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OXYGENATED FURANOSESTERTERPENE TETRONIC ACIDS FROM A SPONGE OF THE GENUS *IRCINIA*

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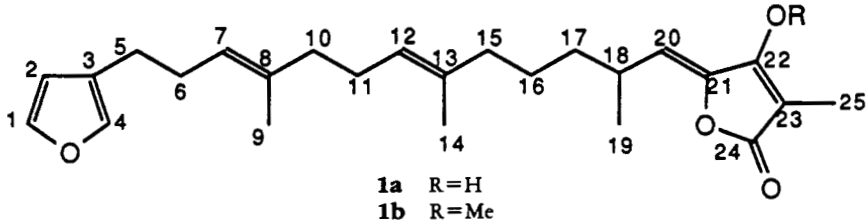
Sponges of the order Dictyoceratida have been a rich source of new sesterterpenes, many of which contain both furan and tetronic acid functional groups (1–3). The prototype of this class of compound, variabilin [**1a**] (4), is a major component in all New Zealand collections of sponges of the genera *Ircinia*, *Psammocinia*, and *Sarcotragus* (5). In one of the *Ircinia* species (*Ircinia* sp. B, family Thorectidae, order Dictyoceratida) the presence of further tetronic acids was noticed (5). Four new furanosesterterpenes oxygenated at C-5 have now been isolated from this species.

An MeOH/toluene extract of the sponge was partitioned between EtOAc and H_2O. Si gel chromatography of the EtOAc partition yielded three sesterterpene-containing fractions. The least polar of these contained variabilin [**1a**] [identified by its uv, ir, ^{1}H-nmr ^{13}C-nmr, and mass spectra (4,6)]. The next fraction contained a mixture of keto sesterterpenes **2a**, **3a**, and **4a** in the ratio 4:2:1, while the most polar fraction contained the hydroxy sesterterpenes **5a** and **6a**.

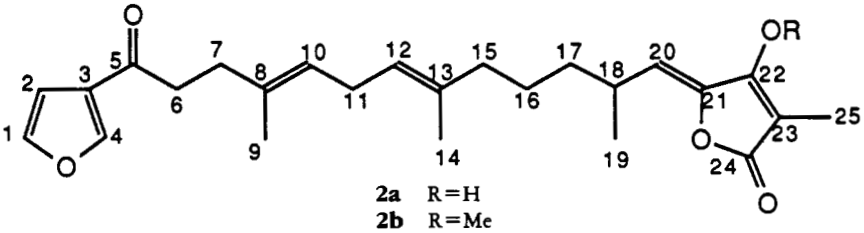
The keto sesterterpenes **2a** and **3a** could not be separated by reversed-phase hplc, and normal phase hplc only partially separated the two minor components **3a** and **4a** from the major component **2a**. A further problem was that the mixture underwent partial decomposition on storage. After a purification step to eliminate the decomposition products, the remaining keto sesterterpene mixture was methylated with CH_2N_2 to decrease the overall molecular polarity and, thereby, enhance the effect of differences in nonpolar regions of these compounds during normal phase chromatography. Separation of these methylated derivatives was achieved using hplc on silica.

The major component **2b** contained the same methylated tetronic acid group as 22-*O*-methylvariabilin [**1b**] (uv, ^{1}H-, and ^{13}C-nmr spectra), but the furan ^{1}H-nmr signals were all shifted downfield from those in **1b** (Tables 1 and 2). Positive ion fabms gave a strong $[MH]^+$ ion at 427 daltons, consistent with the formula $C_{26}H_{34}O_5$ (compound **1b** is $C_{26}H_{36}O_4$). A quaternary carbon signal at 194.64 ppm revealed the presence of a ketone functionality. The furan ^{1}H signals were not coupled to any other signals (by contrast to those in **1b**) so this ketone group was located at C-5. The ^{1}H- and ^{13}C-nmr values for this proposed 3-keto furan unit closely matched those of model compounds (7,8). ^{1}H-^{1}H homonuclear correlation (COSY) and ^{1}H-^{13}C heteronuclear correlation experiments showed that the C-15 to C-26 portion of **2b** matched 22-*O*-methylvariabilin [**1b**]. The COSY spectrum further indicated the substructure CH_3-C=CH-CH_2-CH=C-CH_3 so the two acyclic double bonds were C-8–C-10 and C-12–C-13. The ^{13}C-nmr shifts of C-9 and C-14 suggested that both these double bonds were *E* (9), which left only the absolute stereochemistry at C-18 undetermined for this new compound **2b**.

