Phytochemistry, Vol. 24, No. 9, pp. 2093–2095, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

FLAVONOIDS FROM *ELAEODENDRON BALAE* ROOT BARK

GAMINI WEERATUNGA, LARS BOHLIN*, ROBERT VERPOORTE† and VIJAYA KUMAR‡

Department of Pharmacognosy, University of Uppsala, Box 579, S-751 23 Uppsala, Sweden; †Department of Pharmacognosy, State University of Leiden, Gorlaeus Laboratories, Leiden, The Netherlands; ‡Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

(*Received* 31 *October* 1984)

Key Word Index—*Elaeodendron balae*; Celastraceae; flavonoids; proanthocyanidin; leucoanthocyanidin.

Abstract—The root bark of *Elaeodendron balae* afforded ourateacatechin, ouratea-proanthocyanidin A and a new leucoanthocyanidin derivative elaeocyanidin.

INTRODUCTION

Elaeodendron balae is an evergreen tree found in Sri Lanka. Previous work on *E. balae* included the isolation of friedelane derivatives from its stem and root bark [1, 2] and the isolation of the flavonoid 4′-*O*-methyl-(−)-epigallocatechin [3], previously obtained from the *E. glaucum* [4].

The root bark of *E. balae* contained a new leucoanthocyanidin, elaeocyanidin (5,6α,7,12α-tetrahydro-5,5-dimethyl [2] benzo-pyrano[4,3-b] [1] benzopyran-2,3,4,8,10-pentaol-3-methyl ether) (**1**) and the proanthocyanidins, 4′-*O*-methyl-(−)-epigallocatechin (ourateacatechin) (**2**) and ouratea-proanthocyanidin A (**3**).

RESULTS AND DISCUSSION

The ethyl acetate extract of the root bark of *E. balae* was found to contain three compounds which were thought to be flavonoids as they gave a red colouration with vanillin-HCl with sulphuric acid [5].

Compound **1**, $C_{19}H_{20}O_7$, shows a UV λ_{max} at 280 nm which is characteristic of a catechin. Its IR spectrum showed no carbonyl absorptions. The lowfield region of its ^{1}H NMR spectrum showed signals for four phenolic hydroxyl protons, and for three aromatic protons two of which were *meta*-coupled to each other while the other appeared as a singlet. The spectrum also showed three methyl singlets, two due to aliphatic methyl groups and one due to a methoxy group (Table 1).

Acetylation of **1** gave according to its mass spectrum a tetraacetate (**4**) whose IR spectrum showed no hydroxyl absorption, confirming that only four phenolic hydroxyl groups were present. Since the fifth oxygen is accounted for by the methoxy group, there remains two O atoms which should be present as ether linkages.

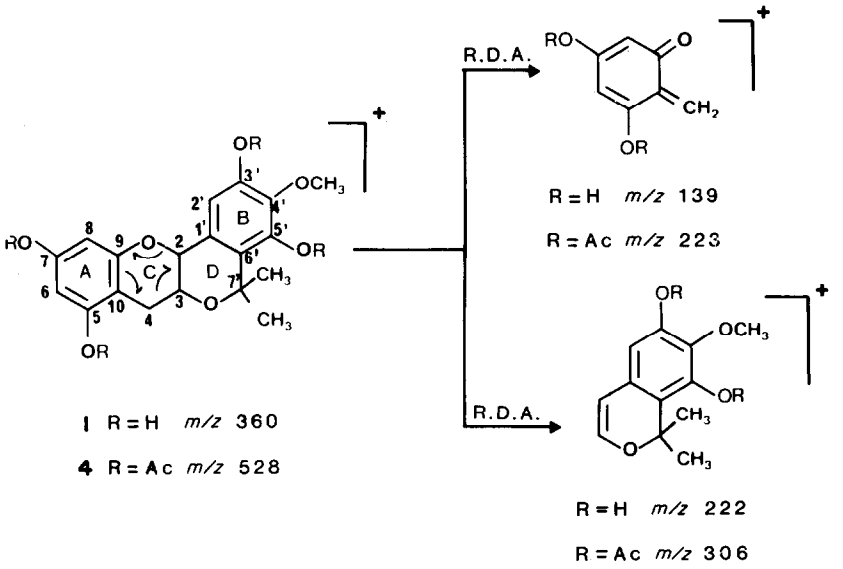
The mass spectrum of **1** showed intense peaks at *m/z* 222 and 139. The formation of these fragment ions could be rationalized in terms of a retro Diels–Alder fission of the chroman heterocyclic nucleus of **1** (Scheme 1). Peaks at *m/z* 345 $[M-Me]^+$ and *m/z* 330 $[M-2Me]^+$ indicated the presence of two methyl groups. A similar fragmentation pattern was observed in the mass spectrum of the acetate (**4**). Thus the acetate with $[M]^+$ at *m/z* 528 gave the corresponding peaks at *m/z* 306, 223 and 291. On the basis of these data structure **1** is proposed, with two phenolic hydroxyl groups in the A ring and two phenolic hydroxyl groups and a methoxy group in the B ring. The two phenolic hydroxyl groups in the A ring could be at C-5 and C-7 or at C-6 and C-8 since the aromatic protons were *meta* coupled. On biogenetic reasoning a C-5, C-7 substitution is preferred.

