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NORSESTERTERPENES FROM THE NORTH ADRIATIC SPONGE *IRCINIA OROS*

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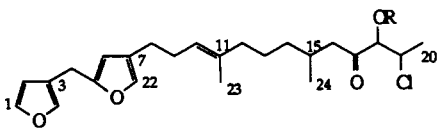
ABSTRACT.—Together with the previously described ircinin 1 [**4**] and ircinin 2 [**5**], two new isomeric linear difurano-norsesterterpenes **1** and **3** have been isolated from the sponge *Ircinia oros*, collected in the Northern Adriatic Sea. The structural elucidation and biological activity of these compounds are reported.

Marine organisms, especially sponges, have provided a large number of sesterterpenoids (1). Acyclic furanosesterterpenes and degraded C-21 furanoterpenes have been isolated from Dictyoceratid sponges (2).

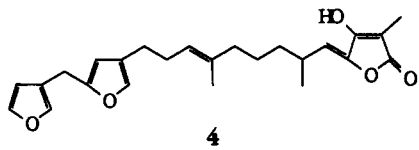
In the course of our search for marine natural compounds that have biological activity we have investigated the marine sponge *Ircinia oros* Schmidt (Dictyoceratida) collected near Rovinj, Yugoslavia, whose extract showed high activity (LD_{50} = 14 μg/ml) in the brine shrimp assay (3). By fractionating the extract, we isolated two isomeric linear difurano-norsesterterpenes **1** and **3**, and two sesterterpenes, ircinin 1 [**4**] and ircinin 2 [**5**], which are responsible for the biological activity. From the same sponge, collected in the Tyrrhenian Sea, other authors have reported the structural determination of two sesterterpenes ircinin 1 [**4**] and ircinin 2 [**5**] as a mixture (4), and of two related C-21 furanoterpenes (5). Recently Manes *et al.* (6) have reported the isolation of **4** and **5** from a Fiji island *Ircinia* sp., their separation by hplc, and the determination of double bond geometry, previously undetermined.

An Me_2CO extract of the sponge was partitioned between Et_2O and H_2O. Si gel chromatography of the Et_2O portion yielded two fractions, each of which was Ehrlich positive and gave a single spot on tlc; each fraction was separated by hplc into two compounds (ratio 7:3) of similar retention time. The least polar fraction contained **1** and **3**, while the most polar fraction contained, as major components, ircinin 1 [**4**] and ircinin 2 [**5**].

Compounds **1** and **3** showed the same molecular formula $C_{24}H_{33}ClO_4$ (hrms); their ir, with bands at 3400 and 1713 cm^{-1} (1715 cm^{-1} for **3**), showed the presence of an al-



1 R=H
2 R=Ac



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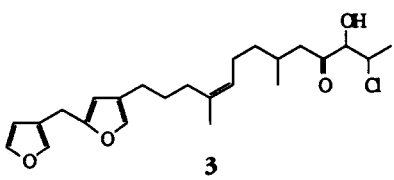

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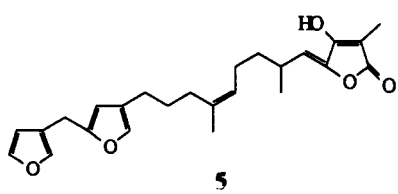
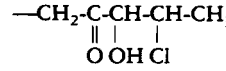


5

cohol and a carbonyl function. The carbon skeletons of **1** and **3** were easily recognized to be acyclic difuranoterpenoids by analysis of both ^{1}H- and ^{13}C-nmr spectra. The presence of a carbonyl group was supported by the ^{13}C-nmr spectrum with a singlet at δ 208.8. The ^{1}H-nmr spectrum of **1** showed two protons at δ 4.30, attributable to two methines by an HETCOR experiment, that correlated these protons to two carbon doublets in the ^{13}C-nmr spectrum at δ 80.2 and 57.5, suggesting the existence of a secondary alcohol and a secondary monochlorinated carbon atom. The COSY-45 spectrum showed that this signal was only correlated with a methyl doublet at δ 1.44 (J = 6.7 Hz). ^{1}H-nmr spectra were recorded in different solvents ($CDCl_3$, C_6D_6, $CDCl_3$ + CD_3OD) to resolve the two signals, but no effects were observed. The ^{1}H-nmr spectrum of the acetyl derivative **2** of **1** showed two different signals, a doublet at δ 5.19 (J = 4.4 Hz) and a multiplet at δ 4.38. The COSY-45 spectrum of **2** showed that the signal at δ 4.38 was correlated with the doublet at δ 5.19 and at the same time with the methyl doublet at δ 1.51 (J = 6.8 Hz). The ^{13}C-nmr spectrum of **2** showed a β acetylation shift for the carbonyl (δ 208.8) and the chloromethine (δ 57.5) and a γ shift for the methylene at δ 47.5 and methyl at δ 19.3. The partial structure **A** was consistent with these data.



