Pergamon

PII: S0031-9422(97)00195-7

Phytochemistry, Vol. 45, No. 8, pp. 1673–1678, 1997

0031-9422/97 $17.00+0.00

PHLOROGLUCINOL DERIVATIVES FROM LEAVES OF *BOSISTOA PENTACOCCA*

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(*Received in revised form* 29 *January* 1997)

Key Word Index—*Bosistoa pentacocca*; *B. pentacocca* var. *connaricarpa*; *B. pentacocca* var. *dryanderensis*; Rutaceae; leaves; phloroglucinol derivatives; pentacoccols.

Abstract—Leaves from four collections of *Bosistoa pentacocca*, two of the variety *connaricarpa* and one each of the type variety and var. *dryanderensis*, have been examined. In each, the major metabolite isolated was characterized as 2-(3,7-dimethyloct-2,5-dienyl)-6-(3-methylbut-2-enyl)phloroglucinol-1-acetate (trivial name pentacoccol). Four other pentacoccol derivatives were obtained as minor components, pentacoccol-5-methyl ether (from var. *pentacocca* and var. *dryanderensis*), 2,2-dimethyl-3ξ,7-dihydroxy-5-acetoxy-6-(3,7-dimethyloct-2,5-dienyl)-3,4-dihydrobenzo-[2*H*]-pyran (3′-hydroxydihydropyranopentacoccol from var. *connaricarpa*), 2-(1-hydroxy-1-methylethyl)-4-acetoxy-5-(3,7-dimethyloct-2,5-dienyl)-6-hydroxybenzo-[2*H*]-furan (2′-(1-hydroxyosopropyl)furanopentacoccol from var. *pentacocca*) and 4-acetoxy-5-(3,7-dimethyloct-2,5-dienyl)-6-hydroxybenzo-[2*H*]-furan (furanopentacoccol from var. *dryanderensis*). All compounds were identified on the basis of their spectroscopic data.

INTRODUCTION

The genus *Bosistoa* F. Muell. ex Benth., as currently delineated by Hartley [1], consists of seven species of small trees found in the rain forests of New South Wales and Queensland. In a series of studies, we have reported on flavonoids from *B. brassii* Hartley [2] and *B. medicinalis* (F. M. Bailey) Hartley [3], a xanthine alkaloid from *B. floydii* Hartley [4] and acridone alkaloids from *B. transversa* [5] and *B. selwynii* Hartley [6].

In the present work, we wish to report the results of a study on the leaves of *B. pentacocca* (F. Muell.) Baillon. Hartley [1] recognized three varieties of this species: var. *pentacocca* occurring in south-east Queensland and north-east New South Wales, var. *connaricarpa* (Domin) Hartley occurring in east central Queensland from Bowen south to Gin-Gin, and var. *dryanderensis* Hartley also occurring in east central Queensland around Mount Dryander and MacKay. A previous investigation on *B. pentacocca*, reported under the illegitimate name *B. sapindiformis*, yielded the pentacyclic triterpenes, taraxerol and taraxerol methyl ether [7]. The only other phytochemical report citing the genus was for *B. euodiiformis*, which yielded acetophenones, furoquinoline alkaloids, a limonoid and the triterpene, bosistoin [8]. *Bosistoa euodiiformis* is now considered [1] to be a species of *Acradenia* rather than *Bosistoa*.

RESULTS AND DISCUSSION

The major compound isolated from each of the four samples gave an ion at *m/z* 395 $[M+Na]^+$ in FAB mass spectra, which corresponded with an empirical formula, $C_{23}H_{32}O_4$. Two major fragments in the HREI mass spectra indicated the loss of C_4H_7 and C_9H_{15} fragments, which suggested the presence of C-prenyl and C-geranyl units, while a major ion at *m/z* 43 was typical of an acetoxyl substituent.

The ^1H NMR spectra (Table 1) confirmed the presence of an acetyl methyl and of 3-methylbut-2-enyl and geranyl moieties. This accounted for all but three protons, which were observed as an aromatic proton singlet at δ 6.36 and two aromatic phenols at δ 8.08 (*br*). The ^{13}C NMR spectrum (Table 1) revealed signals typical of prenyl and geranyl side-chains and the acetoxy, leaving six further carbons, the single aromatic methine (δ 101.3), two equivalent shielded quaternary carbons (δ 113.1) and three deshielded oxygenated aromatics (δ 150.4 and 154.7—double inten-

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Table 1. ^1H and ^{13}C NMR chemical shift values for compounds **1** and **2**

