Journal of Natural Products
Vol. 51, No. 2, pp. 275-281, Mar-Apr 1988

VARIABILIN AND RELATED COMPOUNDS FROM A SPONGE OF THE GENUS *SARCOTRAGUS*

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ABSTRACT.—The bioactivity-directed analysis of the extract from a sponge of the genus *Sarcotragus* led to the isolation of a series of bioactive sesterterpenes, of which variabilin [**1a**] was the major component. The sesterterpenes **2a**, **3**, and **4a**, along with the related C_{21} furanoterpene [**5**], were present in lesser amounts. The unequivocal assignment of the stereochemistry of the 20,21 double bond in variabilin as 20*Z* was achieved through examination of the 22-*O*-methyl derivative **1b** of variabilin and the isolation of the variabilin isomer **2a** with the 20*E* configuration.

Sponges of the order Dictyoceratida have yielded a wide range of new sesterterpenes, many of which contain both furan and tetronic acid functional groups (1,2). Typical of these furanosesterterpene tetronic acids is variabilin [**1a**], which was first isolated from the sponge *Ircinia variabilis* (3). This compound is antimicrobial and has since been reported to be cytotoxic (4). In the original communication, neither the stereochemistry at the double bonds nor the absolute configuration at C-18 was defined. Subsequently, 7*E* and 12*E* configurations were assigned (5), leaving the stereochemistry at the exocyclic double bond unknown.

Variabilin has now been identified as the major component responsible for the bioactivity of an extract from a sponge of the genus *Sarcotragus* (Thorectidae, Dictyoceratida). This isolation arose from a search for potential antiviral and antitumor agents from New Zealand marine invertebrates. Variabilin [**1a**] is a major component in all New Zealand collections of sponges of the genera *Ircinia*, *Psammocinia*, and *Sarcotragus* (6).

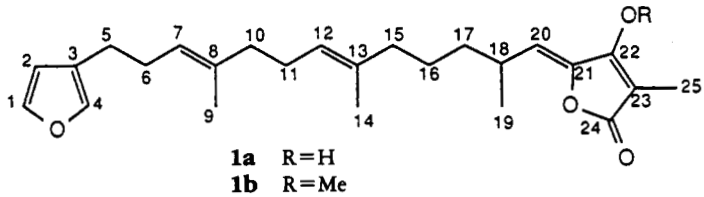
Investigations of the ^{1}H- and ^{13}C-nmr spectra of the 22-*O*-methyl derivative of variabilin have now revealed the stereochemistry of the remaining double bond as 20*Z*. This assignment was aided by the isolation of the 20*E* isomer **2a** as a minor component from the same sponge. Three other terpenes, including another double bond isomer **3**, a hydroxy derivative of variabilin **4a**, and a C_{21} furanoterpene **5** have also been isolated from the *Sarcotragus* sp.

RESULTS AND DISCUSSION

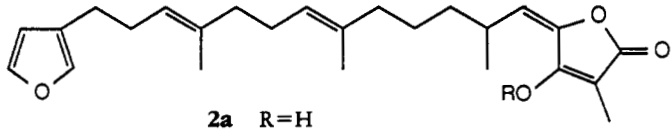
An MeOH/toluene extract of a sponge, identified as *Sarcotragus* sp. I, displayed significant in vitro antiviral activity against *Herpes simplex* Type I and *Polio* Type I, with little cytotoxicity to the tumor cells used in the antiviral assay. Bioassay-directed Si gel chromatography of this extract yielded variabilin [**1a**] as the major bioactive component. However, the pure compound displayed cytotoxic rather than antiviral properties. The cause of this change in bioactivity is not yet understood. The related sesterterpenes **2a**, **3**, and **4a**, along with the related C_{21} furanoterpene **5**, were cytotoxic at similar levels.

