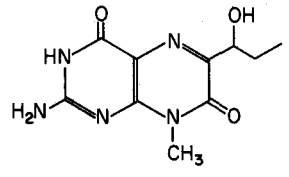
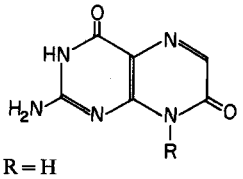
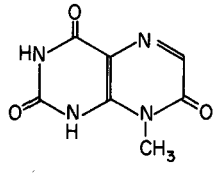
Compound **8** (m.p. > 350 °C; $[a]_D^{20} + 113.1°$, c = 0.011 in methanol; IR (KBr pellet): 3400 - 3300, 2920, 1665, 1640, 1620, 1510, 1450, 1405 cm^{-1}) is strongly fluorescent when viewed under UV-light; its UV-spectrum [λ_{max}^{MeOH} (log ε): pH = 7, 344 (4.17), 295 (3.98), 225 (4.19) nm; pH = 10, 360 (4.18), 278 sh (3.50), 260 (4.04), 220 (4.02) nm] was indicative of a pterin[7]; the molecular formula obtained by high resolution mass spectrometry was $C_{10}H_{13}N_5O_3$ ($M^{+\cdot}$ 251.0657, calculated 251.0663). The EI mass spectrum indicated facile loss of H_2O and C_2H_5. 10 carbon signals were visible in the ^{13}C-NMR-spectrum [80 MHz, DMSO-d_6: δ 175.5 (s), 168.7 (s), 164.9 (s), 160.1 (s), 156.3 (s), 119.8 (s),



8



11 R = H
12 R = CH_3



13

79.4 (d), 36.9 (q), 36.4 (t), 19.4 (q)]. The ^1H-NMR data [90 MHz, DMSO-d_6; δ 0.90 (3,t), 1.85 (2,m), 3.43 (3,s), 4.72 (2,br; + D_2O: 1,dd), 7.25 (2,br; exchange with D_2O)] indicated a 1-hydroxypropyl side chain and an N-methyl grouping. Compound **8** formed a diacetate (acetic anhydride-pyridine, RT) whose ^1H-NMR spectral characteristics were completely consistent with the structural features described above. We propose structure **8** [6-(1′hydroxypropyl)-8-methylisoxanthopterin] for this pterin on the basis of extensive comparison of its spectral data with those reported[7-9] for several similar compounds. It bears close resemblance to the well known isoxanthopterin **11** and its 8-methyl derivate **12**[7], as well as to luciopterin (**13**), reported to occur in *Luciola cruciata,* a Japanese firefly[10]. It differs quite clearly, however, from the as yet incompletely characterized fluorescent compound 'luciferesceine', isolated from *P. pyralis* by Strehler[11].

The characterization of **6** and **7** completes our study of the distasteful principles of *P. pyralis.* It is interesting to note that **6** is the unesterified parent of lucibufagins **1-5.** Although **6** was consistently detected in the HPLC analysis of a large number of fresh extracts of freeze-dried individual specimens and is most likely a natural component, **7** (which was found in much smaller quantities than **1-6**) may be an artifact arising from isomerization during isolation, especially in view of the fact that 11a-hydroxy-12-oxo-bufadienolides are partially isomerized to their 11-oxo-12a-hydroxy counterparts on alumina[6].

1 Acknowledgment. We are grateful to the Fonds National Suisse de la Recherche Scientifique (bourse de relève to M.G.). Partial support of this work by the NIH (grants No. AI 12020 and AI 02908), the NSF (grant No. PCM-77-25807), and the American Heart Association is acknowledged with pleasure. Paper No. 66 of the series Defense Mechanism of Arthropods.

2 To whom reprint requests should be addressed.
3 J. Meinwald, D. Wiemer and T. Eisner, J. Am. chem. Soc. *101,* 3055 (1979).
4 T. Eisner, D. Wiemer, L. Haynes and J. Meinwald, Proc. natl Acad. Sci. USA *75,* 905 (1978).
5 M. Goetz, D. Wiemer, L. Haynes, J. Meinwald and T. Eisner, Helv. chim. Acta *62,* 1396 (1979).
6 K. Huber, H. Linde and K. Meyer, Helv. chim. Acta *50,* 1994 (1967).
7 W. Pfleiderer and E. Taylor eds., Pteridine Chemistry. Macmillan Company, New York 1964.
8 R. Blakley, The Biochemistry of Folic Acid and Related Pteridines. Elsevier, Amsterdam 1969.
9 A. Dieffenbacher, R. Mondelli and W. von Philipsborn, Helv. chim. Acta *49,* 1355 (1966); A. Dieffenbacher and W. von Philipsborn, Helv. chim. Acta *52,* 743 (1969).
10 Y. Kishi, S. Matsuura, S. Inoue, O. Shimomura and T. Goto, Tetrahedron Lett. *1968,* 2847.
11 B. Strehler, Archs Biochem. Biophys. *32,* 397 (1951).

