	TMB	Me	Me	Me
5	Me	Me	Me	TMB
6	TMB	Me	Me	TMB
7	Me	TMB	Me	TMB

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Table 1. ^1H NMR data for (+)-catechin derivatives (300 MHz, $CDCl_3$, TMS as int. standard)

Compound	H(C)-2	H(C)-3	H(C)-4e	H(C)-4a	H(A)-8	H(A)-6	H(B)-2′	H(B)-5′
2	4.54 *d*	3.86–4.13 *m*	3.05 *dd*	2.53 *dd*	6.12 *dd*	6.09 *d*	6.95 *d*	6.88 *d*
2(3-OAc)	5.00 *d*	5.14–5.50 *m*	2.98 *dd*	2.63 *dd*	6.03 *d*	6.15 *d*	6.86 *d*	6.80 *d*
3	4.71 *d*	3.99–4.19 *m*	3.04 *dd*	2.60 *dd*	6.12 *d*	6.10 *d*	7.26 *d*	7.04 *d*
3(3-OAc)	5.08 *d*	5.29–5.37 *m*	2.88 *d*	2.68 *dd*	6.17 *d*	6.09 *d*	7.16 *d*	6.98 *d*
4	4.72 *d*	3.98–4.08 *m*	3.02 *dd*	2.59 *dd*	6.14 *d*	6.10 *d*	7.08 *d*	7.16 *d*
4(3-OAc)	5.12 *d*	5.32–5.38 *m*	2.86 *dd*	2.68 *dd*	6.18 *d*	6.09 *d*	7.01 *d*	7.09 *d*
5	4.70 *d*	4.03–4.12 *m*	3.15 *dd*	2.66 *dd*	6.44 *d*	6.35 *d*	6.98 *d*	6.90 *d*
5(3-OAc)	5.08 *d*	5.34–5.42 *m*	2.96 *dd*	2.74 *dd*	6.49 *d*	6.34 *d*	6.89 *d*	6.84 *d*
6	4.75 *d*	3.99–4.11 *m*	3.11 *dd*	2.65 *dd*	6.45 *d*	6.33 *d*	7.09 *d*	7.16 *d*
6(3-OAc)	5.19 *d*	5.37–5.44 *m*	2.94 *dd*	2.78 *dd*	6.52 *d*	6.37 *d*	7.04 *d*	7.12 *d*
7	4.74 *d*	4.02–4.11 *m*	3.13 *dd*	2.67 *dd*	6.43 *d*	6.35 *d*	7.26 *d*	7.05 *d*
7(3-OAc)	5.13 *d*	5.32–5.40 *m*	2.94 *dd*	2.77 *dd*	6.48 *d*	6.35 *d*	7.16*d*	6.99 *d*

Compound	H(B)-6′	OMe	TMB-2 and 6 protons	TMB-OMe	3-OAc
2	6.99 *dd*	3.67–3.86 (12H, *m*)			
2(3-OAc)	6.89 *dd*	3.78–3.88 (12 H, *m*)			
3	7.33 *dd*	3.75–3.84 (9 H, *t*)	7.46 (2H, *s*)	3.93–3.95 (9 H, *d*)	
3(3-OAc)	7.26 *dd*	3.77–3.82 (9 H, *d*)	7.45 (2 H, *s*)	3.94 (9 H, *s*)	1.98 (3H, *s*)
4	7.05 *dd*	3.74–3.81 (9 H, *t*)	7.44 (2 H, *s*)	3.92 (9H, *s*)	
4(3-OAc)	6.96 *dd*	3.76–3.77 (9H, *d*)	7.43 (2H, *d*)	3.91 (9H, *s*)	1.98 (3H, *s*)
5	7.01 *dd*	3.83–3.91 (9H, *d*)	7.43 (2H, *s*)	3.94 (9H, *s*)	
5(3-OAc)	6.92 *dd*	3.18–3.89 (9H, *t*)	7.44 (2H, *s*)	3.94 (9H, *s*)	1.98 (3H, *s*)
6	7.05 *dd*	3.81–3.82 (6H, *d*)	7.41 (2H, *s*); 7.44 (2H, *s*)	3.92 (18H, *s*)	2.2 (3H, *s*)
6(3-OAc)	6.99 *dd*	3.80–3.82 (6H, *d*)	7.45 (2H, *s*); 7.46 (2H, *s*)	3.93–3.95 (18 H, *d*)	
7	7.35 *dd*	3.83–3.85 (6H, *d*)	7.43 (2H, *s*); 7.46 (2H, *s*)	3.94 (18H, *s*)	
7(3-OAc)	7.25 *dd*	3.80–3.83 (6H, *d*)	7.44 (2H, *s*); 7.46 (2H, *s*)	3.93–3.95 (18H, *d*)	2.0 (3H, *s*)