The identity of variabilin was confirmed by comparison of its uv, ir, ^{13}C- and ^{1}H-nmr spectra with those reported previously (3,5), and from its mass spectrum. Full assignments of the ^{1}H-nmr spectrum (Table 1) and ^{13}C-nmr spectrum (Table 2) were achieved through the use of homonuclear (COSY) and heteronuclear (HETCOR and XCORFE) correlation 2D spectroscopy. These assignments are in agreement with published results for variabilin and other furanosesterterpene tetronic acids (1).

In order to determine the C-20,21 double bond geometry, 22-*O*-methyl variabilin



1a R=H
1b R=Me



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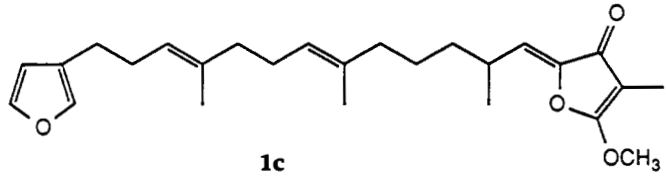
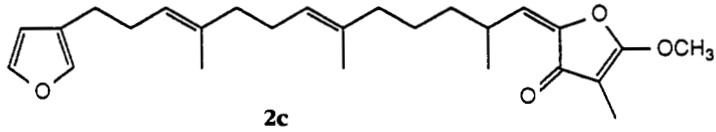
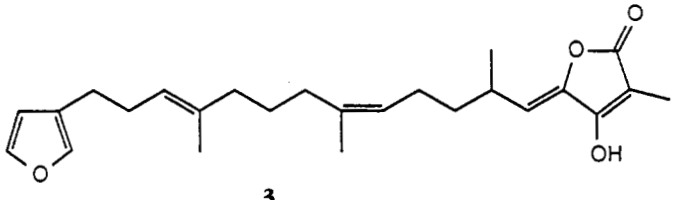
2b R=Me



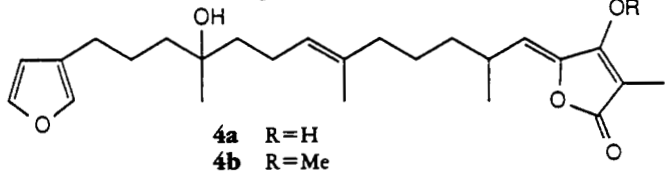
1c



2c



3



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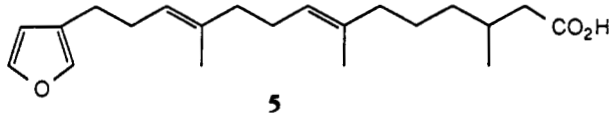
4b R=Me



5

[**1b**] was prepared. Treatment of variabilin with CH_2N_2 gave two methylation products **1b** and **1c** in the ratio 3:1 (7). Difference nOe ^{1}H-nmr experiments on the major methylation product **1b** showed enhancement of the H-20 doublet as well as the tetronic acid methyl signal upon irradiation of the methoxyl signal. Irradiation of the H-20 doublet gave an enhancement of the methoxyl peak intensity. This allowed the exocyclic tetronic acid double bond of the 22-*O*-methyl derivative **1b** and, hence, that of variabilin itself, to be assigned as 20*Z*.

TABLE 1. ^{1}H-Nmr Data for Compounds **1–5**.[a]