	^1H		^{13}C	
C:H	**1**	**2**	**1**	**2**
1			150.4	148.2
2			113.1	111.7*
3 (OH)	8.08 *s*		154.7	156.9
4	6.38 *s*	6.30 *s*	101.3	98.1
5 (OH)	8.08 *s*		154.7	154.1
6			113.1	114.9*
1-OCOMe	2.25 *s*	2.29 *s*	169.3, 20.8	169.5, 20.7
5-OMe		3.74 *s*		55.8
1′	3.08 *d* (6.6)	3.17 *d* (6.7)	23.8*	23.8
2′	5.12 *t* (6.6)	5.20 *t* (6.7)	124.3	122.0
3′			130.7	134.4
3′-Me	1.71 *s*	1.77 *s*	25.9	25.8
3′-Me	1.63 *s*	1.72*s*	17.8	17.9
1″	3.08 *d* (6.6)	3.15 *d* (6.8)	23.9*	23.2
2″	5.12 *t* (6.6)	5.08 *t* (6.8)	124.3	122.8
3″			134.5	134.8
3″-Me	1.72 *s*	1.72 *s*	16.3	16.2
4″	1.94 *m*	1.95 *m*	40.3	39.8
5″	2.08 *m*	2.06 *m*	27.4	26.8
6″	5.12 *t* (6.6)	5.08 *t* (6.7)	125.3	124.5
7″			131.7	131.3
7″-Me	1.63 *s*	1.63 *s*	25.9	25.8
	1.57 *s*	1.57 *s*	17.8	17.8

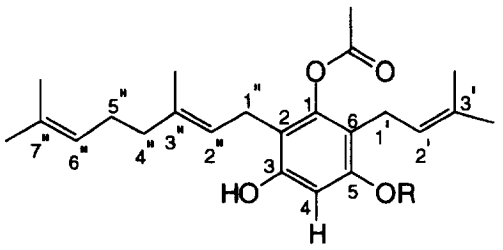
1 in acetone-d_6, **2** in $CDCl_3$.
* Signals interchangeable.

sity). This required the presence of a systematically trioxygenated ring system (e.g. phloroglucinol), in which two of the non-oxygenated carbons were substituted by the 3-methylbut-2-enyl and geranyl groups; one of the oxygenated positions was acetylated.

The positions of the various substituents were established by means of long-range heteronuclear coupling (HMBC) experiments [9] on the basis of the following observations. The single aromatic proton revealed two interactions, with the two equivalent resonances at δ 154.7 (^2J) and with the second equivalent pair at δ 113.1 (^3J). From the two phenolic hydroxyl protons, ^2J interactions were revealed to the carbons resonating at δ 154.7, while there was an intense ^3J interaction to the signals at δ 101.3 and 113.1. These observations required the compound to have the symmetrical structure **1**. Unequivocal assignment of the carbon resonances (Table 1) was confirmed from the full analysis of HMBC and HC-COBI [10] experiments. Compound **1** appears to be novel and has been assigned the trivial name, pentacoccol.

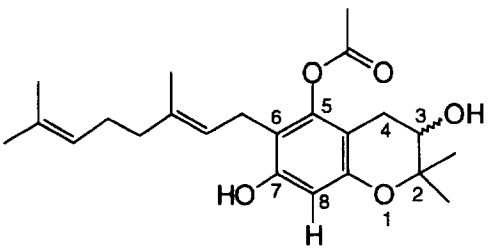
A very similar compound differing only in the methylation of one of the aromatic hydroxyls ($C_{24}H_{34}O_4$ from HREI mass spectrum, δ 3.74 (3H), 55.7) was obtained from *B. pentacocca* var. *pentacocca* and *B. pentacocca* var. *dryanderensis*. The ^{13}C NMR spectrum (Table 1) indicated the phloroglucinol oxygenation pattern and, together with the ^1H NMR spectrum, that the same prenyl and geranyl side-chains were present. The relatively shielded resonance for the methyoxyl carbon at *ca* δ 56 (cf. 60–62 ppm where both *ortho*-positions are substituted) required placement adjacent to the aromatic proton [11]. Assignment to C-5, adjacent to the prenyl substituent, was resolved from a HMBC experiment, where both H-1′ of the prenyl side-chain and the OMe protons showed a ^3J coupling to the carbon at δ 156.9. On this basis, the compound could be characterized as pentacoccol-5-methyl ether (**2**), which is again novel.