The second new sesterterpene **3b** gave uv, ir, and mass spectra similar to those for **2b** which, along with the ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2), suggested that **3b** was a double bond positional isomer of **2b**. A COSY spectrum showed coupling from an isolated methylene doublet at 3.45 ppm (H-6) to a methine triplet at 5.39 ppm (H-7) with further shifts and couplings as for 22-*O*-methylvariabilin [**1b**]. Therefore,



1a R=H
1b R=Me



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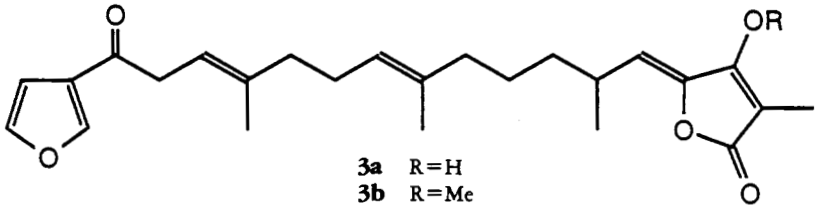
2b R=Me



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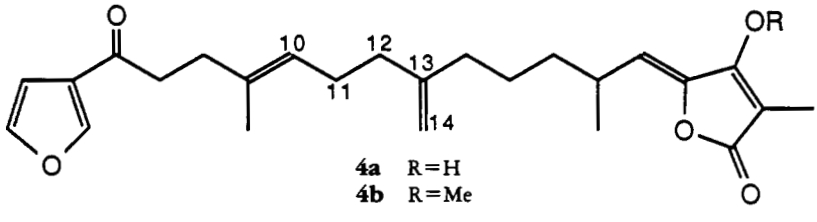
3b R=Me



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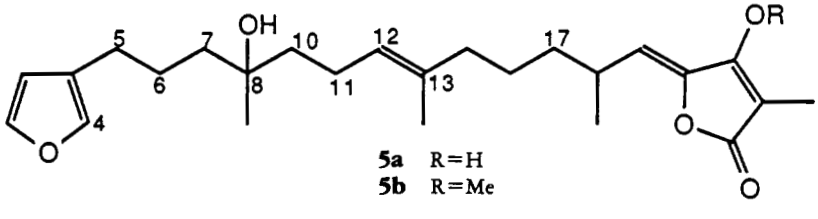
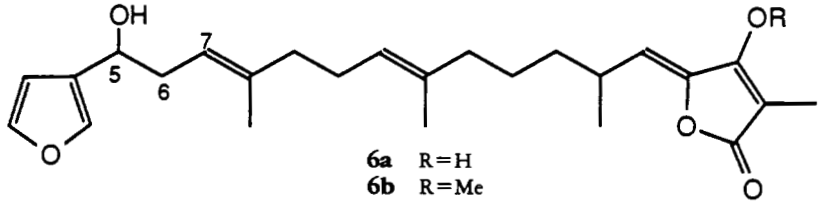
4b R=Me



5a R=H
5b R=Me



6a R=H
6b R=Me

3b differed from **2b** in that the C-8–C-9 double bond was replaced by a C-7–C-8 double bond [*E* by ^{13}C-nmr spectroscopy (9)].