Position	Compound										
	1a	**1a**[b]	**2a**	**1b**	**2b**	**2c**	**3**	**3**[b]	**4a**	**4b**	**5**
1	7.33 t (1.8)	7.27 m	7.33 t (1.8)	7.34 t (1.6)	7.33 t (1.8)	7.33 t (1.8)	7.33 t (1.6)	7.27 m	7.34 t (1.6)	7.34 t (1.6)	7.33 t (1.8)
2	6.27 m	6.24 m	6.28 m	6.28 m	6.27 m	6.28 m	6.28 m	6.24 m	6.26 m	6.27 m	6.28 m
4	7.20 m	7.21 m	7.21 m	7.20 m	7.20 m	7.21 m	7.21 m	7.22 m	7.21 m	7.22 m	7.21 m
5	2.44 t (7.5)	2.47 t (8.1)	2.44 t (7.5)	2.44 t (7.5)	2.44 t (7.5)	2.45 t (7.5)	2.45 t (7.5)	2.48 tt (8.0, 1.5)	2.42 t (7.2)	2.43 t (7.0)	2.45 t (7.5)
6	2.25 q (7.3)	2.32 q (7.3)	2.24 q (7.3)	2.24 q (7.3)	2.24 q (7.3)	2.25 q (7.4)	2.24 q (7.3)	2.33 q (7.1)	1.55 m	1.55 m	2.24 q (7.4)
7	5.16 tq (6.9, 1.0)	5.36 tq (7.0, 1.3)	5.16 tq (6.9, 1.0)	5.16 tq (6.9, 1.0)	5.16 tq (6.0, 1.05)	5.16 tq (6.9, 1.0)	5.16 tq (6.8, 1.1)	5.34 tq (7.0, 1.3)	1.5 m	1.5 m	5.16 tq (6.8, 1.0)
9	1.58 d (0.7)	1.65 d (1.3)	1.58 d (0.7)	1.58 d (0.5)	1.58 d (0.7)	1.58 d (1.8)	1.57 q (0.7)	1.65 d (1.2)	1.19 s	1.17 s	1.59 d (1.0)
10	1.95 m	2.18 t (6.5)	1.95 m	1.95 m	1.95 m	1.95 m	1.93 t (7.5)	2.10 m	1.5 m	1.5 m	1.95 m
11	2.05 m	2.26 t (6.5)	2.05 m	2.05 m	2.05 m	2.05 m	1.4 m	1.62 m	2.05 m	2.05 m	2.05 m
12	5.08 tq (6.2, 0.8)	5.36 tq (7.0, 1.3)	5.08 tq (6.2, 0.8)	5.08 tq (6.2, 0.8)	5.06 tq (6.2, 1.0)	5.08 tq (6.5, 1.0)	1.93 t (7.5)	2.10 m	5.10 tq (6.0, 1.0)	5.11 tq (6.0, 1.05)	5.09 tq (6.0, 1.0)
14	1.55 d (0.7)	1.67 d (1.2)	1.55 d (0.7)	1.56 d (0.5)	1.56 d (0.8)	1.55 d (0.5)	1.65 q (1.0)	1.82 q (1.2)	1.56 d (0.5)	1.56 d (0.5)	1.58 d (1.0)
15	1.95 m	2.10 t (6.8)	1.95 m	1.95 m	1.95 m	1.95 m	5.08 tq (6.2, 1.0)	5.31 tq (7.0, 1.3)	1.95 m	1.95 m	19.5 m
16	1.35 m	1.50 m	1.35 m	1.35 m	1.35 m	1.35 m	1.93 t (7.5)	2.15 m	1.35 m	1.35 m	1.35 m
17	1.35 m	1.38 m	1.35 m	1.35 m	1.35 m	1.35 m	1.40 m	1.47 m	1.35 m	1.35 m	1.35 m
18	2.80 bm	2.97 bm	3.15 bm	2.75 bm	3.02 bm	3.72 bm	2.82 bm	3.00 bm	2.78 bm	2.77 bm	1.95 bm
19	1.05 d (6.4)	1.06 d (6.7)	1.05 d (6.5)	1.03 d (6.4)	1.02 d (7.2)	1.05 d (7.2)	1.06 d (6.8)	1.06 d (6.7)	1.04 d (6.6)	1.03 d (6.6)	0.97 d (7.0)
20	5.44 d (10.3)	5.40 d (10.1)	5.45 d (10.9)	5.15 d (9.7)	5.38 d (11.0)	5.62 d (11.1)	5.30 d (10.1)	5.33 d (10.2)	5.29 d (10.0)	5.15 d (9.9)	2.14 dd (14.9, 8.0) 2.36 dd (14.9, 5.9)
25	1.82 s	1.73 s	1.82 s	2.06 s	2.09 s	1.64 s	1.81 s	1.67 s	1.79 s	2.07 s	
OMe				4.11 s	4.13 s	4.06 s				4.12 s	

[a]Values in ppm, relative to $\delta_H = 0.00$ for $(CH_3)_4Si$ in $CDCl_3$ solutions (coupling constants in Hz).
[b]C_6D_6 solutions.

