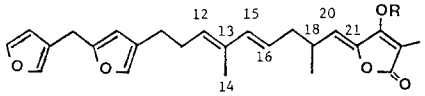
Tetrahedron Letters,Vol.25,No.43,pp 4941-4942,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

BIOACTIVE MARINE METABOLITES V.[1] TWO NEW FURANOSESTERTERPENES, INHIBITORS OF CELL DIVISION OF THE FERTILIZED STARFISH EGGS, FROM THE MARINE SPONGE *CACOSPONGIA SCALARIS*

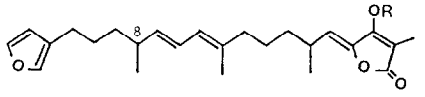
N. Fusetani,* Y. Kato, S. Matsunaga and K. Hashimoto
Laboratory of Marine Biochemistry, Faculty of Agriculture,
The University of Tokyo, Bunkyo-ku, Tokyo (Japan)

Abstract: Two new furanosesterterpenes have been isolated from the marine sponge *C. scalaris* as inhibitors of the cell division of fertilized starfish eggs.

Marine sponges of the family Thorectidae are known to be rich in linear or pentacyclic sesterterpenoids.[2] In the course of our search for biologically active metabolites from Japanese marine invertebrates, we found that the lipophilic extract of a marine sponge *Cacospongia scalaris* (Thorectidae) collected at the Izu Peninsula, 150 km southwest of Tokyo, had significant activity in the starfish egg assay.[3] From the sponge we have isolated two active principles, which were identified a double bond derivative of the ircinins[4] and a double bond isomer of fasciculatin[5] (1 and 2, respectively).



1: R=H
3: R=Ac



2: R=H
4: R=Ac

The ethanol extract of the frozen sponge (1.5 kg) was partitioned between CH_2Cl_2 and water. The organic phase was purified by silica gel and Toyopearl HW-40 (Toyo soda) column chromatographies to obtain the active material. Further separation by HPLC on LS 310 SIL (Toyo soda) yielded 1 and 2, both of which inhibited cell division of the fertilized starfish (*Asterina pectinifera*) eggs at 1.0 μg/mL.

Compound 1 is a colorless oil possessing a conjugated double bond and a conjugated tetronic acid moiety,[4] which were inferred from λ_{max}^{EtOH} 234 (ε 36,000) and 265 sh (ε 22,000) nm, the latter being shifted to 312 nm upon addition of KOH, and by ν_{max}^{KBr} 3500, 1745 and 1635 cm^{-1}. It was easily converted to the monoacetate 3 ($[\alpha]_D = +94.2°$) which was more stable. A molecular formula of $C_{27}H_{30}O_6$ was established for 3 by HRMS (M^+, *m/z* obsd: 450.2049, calcd: 450.2042). The ^1H NMR spectrum of 3[6] revealed three α-protons (δ 7.37,m; 7.29,brs; 7.09,brs) and two β-protons (δ 6.32, brs; 5.91,brs) of two furan rings, a 2-methyl-1,4-disubstituted butadiene moiety [δ 6.04 (d, 15.4 Hz), 5.47 (dt, 15.4, 7.5 Hz), 5.39 (brt, 7.0 Hz), 1.68, brs], an acetate of a conjugated tetronic acid [δ 5.08 (d, 10.0 Hz), 2.35,s; 1.82,s], a doubly allylic isolated methylene (δ 3.73,brs), an allylic methine substituted by a methyl group [δ 2.90,m; 1.70 (d, 7.0 Hz)], and three allylic methylenes (δ 2.42,brt; 2.34,m; 2.18,m). ^1H NMR analyses including double resonance and difference nOe experiments allowed the assignment of all proton signals and secured the structure

of 3. The geometry of the $\Delta^{15,16}$ double bond was determined as *E*, judging from a coupling constant (15.7 Hz) between C-15 and C-16 protons. Irradiation of the C-14 methyl protons enhanced the C-11 methylene signal (δ 2.34) but did not affect the C-12 olefinic proton, thus assigning the $\Delta^{12,13}$ double bond to the *E* configuration, which was also supported by the C-14 methyl signal at δ 12.5q[7] in the ^{13}C NMR. The proton at C-20 appeared at δ 5.32 in 1, but underwent significant upfield shift to δ 5.08 upon acetylation; other signals experienced no fluctuation. This established the *Z* configuration of the $\Delta^{20,21}$ double bond. However, the absolute configuration at C-18 remained unknown.

