Regarding the possibility of compression of the blood vessels in the brood patch, it is seen that, in the case of 4 eggs in the nest, the pressure per cm^2 on the skin equals 14.7 mm Hg, which is far below the ordinary capillary blood pressure of 30 mm Hg. So, during natural incubation in the domestic hen, where the clutch size usually exceeds 4 eggs, the pressure from sitting on the eggs should not interfere with the circulation and the delivery of heat to the brood patch.

In order to assess whether sensory input from the brood patch is involved in controlling the weight that the hen applies to the eggs during incubation, experiments with anesthetized brood patches were made. After intra-cutaneous injection of 1.5–2.0 ml 1% lidocaine along the lateral margins of the brood patch, the incubation weight recorded with 4 eggs in the nest was 293 ± 24 g ($n = 9$), which is significantly higher than the value recorded without local anesthesia ($p < 0.02$). Although cutaneous sensory receptors were blocked, the hens apparently did not lose complete control over the tightness of sit, which suggests that more deeply located receptors in the breast and perhaps also mechanoreceptors in the feet may be involved in the control.

The technique used in the present study was very simple, but the experiments have nevertheless provided some basic knowledge about the weight that the hen applies to her eggs during incubation. By using more elaborate experimental techniques, such as an automatic and continuous recording system, it should be possible to obtain information regarding changes in the tightness of sit during the entire incubation period, or more specifically around the time of hatching. Furthermore, by using artificial eggs, it could also be established whether the shape of the eggs or their surface architecture have any effect on the tightness of sit.

1 Drent, R., in: Avian Biology, vol. 5, pp. 333–420. Eds D.S. Farner and J.R. King. Academic Press, New York 1975.
2 Tøien, Ø., Aulie, A., and Steen, J.B., J. comp. Physiol. *B 156* (1986) 303.
3 Gabrielsen, G., and Steen, J.B., Acta physiol. scand. *107* (1979) 273.
4 Midtgård, U., Sejrsen, P., and Johansen, K., J. comp. Physiol. *B 155* (1985) 703.
5 Davies, S.D., Williams, J.B., Adams, W.J., and Brown, S.L., Auk *101* (1984) 556.
6 Drent, R.H., Postuma, K., and Joustra, T., Behaviour, Suppl. *17* (1970) 237.
7 Haftorn, S., Ornis scand. *10* (1979) 220.
8 White, F.N., and Kinney, J.L., Science *186* (1974) 107.

0014-4754/87/11/121232-02$1.50 + 0.20/0

Isolation of a hexaprenylhydroquinone sulfate from the marine sponge *Dysidea* sp. as an H,K-ATPase inhibitor[1]

N. Fusetani, M. Sugano, S. Matsunaga, K. Hashimoto, H. Shikama*, A. Ohta* and H. Nagano*

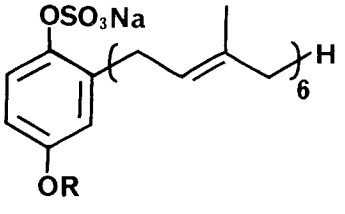
*Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo (Japan), and *Central Research Laboratories, Yamanouchi Pharmaceutical Co. Ltd, Itabashi-ku, Tokyo (Japan), 16 March 1987*

Summary. A hexaprenylhydroquinone sulfate has been isolated as an H,K-ATPase inhibitor from a marine sponge *Dysidea* sp. It also inhibited phospholipase A_2 as well as secretion of gastric acid in rats.
Key words. Marine sponge; *Dysidea*; hexaprenylhydroquinone sulfate; H,K-ATPase inhibitor; gastric secretion.

Little is known of pharmacologically active principles of marine origin, though a high incidence of activity has been reported in the extracts of marine organisms[2–4]. In the course of our search for bioactive substances from Japanese marine invertebrates, we found that the lipophilic extract of a sponge of the genus *Dysidea*, which was collected in Hachijo-jima Island of the Izu Archipelago, inhibited H,K-ATPase in an in vitro assay[5]. From the sponge we have isolated the active principle, which was identified as a hexaprenylhydroquinone sulfate.

The ethanol extract of the frozen sponge (1 kg) was partitioned between water and diethyl ether. The ether soluble materials (9.81 g) were chromatographed on a silica gel column with chloroform-methanol-water (88:12:1, followed by 90:20:1). The active fractions were collected and subjected to HPLC on a YMC-ODS column (Yamamura Chem. Co.) with 30% aq. CH_3CN to yield 144 mg of pure active compound (**1**) as a colorless gum.