A

The HETCOR spectrum of **1** showed that the methylene at δ 47.5 in the ^{13}C-nmr spectrum was correlated with the AB part (δ 2.54, dd J = 17.0, 5.6 Hz and 2.42, dd, J = 17.0, 7.9 Hz) of an ABX system in the ^{1}H-nmr spectrum. Moreover, the ^{1}H-nmr spectrum revealed signals for three furan α protons (δ 7.37, 7.29, and 7.0 g) and two β protons (δ 6.32 and 5.91), suggesting the existence of a β-mono-substituted and an α,β-di-substituted furan ring. These were further substantiated by ^{13}C-nmr data that showed five doublets (δ 142.9, 139.7, 137.4, 111.2, and 107.4) and three singlets (δ 153.9, 125.8, and 121.3). The presence of a vinyl proton (δ 5.13, t, J = 7.0 Hz) and a vinylic methyl (δ 1.56) in the ^{1}H-nmr spectrum suggested the presence of a tri-substituted double bond; this was confirmed by the presence in the ^{13}C-nmr spectrum of a doublet (δ 124.0) and a singlet (δ 135.4). In addition, the ^{1}H-nmr spectrum showed five distinct methylenes: a singlet at δ 3.73 (H-4), a triplet at δ 2.38 (J = 7.8 Hz, H-8), and three multiplets at δ 2.20, 1.95, and 1.35 (H-9, H-13, and H-14 respectively). Finally, a complex signal for three protons (methylene plus methine by the HETCOR spectrum) at δ 2.05 was observed. The COSY-45 spectrum of **1** showed correlation between the methylene singlet at δ 3.73 and the two β furan protons (δ 6.32 and 5.91), and one α furan proton (δ 7.29); also, the β furan proton at δ 6.32 was correlated with the α furan proton at δ 7.37. These correlations suggested that the two furan rings were linked to each other by the methylene. In addition the COSY-45 spectrum showed that the methylene at δ 2.38 was correlated with the β furan proton at δ 5.91 and at the same time with the methylene at δ 2.20, which was correlated with the vinylic proton at δ 5.13. Furthermore, the COSY-45 showed that the vinylic methylene (δ 2.05) was correlated, long-range, with the vinylic proton (δ 5.13) and at the same time with the methylene at δ 1.95, which was also correlated with the remaining methylene (δ 1.35). This last was correlated with the methine at δ 2.08 that was the X part of the ABX system, which was also correlated with the methyl doublet (δ 0.91). Considering these data and the partial structure **A**, we can propose the structure **1**. The HETCOR experiment of **1** allowed us to assign all chemical shifts in the ^{13}C-nmr spec-

TABLE 1. Nmr Spectral Data of **1** and **3** in $CDCl_3$ Solution.[a]

Position	**1**		**3**	
	^{13}C	^1H	^{13}C	^1H
1	142.9 d	7.37 br s	142.9 d	7.37 br s
2	111.2 d	6.32 br s	112.2 d	6.32 br s
3	125.8 s	—	125.8 s	—
4	24.2 t	3.73 br s	24.2 t	3.73 br s
5	153.9 s	—	154.0 s	—
6	107.4 d	5.91 br s	107.2 d	5.91 br s
7	121.3 s	—	121.4 s	—
8	25.4 t	2.38 t (7.8)	24.8 t	2.33 t (7.6)
9	28.3 t	2.20 m	29.7 t	1.60 m
10	124.0 d	5.13 t (7.0)	31.4 t	2.03 m
11	135.4 s	—	135.3 s	—
12	39.6 t	2.05 m	124.8 d	5.10 t (7.1)
13	25.2 t	1.95 m	28.2 t	1.95 m
14	36.4 t	1.35 m	37.1 t	1.35 m
15	28.8 d	2.08 m	28.6 d	2.08 m
16	47.5 t	2.54 dd (17.0, 5.6) 2.42 dd (17.0, 7.9)	47.4 t	2.54 dd (17.0, 5.6) 2.42 dd (17.0, 7.8)
17	208.8 s	—	208.8 s	—
18	80.2 d	4.30 m	80.2 d	4.30 m
19	57.5 d	4.30 m	57.5 d	4.30 m
20	19.3 q	1.44 d (6.7)	19.3 q	1.44 d (6.7)
21	139.7 d	7.29 br s	139.7 d	7.29 br s
22	137.4 d	7.09 br s	137.4 d	7.10 br s
23	15.9 q	1.56 br s	23.3 q	1.67 br s
24	19.9 q	0.91 d (6.7)	19.8 q	0.91 d (6.7)

