such as feeding, might put some circadian aspects (e.g., reproduction) in a non-optimum phase with the environment.

Although a multioscillator/multientrainer system of temporal integration remains the simplest and most straight forward explanation of our results we again caution that other explanations, not addressed by the experimental design of this study, are possible[7,13,14]. For example feeding entrained rhythms could be produced by malleable (food-influenced) intervals between a single oscillator and the rhythms it controls. Or, feeding entrained rhythms may be learning phenomena dependant on a light-dark entrained time-keeping oscillator[13,14]. Studies are presently underway in our laboratory to examine this latter hypothesis.

Finally, researchers working with locomotor rhythms should take into account that the general locomotor pattern is composed of different activities (e.g. agonistic and courtship behaviors) and that these activities may be entrained to different stimuli. Ignoring this fact in experimental studies may confound interpretation of results due to noise introduced by differential phase shifts of the different activity components.

1 We thank Mark Goodrich and Steve Huber for fish maintenance and technical assistance; and Don Dovala for aid in developing the automatic feeder. The research was funded in part by NIH, AM 25191 and NIEHS, ES No.01985.

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0014-4754/87/060621-04$1.50 + 0.20/0

2,3-Dihydrolinderazulene, a new bioactive azulene pigment from the gorgonian *Acalycigorgia* sp.

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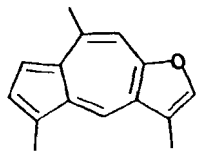
Summary. A new azulene pigment, 2,3-dihydrolinderazulene, has been isolated along with guaiazulene and linderazulene as bioactive metabolites from the gorgonian *Acalycigorgia* sp.

Key words. Gorgonian; *Acalycigorgia* sp.; guaiazulene pigments; antitumor; antifungal.

Gorgonians are rich sources of azulene pigments. Occurrence of linderazulene (**1**) was first reported by Imre et al.[2] from the Marmara Sea gorgonian *Paramuricea chamaeleon*. Fusetani et al.[3] isolated guaiazulene (**2**) from the gorgonian *Euplexaura erecta* collected at Enoshima, Japan. Subsequently, Scheuer's group[4] discovered a number of halogen- and nitrogen-containing guaiazulenes in addition to **1** and **2** from a deep sea gorgonian of Hawaiian waters. In our study of biologically active substances from marine organisms occurring in Okinawan waters, an extract of the gorgonian *Acalycigorgia* sp. showed antitumor activity against P388 mouse leukemia cells. Separation of the extract gave three guaiazulene pigments (**1**–**3**) as active constituents. A sample (600 g) of *Acalycigorgia* sp., collected at Cape Zampa, Okinawa, in April, 1985, was extracted by steeping in acetone. The acetone extract was concentrated, and the resulting aqueous suspension was extracted with ethyl acetate to give 3.4 g of an oil. A part (2.7 g) of the oil was chromatographed on silica gel with heptane-ethyl acetate (10:1) to furnish two portions. The first portion (1.02 g), containing blue pigments, was placed on a bed of reverse phase adsorbent (RP-8) in a sintered glass filter and successively eluted with methanol and acetone. The same filtration was repeated with the methanol eluate to give 320 mg of a mixture containing the pigments. The mixture was then separated on a Lobar Si-60 column with heptane-ethyl acetate (19:1) into 5 fractions. Each of the 3 pigment-containing fractions was further separated by preparative TLC on silica gel (heptane-ethyl acetate 30:1 to 10:1) to give 3 pigments. Purification of these pigments by HPLC (Hibar Si-60, heptane-ethyl acetate 40:1 to 20:1) furnished 93 mg of guaiazulene (**2**) as a blue oil, 7.8 mg of linderazulene (**1**) as purple crystals, m.p. 105.5 °C (lit.[5] m.p. 106–107 °C), and 47 mg of the new compound **3** as a purple oil, $[\alpha]_D$ + 800° (c 0.05, $CHCl_3$).



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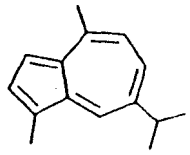

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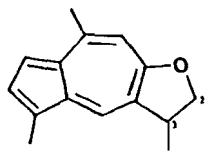

3

Compounds **1** and **2** were identified as linderazulene and guaiazulene, respectively, by comparison of spectral data with those reported for these pigments[2,3]. The molecular formula, $C_{15}H_{16}O$, of the new, optically active pigment (**3**) was deduced from high resolution EIMS[6] at m/z 212.1202 (Δ 0.1 nm). The ^1H NMR signals for the azulene portion [acetone-d_6, δ 8.10 (1 H, s), 7.26 (1 H, d, J = 3.8 Hz), 7.15 (1 H, d, J = 3.8 Hz), 6.69 (1 H, s), 2.73 (3 H, s) 2.57 (3 H, s)] were similar to those of **1**. The remaining resonances, a methyl doublet at δ 1.40 (J = 6.8 H), a methine multiplet at δ 3.72, and methylene double doublets at δ 4.69 (J = 8.8, 8.8 Hz) and 4.14 (J = 8.8, 6.8 Hz), suggested that **3** was 2,3-dihydro-derivative of **1**. The ^{13}C NMR data[6] [δ 77.93 (t, C-2), 39.96 (d, C-3)] also supported this conclusion. Structural confirmation was provided by dehydrogenation of **3** to **1**, as follows. A

mixture of 3 and 10% Pd/C in a long glass tube was heated at 285 °C for 4 min. The product deposited on the tube wall was purified by HPLC to give a purple crystalline compound (yield: 73%) which was identical with linderazulene (1) in all respects. In addition to moderate antitumor activity, compounds 1–3 are antifungal against *Candida albicans*, 3 being most active. Compounds 1 and 3 also show immunostimulatory activity at low concentrations, while 2 exhibits immunodepressant activity.