NOE-difference experiments were used to establish the substitution pattern in ring A and ring B. As no NOE was observed for the δ6.00 or 5.81 (Table 1) doublets upon irradiation at the frequency of the H-4 signals, C-5 must

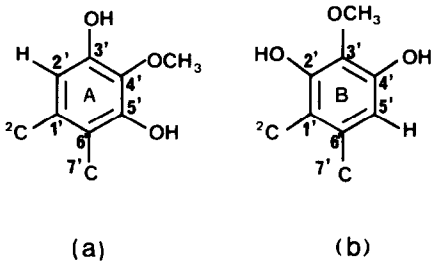
Table 1. ^{1}H NMR data of compounds **1** and **4** (90 MHz, Me_2CO-d_6, δ-values, TMS)

1	**4**	Assignment
8.90 (*s*, 1H)		phenolic
8.16 (*s*, 1H)		hydroxyl
7.94 (*s*, 1H)		groups
7.92 (*s*, 1H)		(5,7,3′,5′)
6.50 (*s*, 1H)	7.20 (*s*, 1H)	H-2′
6.00 (*d*, 1H, $J_{6,8}$ = 2.2 Hz)	6.52 (d, $J_{6,8}$ = 2.2 Hz	H-6/H-8
5.81 (*d*, 1H, $J_{6,8}$ = 2.2 Hz)	6.45 (*d*, 1H, $J_{6,8}$ = 2.2 Hz)	H-6/H-8
4.43 (*s*, 1H)	4.79 (*s*, 1H)	H-2
4.25 (*m*, 1H)	4.41 (*m*, 1H)	H-3
3.82 (*s*, 3H)	3.80 (*s*, 3H)	OMe
2.81 (*m*, 2H)	2.91 (*m*, 2H)	H-4
1.60 (*s*, 3H)	1.54 (*s*, 3H)	7′-Me
1.54 (*s*, 3H)	1.45 (*s*, 3H)	7′-Me
	2.38 (*s*, 3H)	phenolic
	2.35 (*s*, 3H)	acetate
	2.32 (*s*, 3H)	groups
	2.21 (*s*, 3H)	(5,7,3′,5′)

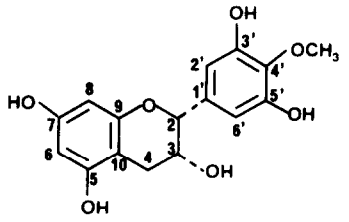
*Author to whom correspondence should be addressed.



Scheme 1. Mass spectral fragmentation of **1** and its corresponding acetate derivative **4**.

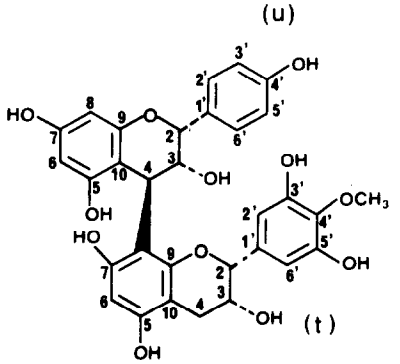


(a) (b)



2

(u)



(t)

3

be substituted and a 5,7-dihydroxy substitution must be present in ring A. Irradiation at the frequency of the *O*-methyl group did not result in any NOE suggesting that the methoxy group should be located between the two hydroxyl groups. This was further supported by the downfield position of the signal of the methoxy group (60.8 ppm) in the ^{13}C NMR spectrum (Table 2). This leaves only two possible substitution patterns for ring B.

