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TWO DIBENZOFURAN DERIVATIVES FROM FRUITS OF *RHODOMYRTUS MACROCARPA*

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Key Word Index—*Rhodomyrtus macrocarpa*; Myrtaceae; Finger Cherry; fruits; dibenzofurans; rhodomyrtoxins; ^{13}C NMR.

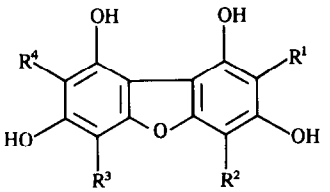
Abstract—Two new isomers of the natural dibenzofuran derivative, rhodomyrtoxin, have been isolated from *Rhodomyrtus macrocarpa* fruits and their structures determined by spectroscopic methods. A third compound, possibly ψ rhodomyrtoxin was isolated in smaller quantities.

INTRODUCTION

The Australian Finger Cherry, *Rhodomyrtus macrocarpa*, which occurs in tropical areas of Australia and New Guinea, has been suspected of causing permanent blindness and of poisoning livestock, though much of the evidence is conflicting [1]. The fruits were first examined chemically by Trippett [2] who isolated from unripe fruits a dibenzofuran derivative which he named rhodomyrtoxin. Subsequently, Anderson *et al.* [3] also examined extracts from unripe fruits and isolated and characterised an isomer of rhodomyrtoxin which they termed ψ rhodomyrtoxin (**1**). The latter authors proposed two possible structures for rhodomyrtoxin (**2** or **3**) and the structure **4** for the semi-synthetic isomer isorhodomyrtoxin. More recently, this has been challenged by Sargent *et al.* [4] who confirmed the structure of ψ rhodomyrtoxin but proposed structure **4** for rhodomyrtoxin and structure **3** for isorhodomyrtoxin. In the present work we have examined extracts of unripe fruits of *R. macrocarpa* and report the isolation of two new isomers of rhodomyrtoxin which we term rhodomyrtoxins B (**5**) and C (**6**) together with lesser amounts of a compound which may be ψ rhodomyrtoxin.

RESULTS AND DISCUSSION

Thin layer chromatography of the petrol (40–60°) soluble fraction from acetone extracts of *R. macrocarpa* fruits on silica gel G developed with 1,2-dichloroethane gave three yellow spots R_f 0.55, 0.48 and 0.03. Repeated column chromatography of the extract on silica gel eluted with 1,2-dichloroethane and dichloromethane–methanol mixtures afforded three crystalline solids with R_f values corresponding to these spots.



1 $R^1 = R^4 = Me$, $R^2 = CO \cdot CH\,Me \cdot C_2H_5$, $R^3 = CO \cdot CH_2 \cdot CHMe_2$
2 $R^2 = R^4 = Me$, $R^1 = R^3 = CO \cdot CH_2CHMe_2$
3 $R^2 = R^3 = Me$, $R^1 = R^4 = CO \cdot CH_2CHMe_2$
4 $R^1 = R^4 = Me$, $R^2 = R^3 = CO \cdot CH_2CHMe_2$
5 $R^2 = R^3 = Me$, $R^1 = R^4 = CO \cdot CH\,Me \cdot C_2H_5$
6 $R^2 = R^3 = Me$, $R^1 = CO \cdot CH\,Me \cdot C_2H_5$, $R^4 = CO \cdot CH_2 \cdot CHMe_2$

Mass spectrometry showed all three to have $[M]^+$ *m/z* 428, and this was also the spectrum base peak. All three compounds had similar fragmentation patterns. The UV spectrum of the compound of R_f 0.03 (λ_{max} 277 nm, 312 nm), was similar to that reported by Anderson *et al.* [3] for ψ rhodomyrtoxin (**1**) whereas the spectra of the other two (λ_{max} 234 nm, 295 nm, 307 nm and 395 nm) were similar to that reported by Trippett [2] for rhodomyrtoxin.