TABLE 2. ^{13}C-nmr Data for Compounds **1–5**.[a]

Carbon	Compound										
	1a	**1a**[b]	**2a**	**1b**	**2b**	**1c**	**3**	**3**[b]	**4a**	**4b**	**5**
1	142.47	143.20	142.51	142.50	142.53	142.5	142.55	143.21	142.75	142.59	142.54
2	111.09	111.76	111.12	111.09	111.09	111.1	111.14	111.74	110.90	110.87	111.09
3	124.98	125.60	125.01	125.00	124.97	125.0	125.04	125.61	124.86	124.89	125.01
4	138.78	139.60	138.82	138.80	138.81	138.8	138.83	139.60	138.85	138.73	138.84
5	25.04	25.92	25.04	25.06	25.04	25.0	25.06	25.88	25.16	25.31	25.08
6	28.44	29.45	28.42	28.46	28.44	28.5	28.42	29.39	24.42	24.51	28.47
7	123.72	124.74[c]	123.75	123.72	123.75	123.8	123.70	124.72[c]	41.26[c]	41.69[c]	123.77
8	135.71	136.07[d]	135.73	135.76	135.71	135.7	135.87[c]	136.13[d]	73.98	72.72	135.77
9	16.04	16.50[e]	16.05	16.05	16.07	16.0	15.96	16.52	26.56	26.92	16.05
10	39.55[c]	40.64	39.54[c]	39.57[c]	39.53[c]	39.5[c]	39.55	40.37	41.21[c]	41.52[c]	39.65[c]
11	26.57	27.56	26.52	26.54	26.56	26.6	25.72	27.18	22.55	22.62	26.55
12	124.41	125.26[c]	124.37	124.30	124.37	124.5	31.39	32.20	124.34	124.30	124.27
13	134.76	135.31[d]	134.89	134.91	134.88	134.7	135.82[c]	136.08[d]	135.63	135.19	134.94
14	15.80	16.69[e]	15.83	15.86	15.86	15.8	23.38	24.11	15.96	15.92	15.89
15	39.68[c]	40.48	39.67[c]	39.69[c]	39.70[c]	39.7[c]	124.52	125.66[c]	39.30	39.56	39.69[c]
16	25.68	26.65	25.70	25.72	25.66	25.7	26.31	26.77	25.52	25.69	25.21
17	36.57	37.42	37.26	36.68	37.26	36.3	37.46	38.41	36.51	36.70	36.24
18	30.95	31.79	30.11	30.81	29.90	30.8	30.79	31.52	30.74	30.80	30.11
19	20.60	21.29	21.62	20.69	21.60	20.3	20.55	21.27	20.59	20.75	19.70
20	117.17	116.85	121.37	115.24	120.79	119.5	116.11	114.68	115.79	115.18	41.21
21	142.96	144.18	142.14	142.70	142.16	146.7	142.67	144.30	142.05	142.59	186.74
22	162.47	163.65	162.29	161.97	163.14	183.8	—[f]	—[f]	162.36	161.84	
23	99.06	100.00	101.61	98.99	101.63	90.1	99.63	100.45	99.27	98.96	
24	172.54	173.2	—[f]	171.10	170.62	175.6	—[f]	—[f]	172.10	171.10	
25	6.06	6.82	6.12	8.57	9.03	4.0	6.06	6.64	6.22	8.70	
OMe				58.80	58.90	56.4				58.85	

[a]Values in ppm, relative to $\delta_c = 0.00$ for (TMS) in $CDCl_3$ solutions.
[b]C_6D_6 solution.
[c–e]Assignments in vertical columns with same superscript may be interchanged.
[f]Signals not observed due to inadequate intensity.