A strong NOE was observed for the singlet at δ6.50 upon irradiation at the frequency of H-2, confirming that the aromatic proton appearing at δ6.50 was located close to H-2. The oxygenation pattern of ring B should therefore be as shown in **a**. Irradiation at the frequency of the two methyl signals at C-7′ did not enhance the intensity of any aromatic proton signal, further confirming the 3′,5′-dihydroxy-4′-methoxy substitution for ring B. On this basis structure **1** can be postulated for the leucoanthocyanidin. The proposed structure was further supported by the ^{13}C NMR data (Table 2).

Since no coupling was observed between H-2 and H-3 of **1**, H-2 appeared as a broad singlet (Table 1) and the

Table 2. ^{13}C NMR data of compound **1** (90 MHz, CD_3OD, δ-values, TMS)

C-2	72.4 (*d*)*	C-1′	129.5 (*s*)
C-3	64.4 (*d*)	C-2′	110.1 (*d*)
C-4	30.7 (*t*)	C-3′	149.7 (*s*)
C-5	157.7 (*s*)†	C-4′	137.7 (*s*)
C-6	96.3 (*d*)	C-5′	147.9 (*s*)
C-7	157.4 (*s*)†	C-6′	122.2 (*s*)
C-8	95.7 (*d*)	C-7′	77.0 (*s*)
C-9	156.5 (*s*)†	7′-Me	28.6 (*q*)
C-10	99.8 (*s*)	7′-Me	24.5 (*q*)
OMe	60.8 (*q*)		

*Multiplicity in the off-resonance decoupled spectrum.

†Assignments may be interchanged.

Table 3. ^{13}C NMR chemical shift (δ-values) of compounds **2** and **3** (90 MHz, Me_2CO-d_6, TMS)

	2			3	
C-2	79.8 (*d*)*	C-1'u	131.4 (*s*)	C-5u, 5t	158.3 (*s*), 158.2 (*s*)
C-3	66.8 (*d*)	C-2'u, 6'u	129.0 (*d*)	C-7u, 7t	157.6 (*s*) 157.4 (*s*),
C-4	28.8 (*t*)	C-4'u	154.2 (*s*)	C-9u, 9t	155.8 (*s*), 155.7 (*s*)
C-5, 7, 9	157.4, 156.8 (*s*)	C-3'u, 5'u	115.4 (*d*)	C-2t	79.1 (*d*)
C-6	96.2 (*d*)†	C-2u	76.8 (*d*)	C-3t	66.4 (*d*)
C-8	95.6 (*d*)†	C-3u	72.7 (*d*)	C-4t	30.7 (*t*)
C-10	99.6 (*s*)	C-4u	36.9 (*d*)	C-6t	96.4 (*d*)
C-1'	135.4 (*s*)†	C-6u	97.1 (*d*)	C-8t	107.0 (*s*)
C-4'	136.1 (*s*)†	C-8u	95.9 (*d*)	C-10t	100.8 (*s*)
C-3', 5'	150.8 (*d*)	C-10u	100.6 (*s*)	C-1't	135.2 (*s*)†
C-2', 6'	106.9 (*s*)			C-2't, 6't	106.7 (*d*)
OCH_3	60.5 (*q*)			C-3't, 5't	150.8 (*s*)†
				C-4't	135.8 (*s*)
				OMe	60.5 (*q*)

*Multiplicity in the off-resonance decoupled spectra.
†Assignments may be interchanged.

configuration at 2,3 was concluded to be *cis*. If the stereochemistry at the ring junction was *trans*, a coupling constant $J_{2,3}$ of *ca* 10 Hz is expected as in peltogynol tetraacetate [6].

Flavonoids **2** and **3** were identified as 3,3',5,5',7-pentahydroxy-4'-methoxy-2,3-*cis*-flavane and ouratea-proanthocyanidin A, respectively, by comparison of physical and spectral data of those compounds reported earlier from *Ouratea* species [7], *Prionostemma aspera* and *Maytenus rigida* [8]. The ^{13}C NMR spectra of the flavonoids **2** and **3** have been assigned (Table 3), by using ^{13}C NMR data of similar proanthocyanidin derivatives [9]. The ^{13}C NMR signals at δ76.8 and δ79.1 for C-2u and C-2t respectively in the flavonoid **3** is evidence for the stereochemistry of the heterocyclic rings of both flavonoid units being similar to that of 4'-*O*-methyl-(−)-epigallocatechin (**2**) [3].