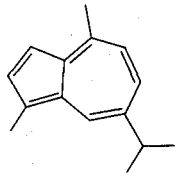
Bioactive marine metabolites I. Isolation of guaiazulene from the gorgonian *Euplexaura erecta*[1]

N. Fusetani, S. Matsunaga and S. Konosu

Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo (Japan), 12 December 1980

Summary. The isolation and identification of guaiazulene from the gorgonian *Euplexaura erecta* is described.

In recent years sesqui- and diterpenes have been isolated from gorgonians and soft corals[2]. Some of them possess antibacterial, antifungal, antineoplastic, or ichthyotoxic activity[3,4]. In the course of our study on antimicrobial substances from Japanese marine invertebrates we found that the ether soluble material from a methanol extract of the gorgonian *Euplexaura erecta* showed mild activity against *Pseudomonas aeruginosa.* We have isolated from this extract guaiazulene (**1**) as the active substance, which is the subject of this paper.



1

Fresh specimens of *E. erecta* (20 g) collected at Enoshima Island, Kanagawa, Japan, were ground and extracted with diethyl ether. The solvent was removed below 30 °C to yield a brownish blue oil which was chromatographed twice on silica gel columns, first with diethyl ether, then with n-hexane. The characteristic blue band was collected as an active fraction to obtain 40 mg of **1**(0.2% wet weight) as a bluish violet oil. It showed a mild activity against fungi, gram-positive and gram-negative bacteria. The high resolution mass spectrum showed the molecular formula $C_{15}H_{18}$ (m/z 198.1452, req. 198.1408). Combustion analysis data

also supported this formula. Compound **1** showed $\lambda_{max}^{CHCl_3}$ 652(sh), 600, 377, 350, 338(sh), 305(sh), 292, 286 nm; ν_{max}^{film} 3040, 2930, 2840, 1900, 1550, 1520, 1455, 1420, 1385, 1360, 1280, 1220, 1190, 1160, 1045, 1015, 985, 955, 915, 810, 770, 705, 645 cm^{-1}; ^{1}H-NMR (100 MHz, δ, $CDCl_3$) 1.35(d, 7 Hz, 6 H), 2.66(s, 3 H), 2.81(s, 3 H), 3.07(m, 1 H), 6.97(d, 11 Hz, 1 H), 7.21(d, 4 Hz, 1 H), 7.38(dd, 1.5, 11 Hz, 1 H), 7.61(d, 4 Hz, 1 H), 8.18(d, 1.5 Hz, 1 H); ^{13}C-NMR(25 MHz, δ, $CDCl_3$) 12.9(q), 24.0(q), 24.8(q, q), 38.2(d), 112.7(d), 125.0(d), 125.1(s), 133.2(d), 134.8(d), 136.1(d), 136.2(s), 137.3(s), 139.8(s), 144.2(s). These spectral data coincide well with the reported data[5,6] for guaiazulene as does its mass spectrum; m/z 198(M^+, 17%), 183(15), 168(10), 165(8), 155(7), 153(10), 141(9), 128(11), 115(12), 69(14), 28(100). Furthermore, our azulene was identical with an authentic sample (Aldrich Chem. Co.) in every respect.

Guaiazulene has so far been found in the essential oils of terrestrial plants[7,8] and in a marine red alga[9]. To our knowledge this is the first isolation of guaiazulene from an animal, though some sesquiterpenes possessing the guaiane skeleton have been reported from gorgonians[2]. *E. erecta* has brilliant blue polyps, which are exceptional among related gorgonians. Apparently this blue color is due to the occurrence of guaiazulene. It is also likely that guaiazulene plays a defensive role in this coral. It is interesting to speculate on the origin and the mode of concentration of the pigment.

1 We are grateful to Professor Paul J. Scheuer, the University of Hawaii, for reading this manuscript, and to Dr K. Aizawa, Department of Agricultural Chemistry of this university, for the measurement of the high resolution mass spectra. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

2 B. Tursch, J.C. Braekman, D. Daloze and M. Kaisin, in: Marine Natural Products, vol.2, p.247. Ed. P.J. Scheuer. Academic Press, New York 1979.
3 L.S. Ciereszko and T.K.B. Karns, in: Biology and Geology of Coral Reefs, vol.2, Biology I, p.183. Ed. O.A. Jones and R. Endean. Academic Press, New York 1973.
4 Y. Hashimoto, Marine Toxins and Other Bioactive Marine Metabolites. Jap. Scient. Soc. Press, Tokyo 1979.
5 D. Meuche, B.B. Molly, D.H. Reich and E. Heilbronner, Helv. chim. Acta *46*, 2483 (1963).
6 J.R. Llinas, D. Roard, M. Derbesy and E.J. Vincent, Can. J. Chem. *53*, 2911 (1975).
7 A.J. Haagen-Smit, Fortschr. Chem. org. NatStoffe *5*, 40 (1948).
8 T. Nozoe and S. Ito, Fortschr. Chem. org. NatStoffe *19*, 32 (1961).
9 B.M. Howard and W. Fenical, Tetrahedron Lett. *1978*, 2453.