The ^{1}H NMR spectrum for the compound with R_f 0.48, which we term rhodomyrtoxin C (Table 1), was superficially similar to that of ψ rhodomyrtoxin reported by Anderson *et al.* [3]. A three proton triplet at δ 0.95 (J = 7.0 Hz), a three proton doublet at δ 1.22 (J = 6.8 Hz) and a one proton multiplet centred near δ 4.0 suggested the presence of a 2-methylbutanoyl moiety whilst doublets at δ 1.00 (6H, J = 6.5 Hz) and δ 3.10 (2H, J = 7.0 Hz) supported a 3-methylbutanoyl residue as in ψ rhodomyrtoxin. However, a major difference was the absence of any hydroxyl signal near δ 4, the only hydroxyl protons being at δ 11.9 (2H singlet) and δ 12.2–13.5. Anderson *et al.* referred peaks in this region to chelated hydroxyl protons, this assignment being supported by the work of Crombie *et al.* [5] on coumarins. Thus ^{1}H NMR suggested structure **6** in which all four hydroxyl protons may form internal hydrogen bonds with the oxo groups. Though not previously reported as a natural product a compound of this structure has recently been synthesized by Sargent *et al.* [4]. Physical data reported by them is consistent with that reported here.

In the ^{1}H NMR spectrum for the compound of R_f 0.55, which we term rhodomyrtoxin B (Table 1), there were no signals attributable to the 3-methylbutanoyl moiety but otherwise it was similar to that of rhodomyrtoxin C, suggesting the symmetrical structure **5**. These observations were supported by the ^{13}C NMR data in Table 2.

Table 1. ^{1}H NMR* chemical shifts for dibenzofurans isolated from *Rhodomyrtus macrocarpa*

Proton(s)	Rhodomyrtoxin B† (5)	Rhodomyrtoxin C‡ (6)	Possible ψ rhodomyrtoxin‡§ (1)
Ar–Me	2.29 (6H, *s*)	2.28 (6H, *s*)	1.98 (*s*)
2′	3.97 (2H, *m*)	3.97 (1H, *m*)	3.46 (*m*)
3′	1.3–2.1 (*br m*)	1.3–2.1 (*br m*)	
4′	0.95 (6H, *t*)	0.95 (*t*)	0.88 (*t*)
5′	1.22 (6H, *d*)	1.22 (*d*)	1.22 (*d*)
2″		3.10 (2H, *d*)	3.16 (*d*)
3″		1.3–2.1 (*m*)	
4″, 5″		1.0 (*d*)	1.0 (*d*)
–OH (1 + 9)	11.8 (2H, *s*)	11.9 (2H, *s*)	4.0–5.4 (*br s*)
–OH (3 + 7)	12.0–13.0 (2H, *br s*)	12.2–13.5 (*v.br s*)	14.10 (*s*), 14.33 (*s*)

*100 MHz, standard TMS.
†Solvent $CDCl_3$–DMSO-d_6 (10:1).
‡Solvent DMSO-d_6.
§Accurate integration of peaks was not obtained with this compound.

Table 2. ^{13}C NMR *chemical shifts (δ) for dibenzofurans isolated from *Rhodomyrtus macrocarpa*

Carbon Atom	Rhodomyrtoxin B† (5)	Rhodomyrtoxin C‡ (6)	Possible ψ rhodomyrtoxin‡ (1)
Ar–Me	8.1 *q*§	8.1 *q*	7.8
1, 3, 4a, 6a, 7, 9	159.6 *s*, 158.8 *s*, 152.2 *s*	159.6 *s*, 158.8 *s*, 152.2 *s*	163.4, 162.7, 152.4
1a, 2, 4, 6, 8, 9a	106.4 *s*, 104.0 *s*, 100.7 *s*	106.7 *s*, 106.4 *s*, 104.0 *s*, 100.7 *s*	108.1, 105.2, 99.3, 98.5
1′	211 *s*	212.1 *s*	203.8 or 199.1
2′	45.2 *d*	46.0 *d*	49.6 or 62.6
3′	26.3 *t*	26.9 *t*	26.2
4′	16.4 *q*	16.5 *q*	18.0
5′	11.7 *q*	11.9 *q*	11.5
1″		207.3 *s*	203.8 or 199.1
2″		53.0 *t*	49.6 or 62.6
3″		24.9 *d*	25.1
4″, 5″		22.9 *q*	22.6

*22.63 MHz.
†Solvent $CDCl_3$–DMSO-d_6 (10:1).
‡Solvent DMSO-d_6.
§Multiplicity obtained by off-resonance technique.