The third compound **4b** from the keto sesterterpene mixture gave uv, ir, and mass spectra similar to those obtained for **2b** and **3b**. ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) showed the presence of a fragment which contained a

TABLE 1. ^{1}H-nmr Data for Methylated Sesterterpenes.[a]

Proton	Compound				
	1b	**2b**	**3b**	**4b**	**6b**
1	7.34 t (1.6)	7.43 t (1.6)	7.42 t (1.7)	7.43 t (1.7)	7.37 d (1.5)
2	6.28 m	6.77 dd (0.9, 1.9)	6.76 dd (0.8, 1.9)	6.77 dd (0.9, 1.9)	6.40 t (1.5)
4	7.20 m	8.03 dd (0.9, 1.5)	8.03 dd (0.8, 1.5)	8.03 m	7.37 d (1.5)
5	2.44 bt (7.5)				4.65 bt (6.5)
6	2.24 bq (7.3)	2.84 m	3.45 bd (7.0)	2.88 m	2.45 bq (6.5)
7	5.16 tq (6.9, 1.0)	2.38 bt (7.9)	5.39 bt (7.0)	2.41 bt (7.5)	5.15 bt (6.5)
9	1.58 d (0.5)	1.66 d (1.2)	1.67 d (1.3)	1.55 d (1.1)	1.62 d (1.4)
10	1.95 m	5.15 tq (7.5, 1.5)	2.00 bt (7.0)	5.08 bt (5.5)	1.93 bt (7.0)
11	2.05 m	2.68 bt (7.5)	2.06 m	2.10 m	2.06 m
12	5.08 tq (6.2, 0.8)	5.06 tq (7.5, 1.5)	5.05 bt (6.5)	2.08 m	5.04 bt (6.0)
14	1.56 d (0.5)	1.57 d (1.2)	1.53 d (1.5)	4.76 q (1.3) 4.73 q (1.4)	1.54 d (1.5)
15	1.95 m	1.94 bt (6.5)	1.91 bt (6.5)	1.93 m	1.93 bt (7.0)
16	1.35 m	1.35 m	1.32 m	1.32 m	1.32 m
17	1.35 m	1.35 m	1.32 m	1.32 m	1.32 m
18	2.75 bm	2.76 bm	2.76 bm	2.76 bm	2.75 bm
19	1.03 d (6.4)	1.03 d (6.7)	1.02 d (6.7)	1.02 d (6.7)	1.02 d (6.7)
20	5.15 d (9.7)	5.15 d (10.1)	5.14 d (10.2)	5.14 d (10.2)	5.14 d (10.2)
25	2.06 s	2.07 s	2.05 s	2.06 s	2.05 s
26	4.11 s	4.12 s	4.11 s	4.11 s	4.11 s

[a]$CDCl_3$ solutions; shifts in ppm downfield of TMS; s = singlet, d = doublet, t = triplet, q = quartet, b = broad; couplings (in Hz) in parentheses.

carbonyl substituted furan along with the C-8–C-10 double bond as in **2b**. A COSY spectrum established the substructure CH_3-C=CH-CH_2-CH_2-C=CH_2, so **4b** differed from **2b** in that the C-12–C-13 double bond was replaced with a terminal C-13–C-14 double bond. [Furanosesterterpenes with C-18–C-19 double bonds are known (10,11).]

The hydroxy sesterterpene mixture was also methylated, and two compounds were then obtained in pure form by silica hplc. The major component **5b** was identified as the known compound 8-hydroxyvariabilin by its uv, ir, nmr, and mass spectra (6,12). The second compound **6b** gave uv and ir spectra similar to those of **5b**, which confirmed the presence of furan and tetronic acid moieties, along with a hydroxyl group. The ei mass spectrum supported the molecular formula $C_{26}H_{36}O_5$ (compound **5b** is $C_{26}H_{38}O_5$). A COSY spectrum showed the substructure -CH.OH-CH_2-CH=C-CH_3 which was assigned as C-5 to C-9 because the ^{1}H- and ^{13}C-nmr spectra of **6b** (Tables 1 and 2) contained signals closely matching those of the C-9 to C-26 portion of **1b**. $NaBH_4$ reduction of **3b** gave a compound with spectral characteristics identical to those for **6b**, confirming the structure of the latter. While it would be expected that the reduction product would be a mixture of diastereoisomers, the separation between the chiral centers at C-5 and C-18 could lead to similar or identical nmr signals for the two diastereoisomers. Indeed, no distinction was observed by ^{1}H-nmr spectroscopy at 300 MHz. The absolute stereochemistries at C-5 and C-18 remain undetermined.