Three further phloroglucinol derivatives were obtained as minor components, each based on cyclization of the prenyl side-chain with the adjacent hydroxyl. Both samples of *B. pentacocca* var. *connaricarpa* yielded a compound which analysed (HREI mass spectrum) for $C_{23}H_{32}O_5$. The NMR spectra (Table 2) revealed the presence of the phloroglucinol system with the acetoxyl and geranyl substituents but the signals for the prenyl unit were not present, being replaced, in the ^1H NMR spectrum, by a CH_2-CH(O)- spin system and two methyls and, in the ^{13}C NMR spectrum, by a methylene, an oxymethine, an oxygenated quaternary sp^3 carbon and two methyls. These resonances could be assigned to a 3-hydroxy-2,2-dimethyldihydropyran system which could be formed by cyclization of a 2,3-dihydroxy-3-methylbutanyl side-chain and the 5-OH with the loss of the elements

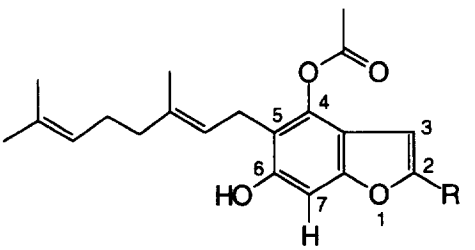


1 R = H

2 R = Me



3



4 R = C(OH)Me$_2$

5 R = H

of water. This was confirmed by an HMBC experiment (Table 2). Thus, this compound is identified as 5-acetoxy-3,4-dihydro-3ξ,7-dihydroxy-6-(3,7-dimethyloct-2,5-dienyl)-2,2-dimethyl-[2H]-benzopyran (**3**), to which we assign the trivial name, 3′ξ-hydroxy-dihydropyranopentacoccol.

A minor isolate from *B. pentacocca* var. *pentacocca* gave a $[M]^+$ for $C_{23}H_{30}O_5$ (HREI mass spectrum). In this case, the signals originating from the modified prenyl group were an aryl methine at δ 6.87 showing a small coupling (0.8 Hz) to the phloroglucinol proton (cf. H-4 in **1**) and geminal methyls resonating at δ 1.64. The ^{13}C NMR spectrum and a HC-COBI experiment revealed that the methine carbon resonated at δ 95.7, the methyls at δ 28.8. An HMBC experiment showed that the methine and methyl protons coupled with quaternary carbons at δ 69.4 and 162.0, which must be oxygen-bonded sp^3 and sp^2 carbons, respectively. There are two plausible structures that could fit these data, a 3-hydroxy-2,2-dimethylpyran or a 2-(1-hydroxyisopropyl)furan. The resonance positions observed were very similar to those noted previously for the acetophenone, furostipitol [12]. In particular, the resonance for the sp^3 quaternary carbon (δ 69.4) was typical of the 1-hydroxyisopropyl group, whereas, in the comparable C-2′ position of benzopyrans, this occurs at *ca* δ 78 (cf. **3**). On this basis, the compound is identified as 2-(1-hydroxy-1-methylethyl)-4-acetoxy-6-hydroxybenzofuran, to which we have assigned the trivial name 2′-(1-hydroxyisopropyl) furanopentacoccol (**4**).

From *B. pentacocca* var. *dryanderensis* another minor compound was obtained which analysed (HREI mass spectrum) for $C_{20}H_{24}O_4$. The ^1H NMR spectrum once again identified the phloroglucinol ring, and geranyl and acetoxyl substituents. The remaining two methines formed a simple spin system (J = 2.2 Hz) typical of a furan ring. Insufficient material was available to obtain a satisfactory ^{13}C NMR spectrum but this compound must be a simple derivative furanopentacoccol (**5**).

A search of the Dictionary of Natural Products [13] failed to show any compounds directly analogous to the pentacoccols. Within the Rutaceae, a number of acetophenones with a phloroglucinol oxygenation pattern substituted with prenyl, geranyl and farnesyl substituents are known, many of which exhibit similarly modified and cyclized side-chains [14]. In *B. pentacocca*, it seems that the phloroglucinol system has been biosynthesized from the combination of three acetate units, whereas, in the acetophenones, four acetate units are involved. The differences in pro-