This stereochemical assignment was confirmed by the isolation of the 20*E* isomer **2b** in 1% yield from the methylation reaction mixture. The spectral properties of **2b** were very similar to those of **1b**, with notable differences being found only for the nmr signals arising from the tetronic acid region (Tables 1 and 2). For this 20*E* isomer **2b**, no nOe effect was observed between the olefinic proton H-20 and the methoxyl protons. Further examination of the methylation mixture led to the isolation of **2c** in 0.4% yield, which was hydrolyzed to the 20*E* isomer of variabilin **2a**. Again, spectroscopic differences between variabilin [**1a**] and **2a** were consistent with their being isomeric at the exocyclic tetronic acid double bond (Tables 1 and 2). The results in Table 2 show a marked downfield shift of the C-20 signal in the 20*E* isomers **2a** and **2b** compared with the corresponding 20*Z* isomers **1a** and **1b**, as expected from data on other tetronic acids (8). Other signals are affected by this change in geometry but only to a small extent. In a recent review, ^{13}C-nmr data are given for eight sesterterpene tetronic acids with exocyclic double bonds of undefined geometry (including variabilin [**1a**]) (1). The C-20 chemical shifts are all in the range 115.8–116.9 ppm. The present results now confirm that all these compounds must have the 20*Z* geometry. Similar conclusions have been reached before but without the 20*E* isomers being available for comparison (9).

Another new sesterterpene **3** was separated from variabilin by reversed-phase liquid chromatography. The uv, ir, and mass spectra of this compound were similar to those for variabilin, while the ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) indicated that it was a geometric or double-bond positional isomer of variabilin. In particular, signals (with appropriate connectivities established by COSY and HETCOR experiments) appeared as for the C-1–C-9 and C-18–C-25 fragments in variabilin [**1a**]. However, the resonance of the remaining allylic methyl carbon occurred at 23.38 ppm, indicating sub-

stitution on a double bond with *Z* stereochemistry. Thus, the new compound was either 7*E*,12*Z*,20*Z*-variabilin or the *Z*-13,15 double bond isomer. Distinction between these two possibilities was achieved through the examination of the ^{1}H- and COSY nmr spectra of **3** obtained with C_6D_6 as solvent. In this solvent, three methylene resonances, which were coincident at 1.93 ppm in $CDCl_3$ solution, were resolved, as were the two methylene resonances coincident at 1.4 ppm in the $CDCl_3$ solution. These separations permitted the observation of the connectivity of H-18 (3.00 ppm)–H_2-17 (1.47 ppm)–H_2-16 (2.15 ppm)–H-15 (5.31 ppm). The remaining high field methylene signal at 1.62 ppm (H_2-11) was coupled to the two allylic methylenes at 2.10 ppm (H_2-10 and H_2-12). These results establish the structure of **3** as having the *Z*-13,15 double bond.

A more polar sesterterpene **4a** from this sponge was separated from the variabilin isomers by Si gel chromatography. Nmr and ms showed that one of the double bonds of variabilin [**1a**] was hydrated in the new compound **4a**. The location of the hydroxyl group at C-8 was established from a COSY experiment and confirmed by the ^{13}C-nmr shifts, which are very close to those for variabilin in the C-12 to C-25 region of the molecule (Table 2). These shifts also established the 12*E*,20*Z* configuration for **4a**. The methoxyl derivative **4b** has previously been prepared from an extract of *Sarcotragus muscarum* (10,11), but the natural product **4a** was not isolated. Hydroxyvariabilin [**4a**] was methylated to give **4b**, whose ^{13}C-nmr signals (Table 2) agreed with those previously reported (10), except for an incorrect shift for C-22.