different from that of tetra-*O*-methyl-3-hydroxy-(+)-catechin (**2**) except for the occurrence of the additional two *meta*-coupled aromatic protons of the 3,4,5-trimethoxybenzoyl (TMB) part of the molecules always at lower field (δ7, 41–7, 46) than the other aromatic protons of the (+)-catechin part of the molecules. Additional methoxy signals at δ3, 91–3, 95 are obvious. On acetylation the *O*-methyl-3-hydroxy-(+)-catechin galloyl esters (**3**–**7**) show the expected downfield shift of the H-2, H-3, H-4 (ax) and shift to higher field of H-4 (eq) (see Table 1).

The deshielding effect of the TMB group on the *ortho* and *para* protons relative to their chemical shifts for tetra-*O*-methyl-(+)-catechin (**2**) (Table 2) is a classical example of how this information can be used to determine the position of the TMB group in the molecule. The protons *ortho* to the TMB group show a downfield shift of 0.24–0.33 ppm while the protons *para* to the group show a shift of 0.34–0.36 ppm (Table 2).

In addition to the deshielding effect on the *ortho* and *para* protons, the exact position of the TMB group was confirmed by NOE experiments (Table 3), thus saving tedious degradation studies on the limited amounts of material available.

High resolution mass spectrometry confirmed the molecular masses of compounds **3**, **4** and **5** to be 526, and the mass spectral fragmentation pattern (Table 4) demonstrated the presence of one TMB-group in each of the compounds [5]. Because of the lability of compounds **6** and **7** no molecular ion could be found when using an electron impact instrument.

EXPERIMENTAL

Melting points: uncorr; IR: $CHCl_3$; ^1H NMR: 300 MHz.

Plant material. The stem bark material of *A. gerrardii* was collected in the Durban area by Mr C. J. Ward of the Department

Table 2. ^1H NMR of aromatic protons (ppm, downfield shift)

Compound	H-2′	H-5′	H-6′	H-6	H-8
2	6.95	6.88	6.99	6.09	6.12
3	7.26 (0.31)	7.04 (0.16)	7.33 (0.34)	6.10 (0.01)	6.12 (0.00)
4	7.08 (0.13)	7.16 (0.28)	7.05 (0.06)	6.10 (0.01)	6.14 (0.02)
5	6.98 (0.03)	6.90 (0.02)	7.01 (0.02)	6.35 (0.26)	6.44 (0.32)
6	7.09 (0.14)	7.16 (0.28)	7.05 (0.06)	6.33 (0.24)	6.45 (0.33)
7	7.26 (0.31)	7.05 (0.17)	7.35 (0.36)	6.35 (0.26)	6.43 (0.31)

Table 3. NOE experiments(%) (300 MHz)

	Compound				
Association	**3**	**4**	**5**	**6**	**7**
7-OMe to H-6	4.5	3.8	None	None	None
7-OMe to H-8	16.2	16.7	None	None	None
5-OMe to H-6	18.0	15.8	13.1	8.4	12.5
4'-OMe to H-5'	17.4	None	9.8*	None	9.2
5'-OMe to H-2'	None	13.6	9.0*	10.1	None

*Approximate values because of close proximity of 3'- to 4'-OMe.