Table 2. ^1H and ^{13}C NMR chemical shift values for compound **3**

	^1H	^{13}C	^2J	^3J
2		77.1		
2-Me	1.34 *s*	25.9	77.1	25.9, 69.5, 152.3
	1.29 *s*	25.9	77.1	25.9, 69.5, 152.3
3	3.75 *t* (5.2)	69.5		105.5
4	2.50 *br d*	26.8		
	2.70 *br d*			
4a		105.5		
5		148.5		
6		112.9		
7		154.8		
8	6.25 *s*	102.9	152.3, 154.8	105.5, 112.9
8a		152.3		
OCOMe	2.31 *s*	169.0, 20.7		
1″	3.17 *d* (6.7)	23.6	112.9, 121.7	138.5, 148.5, 154.8
2″	5.17 *t* (6.7)	121.7	23.6	16.3, 39.9
3″		138.5		
3″-Me	1.81 *s*	16.3	138.5	39.9, 121.7
4″	2.03 *m*	39.9	26.6, 138.5	16.3, 121.7
5″	2.08 *m*	26.6	39.9, 124.0	132.2
6″	5.05 *t* (6.8)	124.0		
7″		132.2		
7″-Me	1.67 *s*	25.9	132.2	17.9, 124.0
	1.59 *s*	17.9	132.2	25.9, 124.0

Spectra in $CDCl_3$.

duction of minor components are not considered to have taxonomic significance; rather, the codominance of pentacoccol is considered to support the close affinity between these taxa proposed by Hartley [1].

EXPERIMENTAL

Extractions were carried out using a Soxhlet apparatus using, sequentially, petrol (bp 60–80°), EtOAc and MeOH as solvents. UV: MeOH. IR: solvent-free film. HREIMS: 70 eV, FABMS: NOBA-matrix. NMR spectra were obtained on a Bruker AMX-400 instrument using standard Bruker microprograms for 2D expts [15].

Plant material. Bosistoa pentacocca var. *pentacocca*: voucher: T. G. Hartley 15170 collected 22 October 1991 on Mount French, Moreton Bay District, Queensland, *B. pentacocca* var. *connaricarpa*: voucher: T. G. Hartley 13201 collected 11 April 1993, State of Forest 50, Glenbar, Wide Bay District, Queensland, voucher: P. Vorster 9398 collected 18 January 1992, Mount Colosseum National Park, Port Curtis District, Queensland, and *B. pentacocca* var. *dryanderensis*: voucher P. Vorster 9411 collected 19 January 1992, Mount Dryander, North Kennedy District, Queensland.

Extraction of B. pentacocca *var.* pentacocca. Dried, milled leaves (230 g) yielded a concentrate (2.5 g) from the petrol extract. CC of this material over Sephadex LH-20, eluting with $CHCl_3$ removed the chlorophylls and yielded a fr. containing **1** as an amorphous solid (1 g). The EtOAc concentrate (5 g) was treated in a similar manner, eluting the Sephadex LH-20 with 10% MeOH in $CHCl_3$ which yielded, in sequence of elution, sitosterol (30 mg), **2** and **5**. Prep. TLC of **2** and **5** (silica gel; petrol–EtOAc, 9:1) yielded pure **2** (100 mg, R_f 0.67) and **4** (4 mg, R_f 0.14).

Extraction of B. pentacocca *var.* connaricarpa (*Hartley* 13201). Dried, milled leaves (290 g) yielded a petrol concentrate (6 g) and an EtOAc concentrate (10 g). These were subjected to identical treatment to *B. pentacocca* var. *pentacocca* to yield, from the petrol extract, **1** (425 mg), and from the EtOAc extract, sitosterol (20 mg) and **3** (3.5 mg, R_f 0.45, petrol–EtOAc, 1:1).

Extraction of B. pentacocca *var.* connaricarpa (*Vorster* 9398). Dried, milled leaves (185 g) yielded a petrol concentrate (2 g) and an EtOAc concentrate (5 g). These were subjected to identical treatment noted above, to yield, from the petrol extract, **1** (1.2 g), and from the EtOAc extract, sitosterol (30 mg) and **3** (5 mg).

Extraction of B. pentacocca *var.* dryanderensis. Dried, milled leaves (400 g) yielded a petrol concentrate (15 g) and an EtOAc concentrate (10 g). These were subjected to identical treatment to that noted above, to yield, from the petrol extract, **1** (1.5 g), and from the EtOAc extract, sitosterol (30 mg), **2** (100 mg) and **5** (4 mg, R_f 0.74 in petrol–EtOAc, 9:1).