Sapintoxin A, a new biologically active nitrogen containing phorbol ester

S.E. Taylor, M.A. Gafur[1], A.K. Choudhury and F.J. Evans

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39, Brunswick Square, London, WC1N 1AX (England), 28 November 1980

Summary. From the unripe fruits of *Sapium indicum*, an irritant compound, sapintoxin, was isolated. Spectroscopic data together with selective hydrolysis and partial synthesis confirmed sapintoxin as 12-O-[N-methylaminobenzoyl]-13-O-acetyl-4-deoxyphorbol.

Sapium indicum, a well-known Indian poisonous plant and piscicidal agent[2] has previously been investigated for its toxic principles[3]. However only the nonbiologically active substance, sapinine, was isolated. Using a new method of purification which involves highspeed centrifugal liquid chromatography (CLC) followed by neutral adsorption TLC and partition chromatography[4] we have been able to isolate a nitrogen containing phorbol ester from this plant. This compound, present in high yield from the unripe fruits, was termed sapintoxin A and represents the first nitrogen containing phorbol derivative to be isolated which demonstrates biological activity in vivo using an erythema skin test[5].

Unripe fruits were powdered and macerated for 2 weeks with acetone at room temperature. The residue left after removal of acetone below 45 °C was dissolved in 40% methanol and the steroids and lipids removed by partition with hexane. Sapintoxin was obtained as an impure resin after extraction of the methanol-phase with ether. The ether soluble resin was fractionated by CLC using a 4-mm porous silica gel disc at a flow rate of 4 ml/min and eluting in a gradient of hexane-toluene-ethylacetate. Fractions were collected at 2-min intervals and monitored by a UV flow-cell, analytical TLC and a biological test[5]. Fractions 54–59 contained sapintoxin together with yellow pigment and were bulked for further purification. Final purification was achieved using 1stly preparativelayer TLC (silica gel G, 500-µm layers, buffered at pH 7.0, eluant, cyclohexane-toluene-ethylacetate-ether 20-15-40-30) (R_f 0.1) and 2ndly partition chromatography (kieselguhr, 500 µm, buffered pH 7.0, coated with 20% diethylene glycol, eluant, cyclohexane-butanone 70-30) (R_f 0.48). Compound **1**, sapintoxin, yield 250 mg, was shown to be a single substance on the basis of its TLC and mass-spectral characteristics. The spectral data for **1** was as follows:

MS: (electron impact, 70 eV, 210°), m/z 523 (M^+, 4%, $C_{30}H_{37}O_7N$), 373 (5%), 313 (6%), 295 (3%), 151 (100%), 81 (56%); UV: λ_{max} (MeOH), 207 (shoulder, $\varepsilon = 46{,}443$), 222 ($\varepsilon = 49{,}791$), 252 ($\varepsilon = 17{,}155$), 356 ($\varepsilon = 9205$) nm. IR: (solvent chloroform), ν_{max} 3480, 1720, 1685, 1580 cm^{-1}; PMR: (250 MHz, $CDCl_3$, TMS = 0.000 ppm), δ 7.842 (d.d., J = 1.8 and 7.8 Hz, 1 H-aromatic), 7.704 (d, J = 5.05 Hz, *H*N− exchangeable with D_2O), 7.572 (s, 1 H-1), 7.420 (t, J = 7.3 Hz, 1 H-aromatic), 6.695 (d, J = 7.8 Hz, 1 H-aromatic), 6.595 (t, J = 7.8 Hz, 1 H-aromatic), 5.685 (s, 1 H-9, exchangeable with D_2O), 5.643 (d, J = 9.6 Hz, 1 H-12), 5.559 (d, J = 4.13 Hz, 1 H-7), 4.037 (s, 2 H-20), 3.275 (m, 1 H-10), 2.932 (d, J = 5.05 Hz, C*H*$_3$N−), 2.814 (m, 1 H-4), 2.441 (m, 2 H-5), 2.131 (s, 3 H-acetyl), 1.739 (m, 3 H-19), 1.32 and 1.254 (6 H-16, 17), 1.224 (d, J = 5.15 Hz, 1 H-14), 0.958 (d, J = 6.43 Hz, 3 H-18). Irradiation at 7.704 ppm caused the 3 H doublet at 2.932 ppm to collapse to a sharp singlet. A similar change in the doublet at 2.932 ppm occurred when the signal at 7.704 ppm was exchanged with D_2O. **1** was hydrolysed by means of methanolic KOH (0.1 M) at room temperature for 20 min. A single mono-ester **2** was