Compound 2 also possessed a conjugated tetronic acid moiety as estimated from the UV spectrum (λ_{max} 265 nm). The ^{1}H NMR spectrum of the acetate 4 ($[\alpha]_D = -34.7°$) included three protons on a furan ring (δ 7.34, brs; 7.20, brs; 6.25, brs), a 1-methyl-1,4-disubstituted butadiene moiety [δ 6.17 (dd, 15.0, 11.1 Hz), 5.76 (d, 11.1 Hz), 5.44 (dd, 15.0, 8.1 Hz), 1.55, s] and an acetate of a conjugated tetronic acid moiety [δ 5.03 (d, 10.0 Hz), 2.35, s; 1.70, s]. Ozonolysis of 4 followed by oxidative work-up and methylation afforded 2-methyl-hexanedioic acid dimethyl ester and 2-methyl-6-oxoheptanoic acid methyl ester, which confirmed the presence of the $\Delta^{10,11}$ and $\Delta^{12,13}$ double bonds. A large coupling constant (15.0 Hz) observed between the C-10 and C-11 protons in 4 suggested *E* geometry of the $\Delta^{10,11}$ double bond. The geometries of the $\Delta^{12,13}$ and $\Delta^{20,21}$ were deduced to be *E* and *Z*, respectively, in the same way as mentioned above; the configurations at C-8 and C-18 are to be studied.

Although several linear sesterterpene tetronic acids have been isolated from sponges of the family Thorectidae,[2] this is the first isolation of this type of compound from the genus *Cacospongia*. Our specimen also contained ircinin-1 and -2, which were reported from the Mediterranean *Ircinia oros*.[4] Compound 1 and 2 inhibited the cell division of starfish eggs at low concentrations without microtubule assembly as reported for variabilin.[8] They also inhibit the growth of *S. aureus*, *P. aeruginosa*, *B. subtilis* and *M. smegmatis*. It is likely that these furanosesterterpenes are involved in defense mechanism in the sponge.

Acknowledgement: Thanks are due to Professor Paul J. Scheuer for reading this manuscript. We are also indebted to Dr. T. Hoshino, the Mukaishima Marine Biological Station of Hiroshima University, for the identification of the sponge and to Prof. N. Otake and Mr. K. Furihata, Institute of Applied Microbiology of this University, for the measurement of the 400 MHz ^{1}H NMR spectra.

REFERENCES and NOTES

1. Part IV: S. Matsunaga, N. Fusetani and S. Konosu, *J. Nat. Prod.*, submitted.
2. P. R. Bergquist and R. J. Wells, *"Marine Natural Products: Chemical and Biological Perspectives"*, vol. 5. P. J. Scheuer ed., Academic Press, New York, 1983, p. 1 ff.
3. S. Ikegami, K. Kawada, Y. Kimura and A. Suzuki, *Agric. Biol. Chem.*, 43, 161 (1979).
4. G. Cimino, S. De Stefano and L. Minale, *Tetrahedron*, 28, 333 (1972).
5. F. Cafieri, E. Fattorusso, C. Santacroce and L. Minale, *Tetrahedron*, 28, 1579 (1972).
6. Acetate 3: ^{13}C NMR ($CDCl_3$): δ 168.5s(C-22), 165.4s(-$OCOCH_3$), 154.3s, 154.0s(C-6, 25), 142.8d(C-1), 142.3s(C-21), 139.7d, 137.5d(C-4, 9), 136.9d(C-15), 133.9s, 130.2s(C-3, 13), 125.5s(C-8), 124.3d(C-12), 121.4d(C-16), 115.8d(C-20), 114.7s(C-23), 111.2d(C-2), 107.3d (C-7), 40.1t(C-17), 31.6d(C-18), 28.6t, 25.0t(C-10, 11), 24.1t(C-5), 20.4q(-$OCOCH_3$), 20.0q(C-19), 12.5q(C-14), 8.4q(C-24).
7. J. M. Clough and G. Pattenden, *J. Chem. Soc. Perkin I*, 3011 (1983).
8. R. S. Jacobs, S. White and L. Wilson, *Fed. Proc.* 40, 26 (1981).

(Received in Japan 25 June 1984)