Finally, a C_{21} furanoterpene **5**, closely related to variabilin, was also isolated from the *Sarcotragus* sp. The uv, ir, and nmr spectra of this compound showed that the tetronic acid group of variabilin [**1a**] was not present. Ms established a molecular formula of $C_{21}H_{32}O_3$. The presence of a carboxylic acid group was indicated by signals in the ir (1715 cm^{-1}) and ^{13}C-nmr spectra (186.74 ppm). This C_{21} carboxylic acid had ^{1}H- and ^{13}C-nmr signals closely matching those of variabilin from C-1 to C-17 (Tables 1 and 2), with those assignments being confirmed by COSY and HETCOR experiments. The presence of the carboxylic acid at C-21 explained the changes in the chemical shifts for C-18 and C-20, which correspond to those of 3-methylpentanoic acid (12). C_{21}-furanoterpenes such as **5** are frequently found to coexist with the corresponding C_{25} tetronic acids and probably arise through oxidative degradation (5). The methyl ester of **5** has been produced previously by oxidative degradation of variabilin to test the validity of this proposed biosynthesis (5), although the free carboxylic acid **5** has not previously been reported as a natural product.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra of films were recorded on a Shimadzu IR 27G instrument. Uv spectra of MeOH solutions (unless otherwise stated) were recorded on a Varian DMS 100 instrument. Nmr spectra of $CDCl_3$ solutions, with TMS as internal standard, were recorded on a Varian XL300 instrument, except for compound **1c** whose ^{1}H- and ^{13}C-nmr spectra were recorded on Varian T60 and CFT20 instruments. Mass spectra were recorded on a Kratos MS 80 instrument. Optical rotations were determined with a Perkin-Elmer 241 polarimeter.

Normal phase liquid chromatography (nplc) was performed on a Shimadzu LC4A series instrument using a Zorbax CN column, while reversed-phase liquid chromatography (rplc) was performed on a Varian 5000 liquid instrument using an Alltech C8 column, 250 × 10 mm. Rotating disk chromatography was performed on a Chromatotron®. All solvents were spectral grade or distilled prior to use.

SPONGE COLLECTION AND TAXONOMY.—The *Sarcotragus* sp. I was collected by scuba diving from Kaikoura, New Zealand, during August 1983. *Sarcotragus* sp. I (University of Canterbury, Department of Chemistry Collection #830801-8) is an undescribed New Zealand sponge of the family Thorectidae Bergquist, order Dictyoceratida. The species is similar in overall morphology and skeletal structure to the type species *Sarcotragus spinosula* Schmidt, as designated by Vacelet (13). The surface is dark gray to black and raised into conules. The skeleton is characterized by uncored primary and secondary fibers. Primary fibers are fasiculate. Numerous knobbed filaments color the sponge matrix red-brown.

EXTRACTION AND ISOLATION OF CONSTITUENTS.—A sample of the sponge (2 kg) was coarsely chopped and soaked in MeOH-toluene (3:1, 1400 ml) for 12 h. After filtration the sample was blended (Waring blender) with further MeOH-toluene (6 × 500 ml) and filtered. The combined extracts were concentrated (200 ml) and partitioned between EtOAc and H_2O. The organic layer was dried and the solvent removed to give a brown oil (12.7 g). Cc of the extract was carried out on silica (200 g) using a hexane-EtOAc gradient. 7*E*,12*E*,20*Z*-variabilin [**1a**], 7*E*,12*E*,20*E*-variabilin [**2a**], the 7*E*,13*Z*,20*Z*-isomer **3**, and the C_{21} furanoterpene **5** eluted together (hexane-EtOAc, 70:30) (fraction A, 5.2 g), while 8-hydroxy-12*E*,20*Z*-variabilin [**4a**] eluted in a later fraction (fraction B, 450 mg).