These new compounds **2b**, **3b**, **4b**, and **6b** are the first examples of furanosesterterpene tetronic acids oxygenated at C-5 (1–3). The combined level of sesterterpenes in this *Ircinia* species was >0.4% of wet wt, with any sterols present at much lower levels. Lawson *et al.* (13) noted that sterol levels in other Dictyoceratida sponges are generally low, with correspondingly high terpene levels, and suggested "...that sponge terpenes have a role in membranes which parallels that of sterols."

Crude extracts of this *Ircinia* species showed in vitro antiviral activity against *Herpes simplex* Type I and *Polio* virus Type

TABLE 2. ^{13}C-nmr Data for Methylated Sesterterpenes.[a]

Carbon	Compound				
	1b	**2b**	**3b**	**4b**	**6b**
1	142.50	143.91	143.91	143.96	142.92
2	111.09	108.48	108.80	108.49	108.50
3	125.00	127.52	127.47	127.53	128.53
4	138.80	146.87	147.13	146.82	138.78
5	25.06	194.64	[b]	194.59	66.66
6	28.46	39.23[c]	40.52	39.46	36.74[c]
7	123.72	33.92	115.94	33.92	119.16
8	135.76	133.30[d]	133.96[c]	135.12	139.20
9	16.05	16.20	16.67	15.90	16.34
10	39.57[c]	123.88	39.58[d]	123.70	39.50[d]
11	26.54	26.88	26.45	26.25	26.34
12	124.30	122.71	123.85	36.58[c]	123.84
13	134.91	134.92[d]	135.12[c]	143.92	135.07
14	15.86	15.88	15.94	115.02	15.86
15	39.69[c]	39.45[c]	39.68[d]	38.78	39.76[d]
16	25.72	25.63	25.76	25.64	25.70
17	36.68	36.65	36.72	36.34[c]	36.65[c]
18	30.81	30.73	30.86	30.74	30.77
19	20.69	20.68	20.79	20.68	20.71
20	115.24	115.06	115.21	115.08	115.11
21	142.70	142.47	142.58	142.47	142.49
22	161.97	161.73	[b]	161.72	161.73
23	98.88	98.83	98.96	98.84	98.87
24	171.10	170.86	[b]	170.84	170.87
25	8.57	8.58	8.71	8.59	8.60
26	58.80	58.73	58.85	58.74	58.75

[a]$CDCl_3$ solutions; shifts in ppm downfield of TMS.
[b]Signal not observed due to inadequate intensity.
[c,d]Assignments with the same superscript in the same vertical column may be interchanged.

I, with some cytotoxicity to the BSC host cells. For a description of this assay system see Munro *et al.* (14). Pure variabilin **[1a]** is cytotoxic in this assay at 2 μg/disc but shows varying antiviral effects (6,14). 22-*O*-Methylvariabilin **[1b]** is inactive at 20 μg/disc. Parallel results were obtained with the hydroxy compound **5a** and its 22-*O*-methyl derivative **5b**. The 22-*O*-methyl keto compounds **2b**, **3b**, and **4b** were cytotoxic, without antiviral activity, at 2 μg/disc. The 22-*O*-methyl hydroxy compound **6b** was marginally cytotoxic at this level.

EXPERIMENTAL

Ir (film) and uv (MeOH) spectra were recorded on Shimadzu IR 27G and Varian DMS 100 spectrometers, respectively. All nmr data (Tables 1 and 2) were recorded on a Varian XL300 instrument for $CDCl_3$ solutions with TMS as internal standard. Mass spectra were recorded on Kratos MS 80 or JEOL HX 110 spectrometers. Normal phase and silica hplc were carried out on a Shimadzu LC4A instrument using Zorbax CN or Alltech silica columns. Reversed-phase hplc was performed on a Varian 5000 instrument using an Alltech C8 column.