Compound **1** had a molecular formula of $C_{36}H_{53}O_5SNa$ which was established by FABMS[$(m/z$ 643$(M + Na)^+$, 659$(M + K)^+$] as well as combustion analysis (C, 67.66; H, 8.32; S, 5.74%). The UV spectrum[λ_{max} 281nm(ε 2200)] was reminiscent of a hydroquinone chromophore[6], while the IR absorption at 1240 and 840 cm^{-1} indicated that it contained a sulfate moiety[7]. The ^{1}H-NMR and ^{13}C-NMR spectra implied the presence of a monosubstituted hydroquinone[δ 7.19(1H,d,J = 8.0Hz), 6.65 (1H,d,J = 3.0Hz) 6.55(1H,dd, J = 8.0,3.0Hz); 153.7s(C–4), 121.0s(C–2), 113.6d(C–5), 142.8s(C–1), 116.8d(C–3), 131.1d(C–6)][8], and an hexaprenyl moiety [δ 138.0s, 136.6s, 135.4s, 135.1s, 134.9s(2C), 124.3d(3C), 124.0d(2C), 123.1d, 39.8t(6C), 26.9t(5C), 25.7t, 17.7q, 16.0q(5C)][9]. These structural features were further established by EI mass fragment ions at m/z 518, 449, 381, 313, 245, 177 and 109. Detailed NMR studies including NOE and LSPD[10] experiments led to unambiguous assignment of



1 R = H
2 R = Me

the hexaprenylhydroquinone sulfate structure. To determine the position of the sulfate, **1** was treated with CH_3I and anhydrous potassium carbonate in acetone[8] to give rise to the methyl ether (**2**) which was studied by nuclear Overhauser enhancement difference spectroscopy. Irradiation of the O-methyl signal at δ 3.75(s, 3H) enhanced the *ortho* protons at C–3 (δ 6.70) and C–5 (δ 6.65), indicating that the sulfate must

be placed at C-1. Incidentally, non-sulfated polyprenylhydroquinones were isolated from the Mediterranean sponge *Ircinia spinosula*[11].

Compound **1** is inhibitory not only against H,K-ATPase with an IC_{50} of 4.6×10^{-6} M but also against secretion of gastric acid in rats (po. 54% inhibition at 300 mg/kg). However, no antiulcer activity in rats was observed at 300 mg/kg. It should also be noted that **1** inhibits phospholipase A_2 with an IC_{50} of 1.8×10^{-6}M[12].

Acknowledgments. We thank Professor Paul J. Scheuer, University of Hawaii, for reading this manuscript. We are indebted to Dr T. Hoshino of the Mukaishima Marine Biological Station of Hiroshima University for identification of the sponge, and to Messrs M. Shimizu and H. Kaniwa of the Central Research Laboratories of Yamanouchi Pharmaceutical Co. Ltd. for measurements of HREIMS and NMR spectra, respectively.

1 Bioactive Marine Metabolites XVIII; Part XVII: Fusetani, N., Yasukawa, K., Matsunaga, S., and Hashimoto, K., Tetrahedron Lett. *28* (1987) 1187.
2 Kaul, P.N., Kulkarni, S.K., Weinheimer, A.J., Schmitz, F.J., and Karns, T.K.B., Lloydia *40* (1977) 253.
3 Endo, M., Nakagawa, M., Hamamoto, Y., and Ishihama, M., Pure appl. Chem. *58* (1986) 387.
4 Andersson, L., Lingren, G., Bohlin, L., Magni, L., Ogren, S., and Afzelius, L., Acta pharm. swed. *20* (1983) 401.
5 Fiske, C.H., and Subbarow, Y., J. biol. Chem. *66* (1975) 375.
6 Scott, A.I., in: Interpretation of the Ultra-Violet Spectra of Natural Products, p. 91. Pergamon Press, London 1964.
7 Pouchert, C.J., The Aldrich Library of Infrared Spectra, 2nd edn., p. 481. Aldrich Chem. Co., Milwaukee 1975.
8 Ochi, M., Kotsuki, H., Muraoka, K., and Tokoroyama, T., Bull. chem. Soc. Japan *52* (1979) 629.
9 Bowden, B.F., and Coll, J.C., Aust. J. Chem. *34* (1981) 2667.
10 Seto, H., Sasaki, T., Yonehara, H., and Uzawa, J., Tetrahedron Lett. *1978*, 923.
11 Cimino, G., De Stefano, S., and Minale, L., Tetrahedron *28* (1972) 1315.
12 Rothhut, B., Russo-Marie, F., Wood, J., DiRosa, M., and Flower, R.J., Biochem. biophys. Res. Commun. *117* (1983) 878.