SEPARATION OF 7*E*,12*E*,20*Z*-VARIABILIN [**1a**] AND 7*E*,12*E*,20*E*-VARIABILIN [**2a**].—A subsample of fraction A (2.0 g) was methylated using CH_2N_2 in Et_2O (7). The methylation products **1b**, **1c**, **2b**, and **2c** were obtained in relative yields 100:40:2.5:1. An initial separation of these derivatives was achieved on a spinning silica disc, with further purification involving the use of semipreparative rplc. The methylated derivatives **1c** and **2c** were converted back to the unmethylated species **1a** and **2a** in the presence of MeOH-H_2O-TFA (80:20:0.01) when left at 50° for 1 h.

7*E*,12*E*,20*Z*-VARIABILIN [**1a**].—Uv, ir, and ms data are in agreement with literature values (3). For ^{1}H nmr and ^{13}C nmr see Tables 1 and 2. $[\alpha]_D$ −4° ($CHCl_3$, c = 1.0).

7*E*,12*E*,20*E*-VARIABILIN [**2a**].—Uv λ max (MeOH) 256 nm (ε 12,700); λ max (MeOH/OH^-) 250 (ε 13,300), 310 nm (ε 7600); ir ν max 3100, 2950, 1815, 1740, 1640, 1570, 1450, 1385, 1285, 1175, 1150, 1120, 1050, 1020, 870, 780, 760, 600 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2; ms *m/z* 398.2465 ($C_{25}H_{34}O_4$ requires 398.2459).

22-*O*-METHYL-7*E*,12*E*,20*Z*-VARIABILIN [**1b**] (14).—Uv λ max 266 nm (ε 10,000); ir ν max 2950, 1760, 1640, 1455, 1390, 1370, 1340, 1280, 1205, 1130, 1055, 1020, 980, 885, 780, 760, 605 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2; ms *m/z* 412.2621 ($C_{26}H_{36}O_4$ requires 412.2615).

24-*O*-METHYL-7*E*,12*E*,20*Z*-VARIABILIN [**1c**] (14).—Uv λ max 250 nm (ε 8,000); ir ν max 2950, 1620, 1475, 1390, 1370, 1110, 1020, 875, 760, 605 cm^{-1}; ^{1}H nmr δ 7.33 (m, 1H), 7.21 (m, 1H), 6.24 (m, 1H), 5.68 (d, J = 11.0 Hz, 1H), 5.15 (m, 2H), 4.10 (s, 3H), 1.2–3.0 (21H), 1.65 (s, 3H), 1.05 (d, 7.2 Hz, 3H); ^{13}C nmr see Table 2; ms *m/z* 412.2613 ($C_{26}H_{36}O_4$ requires 412.26150).

22-*O*-METHYL-7*E*,12*E*,20*E*-VARIABILIN [**2b**].—Uv λ max 268 nm (ε 14,000); ir ν max 2950, 1760, 1635, 1450, 1400, 1280, 1120, 1060, 1020, 980, 870, 780, 750, 680, 600 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2; [α] λ = 0.0 (589), 0.0 (578), 0.4 (546), 2.0 (436), 6.2° (365 nm); ms *m/z* 412.2599 ($C_{26}H_{36}O_4$ requires 412.2615).

24-*O*-METHYL-7*E*,12*E*,20*E*-VARIABILIN [**2c**].—For ^{1}H nmr see Table 1.

ISOLATION OF 7*E*,13*Z*,20*Z*-VARIABILIN [**3**].—A subsample of fraction A (80 mg) was passed through semipreparative rplc to give a clear oil (10 mg). Uv λ max (MeOH) 253 nm (ε 9000); λ max (MeOH/OH^-) 306 (ε 5000), 249 nm (ε 9000); ir ν max 3150, 2950, 1730, 1630, 1450, 1420, 1310, 1065, 1025, 875, 780, 760, 600 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2; [α] λ = −4.4 (589), −4.9 (578), −5.5 (546), −12.4 (436), −50.0° (365 nm) ($CHCl_3$, c = 0.9); ms *m/z* 398.2460 ($C_{25}H_{34}O_4$ requires 398.2459).