SPONGE COLLECTION AND TAXONOMY.—This sponge was collected by scuba diving from Kaikoura, New Zealand, in December 1983. *Ircinia* sp. B, Family Thorectidae, Order Dictyoceratida remains undescribed in the literature. The sponge resembles other New Zealand *Ircinia* species, being dark gray in color, of massive morphology, and possessing a tough, wrinkled surface. It is distinct in that the skeleton incorporates very fine unbeaded filaments. The type specimen #831202-6 is deposited with the University of Canterbury Marine Chemistry Group's collection.

EXTRACTION AND ISOLATION OF CONSTITUENTS.—The sponge (40 g) was extracted with MeOH-toluene (3:1) (2 × 100 ml). After filtration the combined extracts were concentrated and partitioned between EtOAc and H_2O. The organic layer was dried and the solvent removed to give a brown oil (480 mg). Column chromatography on silica (100 g) using a hexane/EtOAc gradient gave *7E,12E,20Z*-variabilin [**1a**] (70 mg) eluted with hexane-EtOAc (1:1), then the keto sesterterpenes **2a**, **3a**, and **4a** (80 mg) with hexane-EtOAc (2:3), then the hydroxy sesterterpenes **5a** and **6a** (20 mg) with hexane-EtOAc (1:4).

SEPARATION OF **2b**, **3b**, AND **4b**.—The green oil (80 mg) containing **2a**, **3a**, and **4a** could not be separated by either rplc or nplc. This mixture was unstable, and after 30 days in the freezer only 25% was intact. After nplc purification the remaining sample (20 mg) was methylated using CH_2N_2 in Et_2O (15) and separated by silica hplc [hexane-iPrOH (999:1)], although 50% losses occurred due to column acidity.

22-O-Methyl-5-oxo-8(10)E,12E,20Z-variabilin [**2b**].—Colorless oil: λ max (ε) 266 nm (11000); ν max 3150, 2910, 2850, 1760, 1680, 1640, 1460, 1395, 1360, 1160, 980, 870, 760, 740, 600 cm^{-1}; fabms *m/z* $[MH]^+$ 427.

22-O-Methyl-5-oxo-7E,12E,20Z-variabilin [**3b**].—Colorless oil: λ max (ε) 266 nm (11000); ν max 2910, 2850, 1760, 1680, 1640, 1460, 1360, 1160, 980, 870, 760, 600 cm^{-1}; fabms *m/z* $[MH]^+$ 427.

22-O-Methyl-5-oxo-8(10)E,13,20Z-variabilin [**4b**].—Colorless oil: λ max (ε) 266 nm (11000); ν max 3160, 2910, 2850, 1760, 1680, 1640, 1460, 1395, 1360, 1155, 1060, 980, 860, 755, 600 cm^{-1}; fabms *m/z* $[MH]^+$ 427.3475 ($C_{26}H_{35}O_5$ requires 427.3484).

SEPARATION OF **5b** AND **6b**.—The mixture of alcohols **5a** and **6a** (20 mg) was methylated using CH_2N_2 in Et_2O (15), then separated by nplc [hexane-iPrOH (96:4)] to give 22-*O*-methyl-8-hydroxy-12*E*,20*Z*-variabilin [**5b**] (4 mg) and **6b** (2 mg).

22-O-Methyl-5-hydroxy-7E,12E,20Z-variabilin [**6b**].—Colorless oil: λ max (ε) 266 nm (10000); ν max 3500, 2950, 2870, 1760, 1640, 1460, 1400, 1360, 1210, 1160, 1150, 1060, 980, 870, 760, 600 cm^{-1}; fabms *m/z* $[MNa]^+$ 451.2486 ($C_{26}H_{36}O_5Na$ requires 451.2461).

REDUCTION OF KETO SESTERTERPENE **3b**.—Excess $NaBH_4$ (4 mg) was added to a solution of **3b** (1 mg) in MeOH (0.5 ml). After stirring (10 min, room temperature), the MeOH was removed under reduced pressure and the residue partitioned between HCl (1 M) and EtOAc. The organic layer was washed with $NaHCO_3$ solution and the solvent removed under reduced pressure to yield alcohol **6b** (1 mg).

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