Table 4. Mass spectral fragmentation pattern (%)

	Compound		
Fragment	**3**	**4**	**5**
$[M]^+$	7.4	3.5	15.0
$[M-(MeO)_3C_6H_2CO]^+$	0.7	—	2.5
$[M-(MeO)_3C_6H_2COOH]^+$	1.3	—	0.9
$[(MeO)_3C_6H_2CO]^+$	100	100	100

CD spectra of the methyl ether acetate derivatives of the galloyl esters **3**–**7** were identical with that of tetra-*O*-methyl-3-acetoxy-(+)-catechin thus confirming the (2*R*,3*S*)-(+)-catechin configuration of the galloyl esters. IR spectra of **3**–**7** indicated the presence of an ester carbonyl at 1760 cm^{-1}.

of Botany and a specimen is deposited at the Herbarium at the University of Durban-Westville.

Extraction and isolation. Air-dried milled stem bark material was defatted with hexane followed by extraction with Me_2CO and the extract evaporated to dryness under reduced pressure. The Me_2CO extract (72 g) was chromatographed over kieselgel 60 (Merck 7734) and eluted with a C_6H_6–Me_2CO (2:1) mixture and 30 fractions of 100 ml each were collected. Fraction 12 and above were selectively combined to eventually result in 5 combined fractions which after vacuum-drying was then alkylated by methods already described [6]. The *O*-methylated fractions were separated by prep. TLC (C_6H_6–EtOAc–Me_2CO, 7:2:1) and compounds **2**–**7** were obtained. The 3-OAc derivatives of compounds **2**–**7** were then prepared by known procedures [6].

5,7,3',4'-*Tetra-O-methyl-(+)-catechin* (**2**) and its 3-OAc derivative were identical in all respects to an authentic sample.

Hexa-O-methyl-(+)-catechin-3'-gallate (**3**). Non-crystalline, 20 mg (R_f 0.55), mp 75–77°. Found: C, 63.64; H, 5.70% ($C_{28}H_{30}O_{10}$ requires: C, 63.85; H, 5.74%). The 3-OAc derivative (11 mg), mp 72–73°. Found *m/z* 568.1945 ($C_{30}H_{32}O_{11}$ requires 568.1941).

Hexa-O-methyl-(+)-catechin-4'-gallate (**4**). Non-crystalline, 25 mg (R_f 0.57), mp 76–78°. Found: C, 63.91; H, 5.72% ($C_{28}H_{30}O_{10}$ requires: C, 63.85; H, 5.74%). The 3-OAc derivative (12 mg), mp 70–73°. Found *m/z* 568.1945 ($C_{30}H_{32}O_{11}$ requires 568.1941).

Hexa-O-methyl-(+)-catechin-7-gallate (**5**). Non-crystalline, 11 mg (R_f 0.41), mp 77–80°. Found: C, 63.66; H, 5.64%. ($C_{28}H_{30}O_{10}$ requires: C, 63.85; H, 5.74%). The 3-OAc derivative (4 mg), mp 63–65°. Found *m/z* 568.1943 ($C_{30}H_{32}O_{11}$ requires 568.1941).

Penta-O-methyl-(+)-catechin-4',7-digallate (**6**). Non-crystalline, 35 mg (R_f 0.51), mp 100–102°. Found: C, 62.81; H, 5.46%. ($C_{37}H_{38}O_{14}$ requires: C, 62.68; H, 5.42%). The 3-OAc derivative (3 mg), mp 80–83°.

Penta-O-methyl-(+)-catechin-3',7-digallate (**7**). Non-crystalline, 11 mg (R_f 0.45), mp 85–87°. Found: C, 62.86; H, 5.42%. ($C_{37}H_{38}O_{14}$ requires: C, 62.86; H, 5.42%). The 3-OAc derivative (6 mg), mp 79–82°.

Acknowledgements—This investigation was supported by a grant from the Research Committee of the University of Durban-Westville. We thank Prof. D. Ferreira and Dr D. A. Young of the University of the Orange Free State for their invaluable assistance with the NMR spectra. Sections of the wood of *Acacia gerrardii* were kindly collected and identified by Mr C. J. Ward of the Department of Botany at University of Durban-Westville.

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