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6 Low resolution EIMS m/z 212 (M^+, 100), 197 (60), 182 (22), 169 (19), 165 (9), 154 (11), 153 (11), 141 (8), 128 (8), 115 (8), and 106 (6 rel. %). ^{13}C NMR (acetone-d_6) δ 166.23 (s), 146.14 (s), 134.16 (s), 132.68 (s), 131.97 (d), 129.54 (d), 126.75 (s), 124.47 (s), 115.74 (d), 109.53 (d), 77.93 (t), 39.96 (d), 24.40 (q), 20.68 (q), and 12.83 (q).

* We thank Drs M. Lui, P. McCarthy, and G. Faircloth for the biological testings.

0014-4754/87/060624-02$1.50 + 0.20/0

Circadian fluctuation of susceptibility to haloperidol under constant conditions

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Summary. An endogenous circadian rhythm of the sedative and antiapomorphine effects of haloperidol was observed under constant conditions for 7 days as well as under an entrained light-dark cycle.
Key words. Haloperidol; circadian rhythm; sedation; antiapomorphine effect; constant condition.

A circadian rhythm of susceptibility to haloperidol[1], chlorpromazine[2], tetrabenazine[3] and apomorphine[4] has been shown to exist. The rhythm is synchronized to the light-dark (LD) cycle, since it is reversed following the reversal of the cycle[2].

But this alone does not clarify whether this rhythm is due to endogenous causes or is merely a response to the external light-dark cycle. This can be determined by studying whether it persists when conditions such as light, temperature, and humidity, are kept constant. We carried out such a study of the sedative and additionally antiapomorphine effects of haloperidol.

Method. Male S.D. rats weighing 300–400 g were used. They were kept in a quiet dark animal house under controlled lighting conditions with 12 h of artificial light between 19.30 h and 07.30 h and 12 h of complete darkness. For at least 5 weeks before the experiment the temperature of the room was maintained at 25±1 °C, humidity at 55%. Food and water were supplied ad libitum. A dim red light was used during injection.

1) Circadian fluctuation of the sedative effect of haloperidol under constant conditions. The activity of one rat placed in a completely dark sound-proof room was measured with Animex DS (AB FARARD). Haloperidol 0.5 mg/kg (0.05% solution) at a temperature of 24 °C was administered i.p. with the utmost care not to excite the rats. After the administration the sedation period was assessed by the method of Nagayama et al.[3]. The sedation period is defined as the time from the beginning of sedation due to the administration of haloperidol to the time the Animex reads 90 counts/10 min. Haloperidol was administered 0–168 h after rats were placed under constant conditions at one of the following times: 01.30, 07.30, 13.30, 19.30 h. Each rat was used only once in the experiment.

With the control group, under 12:12 LD conditions, a similar experiment was conducted in the sound-proof room. At each administration of haloperidol or saline, a group of 4 rats were used.

2) Circadian fluctuation of the antiapomorphine effect of haloperidol under constant conditions. Under the same conditions as in (1), haloperidol 0.5 mg/kg was administered i.p. to one group, and to the other saline i.p. To both groups, apomorphine 5 mg/kg was administered s.c. 1 h later and the duration of AISB (apomorphine-induced stereotyped behavior) assessed according to the method of Nagayama et al.[4].

Haloperidol or saline was administered 42–60 h after rats were placed under constant conditions at one of the following times: 01.30, 07.30, 13.30, 19.30 h. Each rat was used only once in the experiment (N = 6/group). The AISB duration was compared between the saline- and haloperidol-treated groups, to calculate the AISB inhibition percentage, which was used as an index of the antiapomorphine effect of haloperidol.

With the control group, under 12:12 LD condition, a similar experiment was conducted in the same sound-proof room.

Results and discussion. A significant diurnal fluctuation in the sedative effects of haloperidol was observed under 12:12 LD with the peak at 19.30 and nadir at 07.30 h. A similar rhythm was also observed daily for 7 days under constant conditions (fig 1).

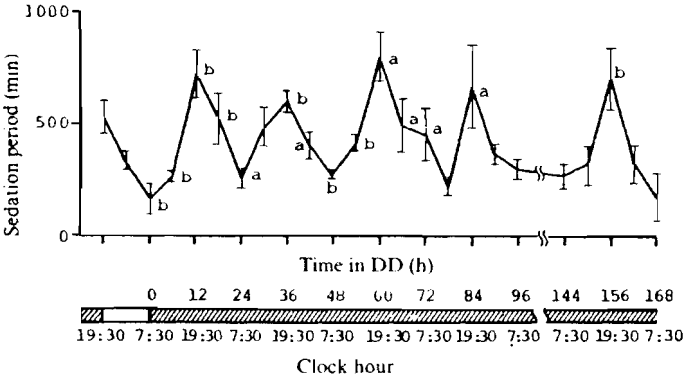

Figure 1. Rhythm of sedative effect of haloperidol under constant conditions. Significant differences were observed in 24 h covering 5 administration times centering on *a* ($p < 0.05$) or *b* ($p < 0.01$) (one-way ANOVA).