[a]Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses.

trum. The configuration of the trisubstituted double bond was assigned as *E* by ^1H- and ^{13}C-nmr chemical shifts (δ 1.56 and 15.9 for ^1H and ^{13}C, respectively) of vinyl methyl.

With the exception of groups in the vicinity of the C-11 double bond, the ^1H- and ^{13}C-nmr spectra of **1** and **3** were very similar. The COSY-45 spectrum of **3** showed that the C-8 methylene (δ 2.33) was correlated with the methylene at δ 1.60, which was also correlated with the vinylic methylene at δ 2.03 and long-range correlated with the vinylic proton (δ 5.10), suggesting that the double bond was between the C-11 and the C-12. The vinyl methyl of **3** had ^1H- and ^{13}C-nmr shifts of δ 1.67 and 23.3, which indicated that the double bond geometry must be *Z*.

Compounds **4** and **5** showed the same molecular formula $C_{25}H_{30}O_5$ (hrms); their ir (3148, 1736, 1630 cm^{-1} for **4**; 3150, 1735, 1630 cm^{-1} for **5**) and uv (264 nm for **4**; 266 nm for **5**) spectra showed the presence, in both compounds, of a tetronic acid moiety. All spectral data of **4** and **5** are in excellent agreement with published values (4,6).

The toxicity of compounds **1–5** was tested in the *Artemia salina* shrimp bioassay (3). The norsesterterpenes (**1** and **3**) showed less activity (LD_{50} 8.2, 8.6 μg/ml) than ircinin 1 and ircinin 2 (LD_{50} 2.4 and 2.7 μg/ml, respectively).

The finding of these norsesterterpenes supports the biogenetic hypothesis (7) that the C-21 furanoterpenes are derived from sesterterpenes by loss of four C atoms. In fact, we can suppose that **1** and **3** are the first stage of degradation of the sesterterpenes **4** and **5**, through introduction of a chlorine atom by action of a chloroperoxidase, then hydro-

lysis of the lactone ring and subsequent decarboxylation to produce keto-chlorohydrins, which can be easily degraded giving the C-21 terpenes.

The finding of halogenated terpenes is usual in algae, while extremely rare in marine sponges. To our knowledge there are only a few reports of chlorinated terpenes from sponges, all isolated from the sponge *Acanthella* sp. (8–10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained on a Varian DMS 90 spectrophotometer. Ir spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter, using a 10-cm microcell. Low-resolution and high-resolution mass spectra were recorded on an AEI MS-50 spectrometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker WM 500 instrument, under Aspect 2000 control. The 2D nmr spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Preparative hplc purifications were carried out on a Waters apparatus equipped with two μ-Porasil columns (7.8 mm i.d. × 30 cm) and with a refractive index detector.

EXTRACTION AND ISOLATION OF COMPOUNDS.—The *I. oros*, collected by hand at about 8 m depth at Rovinj, Yugoslavia, in May 1988 and 1989, was frozen at −20° until extracted. A voucher specimen is maintained in the collection of the Italian institute. The frozen sponge (300 g dry wt after extraction) was extracted with Me_2CO, and after elimination of the solvent in vacuo, the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The extracts were submitted to the brine shrimp assay (3). The active Et_2O extract was evaporated in vacuo to obtain a brown oil (13 g), which was applied on a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40–70°) to Et_2O.

Fractions with the same tlc profile were combined. Two Ehrlich-positive fractions were recovered. The less polar fraction was subjected to preparative hplc (*n*-hexane), yielding **1** (18 mg) and **3** (8 mg), while the second fraction (4.8 g) was only in part subjected to preparative hplc [*n*-hexane–Et_2O (9:1)], yielding **4** and **5**.