0014-4754/87/11/121233-02$1.50 + 0.20/0

(+)-Curcuphenol and dehydrocurcuphenol, novel sesquiterpenes which inhibit H,K-ATPase, from a marine sponge *Epipolasis* sp.[1]

N. Fusetani, M. Sugano, S. Matsunaga and K. Hashimoto

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo (Japan), 21 April 1987

Summary. Two H,K-ATPase inhibitors and an inactive related compound have been isolated from a marine sponge *Epipolasis* sp. They are aromatic sesquiterpene α-curcumenes.
Key words. Marine sponge; *Epipolasis;* curcuphenol; H,K-ATPase inhibitor; gastric secretion.

In the course of our search for bioactive metabolites from Japanese marine invertebrates, we found that the lipophilic extract of a marine sponge *Epipolasis* sp.[2] collected on Hachijojima Island of the Izu Archipelago showed strong inhibitory activity against H,K-ATPase[3]. From this sponge we have isolated two active substances, (+)-curcuphenol (**1**) and dehydrocurcuphenol (**3**), and a closely-related inactive sesquiterpene (**4**).

The ethanol extract of the frozen animal (1 kg) was partitioned between ether and water. The ether layer was subjected to silica gel column chromatography (*n*-hexane/ethyl acetate) followed by gel-filtration on Toyopearl HW-40S (Toyo Soda Co.) with $CHCl_3$-MeOH (1:1). The main active fraction was purified by reversed-phase HPLC on a YMC ODS column (Yamamura Chem. Co.) with 30% aq CH_3CN to give **1** (300 mg) and **3** (13 mg), both as yellowish oils.

Compound **1**, $[\alpha]_D^{23}$ +29.1°, (*c* 3.13, $CHCl_3$), possessed the molecular formula $C_{15}H_{22}O$ which was established by high resolution EIMS (*m/z* 218.1662, *Δ* −0.7 nm). An IR band at 3450 cm^{-1} together with characteristic UV absorption at λ_{max}(MeOH) 278 nm (ε 2500) indicated the presence of a phenol[4]. The ^1H NMR spectrum consisted of a secondary methyl (*δ* 1.21, 3H, br d, *J* = 7 Hz), two olefinic methyls (*δ* 1.65, 1H, s; 1.50, 1H, s), two methylenes (*δ* 1.90, 4H, m), a methine (*δ* 3.00, 1H, m), a trisubstituted double bond (*δ* 5.15, 1H, dd, *J* = 7, 7 Hz) and three aromatic signals for a 1,2,4-trisubstituted benzene ring (*δ* 6.60, 1H, br s; 6.70, 1H, br d, *J* = 7 Hz; 7.05, 1H, d, *J* = 7 Hz), which were supported by the ^{13}C NMR data (table). These structural features were in good agreement with those reported for (−)-curcuphenol (**2**)[5], an antimicrobial sesquiterpene, isolated from a Caribbean gorgonian *Pseudopterogorgia rigida,* $[\alpha]_D$ −7.0°. Recently, an $[\alpha]_D$ value of −23.6° was reported for synthetic (−)-curcuphenol[6], which indicates that our compound must possess the opposite (*S*) configuration at C-7. Therefore, **1** is (*S*) or (+)-curcuphenol.

Compound **3**, $[\alpha]_D^{23}$ −1.2° (*c* 0.48, $CHCl_3$), showed λ_{max}(MeOH) 232 (ε 9600) and 278 nm (ε 2500), indicating the presence of a diene chromophore in addition to a phenol system[4]. The molecular ion peak at *m/z* 216 in EIMS together with other spectral data suggested the dehydrocurcuphenol structure. The ^1H NMR spectum (table) including intensive decoupling experiments led to 8,9-dehydrocurcuphenol for