The compound of R_f 0.03 was obtained in an insufficiently pure state for unambiguous NMR data to be obtained. However, the ^{1}H NMR spectrum showed the presence of both 2-methylbutanoyl and 3-methylbutanoyl residues and a broad hydroxyl band at δ 4.0–5.3. In addition the UV spectrum of this compound was similar

to that of ψ rhodomyrtoxin and unlike that of rhodomyrtoxins B and C. Thus this compound may be identical to the ψ rhodomyrtoxin (**1**) of Anderson *et al.*

EXPERIMENTAL

Unripe fruits of *R. macrocarpa* Benth. collected in the wet season near Townsville, Queensland, Australia by Dr. W. Griffin, University of Queensland (voucher specimens, Herbarium Australiensis WTJ 2287, bulk sample 7229), were extracted by the method of ref. [2]. Separation of the crude extract was carried out by CC on silica gel (100–200 μm) eluted with $CHCl_3$ and $CHCl_3$–MeOH (4 : 1). The $CHCl_3$ fraction was further separated by CC on silica gel H (TLC grade) and eluted with 1,2-dichloroethane to yield pure samples of rhodomyrtoxin B (**5**) and rhodomyrtoxin C (**6**). The $CHCl_3$–MeOH fraction was subjected to repeated CC on silica gel H and eluted with CH_2Cl_2 and CH_2Cl_2–MeOH mixtures to yield a small quantity of a compound which may be ψ rhodomyrtoxin (**1**).

Rhodomyrtoxin B (1,3,7,9-*tetrahydroxy*-4,6-*dimethyl*-2,8-*di*(2-*methylbutanoyl*)*dibenzofuran*, **5**). Yellow crystals, mp (uncorr.) 178–179°. EIMS 70 eV, *m/z* (rel. int.) 428 $[M]^+$ (100), 410 $[M-H_2O]^+$ (20) supported by m* at 392.8, 371 $[M-C_4H_9]^+$ (88) supported by m* at 321.6, 353 $[M-C_4H_9-H_2O]^+$ (55) supported by m* at 335.9, 314 $[M-C_4H_9-C_4H_9]^+$ (8). UV λ_{max}^{EtOH} nm (log ε): 234 (4.38), 295 (4.56), 307 (4.47), 395 (3.62). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650 (free –OH), 3300 (chelated –OH), 1610 (chelated C=O). ^1H NMR signals are given in Table 1 and ^{13}C NMR signals in Table 2.

Rhodomyrtoxin C (1,3,7,9-*tetrahydroxy*-4,6-*dimethyl*-2-(2-*methylbutanoyl*)-8(3-*methylbutanoyl*)*dibenzofuran*, **6**). Yellow needles from CH_2Cl_2–petrol (40–60°), mp (uncorr.) 200–201°. EIMS 70 eV, *m/z* (rel. int.) 428 $[M]^+$ (100), 410 (22), 371 (92), 353 (35), 314 (**5**). Assignment of fragments as for rhodomyrtoxin B. UV λ_{max}^{EtOH} and IR as for rhodomyrtoxin B. ^1H NMR (Table 1) and ^{13}C NMR (Table 2).

Polar component. Possibly ψ rhodomyrtoxin (1,3,7,9-tetrahydroxy-2,8-dimethyl-4-(2-methylbutanoyl)-6-(3-methylbutanoyl)dibenzofuran) (**1**). Yellow solid. EIMS 70 eV, *m/z* (rel. int.) 428 $[M]^+$ (100), 410 (12), 371 (96), 353 (50), 314, (6). Assignment of fragments as for rhodomyrotoxin B. UV λ_{max}^{EtOH} nm (log ε): 277 (4.47), 312 (4.39). IR as for rhodomyrtoxin B. ^1H NMR: Table 1. ^{13}C NMR: Table 2.

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