Compound **1**.—$[\alpha]^{25}D$ +34 (c = 1.3, $CHCl_3$); ir ν max (liquid film) 3400, 1713, 1545, 1380, 1150, 1115, 1023 cm^{-1}; eims *m/z* (%) $[M+2]^+$ 422 (12), $[M]^+$ 420.2083 ($C_{24}H_{33}ClO_4$ requires 420.2082) (49), $[M-HCl]^+$ 384 (8), 341 (6), $[M-C_5H_5O]^+$ 339 (13), 303 (6), 215 (30), 175 (12), 162 (100), 161 (14); ^{1}H and ^{13}C nmr see Table 1.

Compound **3**.—$[\alpha]^{25}D$ +40 (c = 0.7, $CHCl_3$); ir ν max (liquid film) 3400, 1715, 1545, 1383, 1148, 1114, 1022 cm^{-1}; eims *m/z* (%) $[M+2]^+$ 422 (10), $[M]^+$ 420.2084 ($C_{24}H_{33}ClO_4$ requires 420.2082) (47), $[M-HCl]^+$ 384 (7), 341 (5), $[M-C_5H_5O]^+$ 339 (13), 303 (5), 229 (20), 188 (18), 162 (100), 161 (15); ^{1}H and ^{13}C nmr see Table 1.

Ircinin 1 [**4**].—Uv λ max (MeOH) 264 (ε 11,800); ir ν max (liquid film) 3148, 1736, 1630 cm^{-1}; eims *m/z* $[M]^+$ 410.2096 ($C_{25}H_{30}O_5$ requires 410.2094).

Ircinin 2 [**5**].—Uv λ max (MeOH) 266 (ε 11,500); ir ν max (liquid film) 3150, 1735, 1630 cm^{-1}; eims *m/z* $[M]^+$ 410.2095 ($C_{25}H_{30}O_5$ requires 410.2094).

Acetylation of **1**.—A solution of **1** (8 mg) in pyridine (2 ml) and Ac_2O (0.2 ml) was kept at room temperature overnight. The excess reagents were removed in vacuo, and the residue was partitioned between H_2O and Et_2O. The Et_2O extract was dried over anhydrous Na_2SO_4, and the solvent was evaporated. The residue was purified by Si gel column [petroleum ether-Et_2O (95:5)], yielding **2** (6 mg): ir ν max (liquid film) 1750, 1713, 1545, 1460, 1380 cm^{-1}; eims *m/z* (%) $[M+2]^+$ 464 (10), $[M]^+$ 462 (30), 426 (6), 404 (5), 402 (15), 337 (8), 215 (15), 175 (6), 162 (100), 161 (15); ^{1}H-nmr ($CDCl_3$) δ 7.37 (1H, br s), 7.29 (1H, br s), 7.09 (1H, br s), 6.32 (1H, br s), 5.91 (1H, br s), 5.19 (1H, d, J = 4.4 Hz), 5.13 (1H, t, J = 7.0 Hz), 4.38 (1H, m), 3.73 (2H, s), 2.57 (1H, dd, J = 17.8, 5.3 Hz), 2.39 (2H, t, J = 7.3 Hz), 2.31 (1H, dd, J = 17.8, 7.8 Hz), 2.20 (2H, m), 2.16 (3H, s), 2.04 (3H, m), 1.95 (2H, m), 1.56 (3H, br s), 1.51 (3H, d, J = 6.8 Hz), 1.35 (2H, m), 0.88 (3H, d, J = 6.6 Hz); ^{13}C nmr ($CDCl_3$) δ 204.7 (s), 169.9 (s), 153.9 (s), 142.9 (d), 139.7 (d), 137.4 (d), 135.6 (s), 125.8 (s), 123.9 (d), 121.3 (d), 111.2 (d), 107 (d), 80.6 (d), 54.5 (d), 48.1 (t), 39.7 (t), 36.4 (t), 28.4 (t), 28.2 (d), 25.4 (t), 25.2 (t), 24.2 (t), 20.6 (q), 20.1 (q), 19.8 (q), 14.1 (q).

BIOLOGICAL EVALUATIONS.—The brine shrimp lethality assay, performed as described by Meyer *et al.* (3) in the Naples laboratory, gave **1** LD_{50} = 8.2 μg/ml, **3** LD_{50} = 8.6 μg/ml, ircinin 1 [**4**] LD_{50} = 2.4 μg/ml, and ircinin 2 [**5**] LD_{50} = 2.7 μg/ml.

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