PURIFICATION OF 8-HYDROXY-12*E*,20*Z*-VARIABILIN [**4a**].—Fraction B (450 mg) was further chromatographed on silica to give a green oil (385 mg). A portion of this (100 mg) was further purified by semipreparative rplc, 75:25 MeOH-H_2O (0.05% trifluoroacetic acid), to give a clear oil (80 mg). Uv λ max (MeOH) 253 nm (ε 10,000); λ max (MeOH/OH^-) 248 (ε 10,000), 308 nm (ε 7000); ir ν max 3440, 2950, 1815, 1745, 1640, 1380, 1315, 1260, 1060, 880, 760, 600 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2; $[\alpha]_D$ −24.5° (MeOH, c = 0.22); fabms *m/z* 417.2658 $[M+1]^+$ ($C_{25}H_{37}O_5$ requires 417.2654).

8-HYDROXY-22-*O*-METHYL-12*E*,20*Z*-VARIABILIN [**4b**].—CH_2N_2 methylation (as above) of **4a** gave **4b** with uv, ir, ms, and nmr data (Tables 1 and 2) in agreement with literature values (10).

ISOLATION OF THE C_{21}-FURANOTERPENE **5**.—A subsample of fraction A (2.2 g) was applied to a spinning silica disc and the relevant fraction (5 mg) purified on nplc to give a clear oil (2 mg). Ir ν max 3100, 2950, 1715, 1510, 1460, 1390, 1370, 1150, 1060, 1020, 875, 780, 600 cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2; ms *m/z* 332.2349 ($C_{21}H_{32}O_3$ requires 332.2351).

ACKNOWLEDGMENTS

Grants from the New Zealand University Grants Committee and the Canterbury Medical Research Foundation are gratefully acknowledged. We thank Professor P.R. Bergquist, University of Auckland,

Dr. C.N. Battershill, and Dr. G.D. Fenwick for sponge identifications; Dr. D. Crump and Dr. S. Bloor, DSIR, Wellington, for optical rotation measurements; Mrs. V.L. Calder for bioassays; and Dr. R.J. Lake for assistance with the characterization of 8-hydroxy-12*E*,20*Z*-variabilin [**4a**].

LITERATURE CITED

1. P. Crews and S. Naylor, *Prog. Chem. Org. Nat. Prod.*, **48**, 203 (1985).
2. P.R. Bergquist and R.J. Wells, in: "Marine Natural Products." Ed. by P.J. Scheuer, Vol. V, Academic Press, New York, 1983, p. 1.
3. D.J. Faulkner, *Tetrahedron Lett.*, 3821 (1973).
4. R.S. Jacobs, S. White, and L. Wilson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **40**, 26 (1981).
5. A.G. Gonzalez, M.L. Rodriguez, and A.S.M. Barrientos, *J. Nat. Prod.*, **46**, 256 (1983).
6. N.B. Perry, C.N. Battershill, J.W. Blunt, G.D. Fenwick, M.H.G. Munro, and P.R. Bergquist, *Biochem. Syst. Ecol.*, **15**, 373 (1987).
7. G. Alfano, G. Cimino, and S. De Stefano, *Experientia*, **35**, 1136 (1979).
8. A. Pelter and M.T. Ayoub, *J. Chem. Soc., Perkin Trans. 1*, 1173 (1981).
9. L.V. Manes and P. Crews, *J. Nat. Prod.*, **49**, 787 (1986).
10. A.G. Gonzalez, D. Estrada, M.L. Rodriguez, and A. San Martin, *An. Quim., Ser. C*, **79**, 69 (1983).
11. P.R. Bergquist, *N.Z. J. Zool.*, **7**, 443 (1980).
12. P.A. Couperus, A.D.H. Clague, and J.P.C.M. van Dongen, *Org. Magn. Reson.*, **11**, 590 (1978).
13. J. Vacelet, *Recueil de Travaux de la Station Marine d'Endoume*, **26** (Bull. 16), 92 (1959).
14. I. Rothberg and P. Shubiak, *Tetrahedron Lett.*, 769 (1975).

Received 30 September 1987