EXPERIMENTAL

UV were recorded in EtOH and IR in KBr discs. MS were measured at 70 eV. ^1H NMR and ^{13}C NMR spectra were obtained using TMS as int. standard. Optical rotations were determined in EtOH soln.

Isolation of proanthocyanidin. Root bark of *E. balae* (1 kg), collected by A. J. Kostermans at Yala in the Hambatota district of Sri Lanka was dried, pulverized and extracted by refluxing with EtOAc after initial extraction with petrol and C_6H_6. Concn of the EtOAc extract gave a reddish brown amorphous solid (8 g). The solid was chromatographed on silica gel and eluted with $CHCl_3$ and $CHCl_3$–MeOH.

Elution of the column with $CHCl_3$–MeOH (19:1) afforded compound **1** (20 mg) as a colourless amorphous solid. Further elution of the column with $CHCl_3$–MeOH (9:1) gave the major constituent **2** (5.2 g) which crystallized as needles from H_2O and compound **3** (110 mg) was obtained by elution of the column with $CHCl_3$–MeOH (4:1).

Elaeocyanidin (**1**). Mp 149°; $[\alpha]_D$ +205° (*c* = 1; Me_2CO); UV λ_{max} 280 nm (log ε 5.23); IR ν_{max}^{KBr} cm^{-1}: 3413, 1630 and 813; MS *m/z* (rel. int.): 360 $[M]^+$ (42%), 345 (31), 330 (05), 222 (22), 207 (100), 192 (17) and 139 (29).

Elaeocyanidin tetraacetate (**4**). Mp 101–102°; $[\alpha]_D$ +127° (*c* = 1; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1776, 1624, 1595 and 800; MS *m/z* (rel. int.): 528 $[M]^+$ (68%), 513 (26), 486 (19), 471 (55), 429 (61), 387 (09), 306 (35), 264 (22), 249 (49), 223 (3) and 207 (56).

4'-O-Methyl-(−)-epigallocatechin (**2**). Mp 143–145° (lit. [4] mp 142–144°); $[\alpha]_D$ = −60° (EtOH); UV λ_{max}^{EtOH} 278 nm (log ε 3.2); IR ν_{max}^{KBr} cm^{-1}: 3415, 1630, 1603 and 812 cm^{-1}; MS *m/z* (rel. int.): 320 $[M]^+$ (35%) 302 (8), 182 (65), 167 (50), 163 (25), 153 (20) and 139 (100).

Ouratea-proanthocyanidin A (**3**). Amorphous solid ($[M]^+$ 592.439, $C_{31}H_{28}O_{12}$ requires 592.158); $[\alpha]_D$ +54° (Me_2CO) (lit. [7], $[\alpha]_D$ +54°); IR ν_{max}^{KBr} 3420, 1606, 1571 and 805 cm^{-1}.

Acknowledgements—We are grateful to Professor S. Balasubramaniam, University of Peradeniya, Sri Lanka, for collection and identification of plant material. One of the authors (G.W.) is grateful to the International Seminar in Chemistry, University of Uppsala, Sweden, for financial support.

REFERENCES

1. Weeratunga, G., Kumar, V., Sultanbawa, M. U. S. and Balasubramaniam, S. (1982) *J. Chem. Soc. Perkin Trans* 1, 2457.
2. Weeratunga, G. and Kumar, V. (1984) *Aust. J. Chem.* (submitted).
3. Weeratunga, G., Bohlin, L., Sandberg, F. and Kumar, V. (1984) *Acta Pharm. Suec.* **21**, 73.
4. Anjaneyulu, A. S. R. and Rao, M. N. (1979) *Indian J. Chem.* **18B**, 292.
5. Venkataraman, K. (1962) *The Chemistry of Flavonoid Compounds* (Geissman, T. A., ed.), p. 72. Pergamon Press, Oxford.
6. Haslam, E. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T., eds), p. 431. Chapman & Hall, London.
7. Delle Monache, F., d'Albuquerque, L., Ferrari, F. and Marini-Bettolo, G. B. A. (1967) *Tetrahedron Letters* 4211, 4214.
8. Delle Monache, F., Pompani, M., Marini-Bettolo, G. B. A., d'Albuquerque, L. and Goncalves de Lima, O. (1976) *Phytochemistry* **15**, 573.
9. Markham, K. R. and Mohanchari, V. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T., eds), p. 19. Chapman & Hall, London.