Pentacoccol (**1**). Amorphous solid. FABMS: *m/z*

Table 3. ^1H and ^{13}C NMR chemical shift data and long-range H-C coupling for compounds **4**, and ^1H NMR data for **5**

H	^1H	^{13}C	^2J	^3J	**5**
2		162.0			7.47 *d* (2.2)
3	6.87 *d* (0.8)	95.9**	115.9, 162.0	141.1, 154.4	6.52 *br d* (2.2)
3a		115.9			
4		141.1			
5		115.7*			
6		153.3			
7	6.32 *d* (0.8)	95.7**	153.3, 154.4	115.7, 115.9	6.96 *br s*
7a		154.4			
OCOMe	2.42 *s*	169.1, 20.9		169.1	2.40 *s*
1″		69.4			
1″-Me	1.64 *s*	28.8	69.4	28.8, 162.0	
	1.64 *s*	28.8	69.4	28.8, 162.0	
1″	3.30 *d* (6.8)	23.7	115.7, 121.5	138.7, 141.1, 153.3	3.36 *d* (6.6)
2″	5.19 *t* (6.8)	121.5			5.21 *t* (6.6)
3″		138.7			
3″-Me	1.83 *s*	16.4	138.7	39.8, 121.5	1.80 *s*
4″	2.10 *m*	39.8	26.6, 138.7	16.4, 123.9	2.06 *m*
5″	2.16 *m*	26.6	39.8, 123.9	132.2	2.12 *m*
6″	5.03 *t* (6.6)	123.9			5.05 *t* (7.0)
7″		132.2			
7″-Me	1.72 *s*	25.8	132.2	17.9, 123.9	1.68 *s*
	1.59 *s*	17.9	132.2	25.8, 123.9	1.60 *s*

Spectra in $CDCl_3$.

*/** Signals with same number of * are interchangeable.

395 $[M+Na]^+$. UV λ_{max} nm (log ε): 208 (4.18), 225 (3.99), 273 (3.63). IR ν_{max} cm^{-1}: 3436, 2924, 1744, 1633, 1598, 1466, 1370, 1199, 1050. NMR: Table 1. EIMS *m/z* (rel. int.): 249 (35), 207 (70), 151 (52), 123 (100).

Pentacoccol-5-methyl ether (**2**). Oil. FABMS: *m/z* 409 $[M+Na]^+$. HREIMS: found $[M]^-$ *m/z* 386.2485; $C_{24}H_{34}O_4$ requires 386.2458. UV λ_{max} nm (log ε): 210 (4.28), 226 (4.05), 274 (3.70). IR ν_{max} cm^{-1}: 3436, 2916, 1735, 1620, 1487, 1433, 1368, 1208, 1164, 1001. NMR: Table 1. EIMS *m/z* (rel. int.): 363 (33), 247 (12), 221 (48), 219 (100), 166 (8), 123 (43).

3′-Hydroxydihydropyranopentacoccol (**3**). Oil. $[\alpha]_D$ −7.5 (*c* 0.002, $CHCl_3$). HREIMS: found $[M]^+$ *m/z* 388.2196; $C_{23}H_{32}O_5$ requires 388.2250. UV λ_{max} nm (log ε): 209 (4.47), 225 (4.17), 285 (3.87). IR ν_{max} cm^{-1}: 3393, 2921, 1738, 1627, 1599, 1447, 1369, 1201, 1117, 1063. NMR: Table 2. EIMS *m/z* (rel. int.): 388 (56), 265 (63), 223 (100).

2′-(1-Hydroxyisopropyl)furanopentacoccol (**4**). Oil. HREIMS: found $[M]^+$ *m/z* 386.2057; $C_{23}H_{30}O_5$ requires 386.2094. UV λ_{max} nm (log ε): 210 (4.32), 249 (3.95), 295 (3.81). IR ν_{max} cm^{-1}: 3360, 2924, 1742, 1633, 1467, 1369, 1208, 1163, 1105, 1074. NMR: Table 3. EIMS *m/z* (rel. int.): 386 (40), 344 (39), 327 (27), 221 (100), 123 (31).

Furanopentacoccol (**5**). Oil. HREIMS: found $[M]^-$ *m/z* 328.1665; $C_{20}H_{24}O_4$ requires 328.1675. UV λ_{max} nm (log ε): 213 (4.47), 228 (4.10), 273 (3.93). IR ν_{max} cm^{-1}: 3452, 2919, 1735, 1623, 1469, 1445, 1370, 1164, 1049, 1043. NMR: Table 3. EIMS *m/z* (rel. int.): 328 (86), 286 (84), 163 (100).

Acknowledgements—A. A. wishes to thank the Libyan Government for the award of a scholarship. The Carnegie Trust for the Universities of Scotland and the Royal Society are thanked for their support to P. G. W. NMR studies were performed in the Strathclyde University NMR Laboratory.

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