cates that the inhibitory effect of the compounds is reversible. On the other hand, the inhibitory effect of a typical cationic detergent, dodecylbenzyltrimethylammonium chloride, was irreversible. Sperm inactivated by pretreatment with the detergent at 0.5 mM was unable to recover its ability to fertilize the eggs even when the detergent concentration was diluted 17-fold. Thus the mechanism of the inhibitory effect of methyl ester derivatives of bestatin and its analogs seems to be different from that of the cationic detergent.

Christen et al.[14,15] have reported that both respiration and motility of sea urchin sperm were inhibited when the intracellular pH was decreased. In a preliminary experiment, we observed that motility of the sperm was inhibited by the addition of bestatin methyl ester. The accumulation in the sperm cell of methyl ester derivatives of bestatin and its analogs, which are cationic in nature like NH_4^+ under physiological conditions, would not decrease the intracellular pH, because NH_4^+ has been reported to elevate this pH[14,15]. It seems probable, therefore, that the action of the compounds used in this study on sperm respiration and motility, resulting in the depression of fertilization, is due to their inhibitory effect on sperm aminopeptidase. Further study will be necessary in order to elucidate the precise function of aminopeptidase in the sperm.

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Gonyauline: A novel endogenous substance shortening the period of the circadian clock of a unicellular alga

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Summary. The circadian clock in the unicellular alga *Gonyaulax polyedra* is accelerated by a substance in extracts from the cells themselves. The extracts have been fractionated using the circadian rhythm of bioluminescence as bioassay. The active substance, termed gonyauline, has been isolated and characterized as a novel low molecular weight cyclopropanecarboxylic acid (*S*-methyl-*cis*-2-(methylthio) cyclopropanecarboxylic acid). Synthetic gonyauline has a similar shortening effect on the period of the circadian clock.
Key words. Circadian rhythm; period length; creatine; *S*-methyl-*cis*-2-(methylthio) cyclopropanecarboxylic acid; *Gonyaulax polyedra.*

Many biological phenomena occur predominantly at a certain time of day. In the unicellular marine dinoflagellate, *Gonyaulax polyedra*, several functions fall into this category. For example, cell division, photosynthesis, bioluminescence, motility and pattern formation show circadian rhythmicity which may persist under constant conditions with a precise period of about 24 h (see controls in fig. 1)[1-4]. The length of the free-running circadian period (τ) can be altered by light intensity[5] and by chemicals including propanol extracts of several eukaryotic organisms[6], and these τ-effects can depend on the spectral composition of the background light[7]. We have recently isolated and identified creatine from mammalian muscle as an effective period-shortening substance in *Gonyaulax*[7].

Here we report the isolation and structural identification of an endogenous period-shortening substance obtained from extracts of *G. polyedra*, termed gonyauline. Synthetic gonyauline has a comparable τ-shortening activity. Along with the proteoglycan gene product of the per-lo-

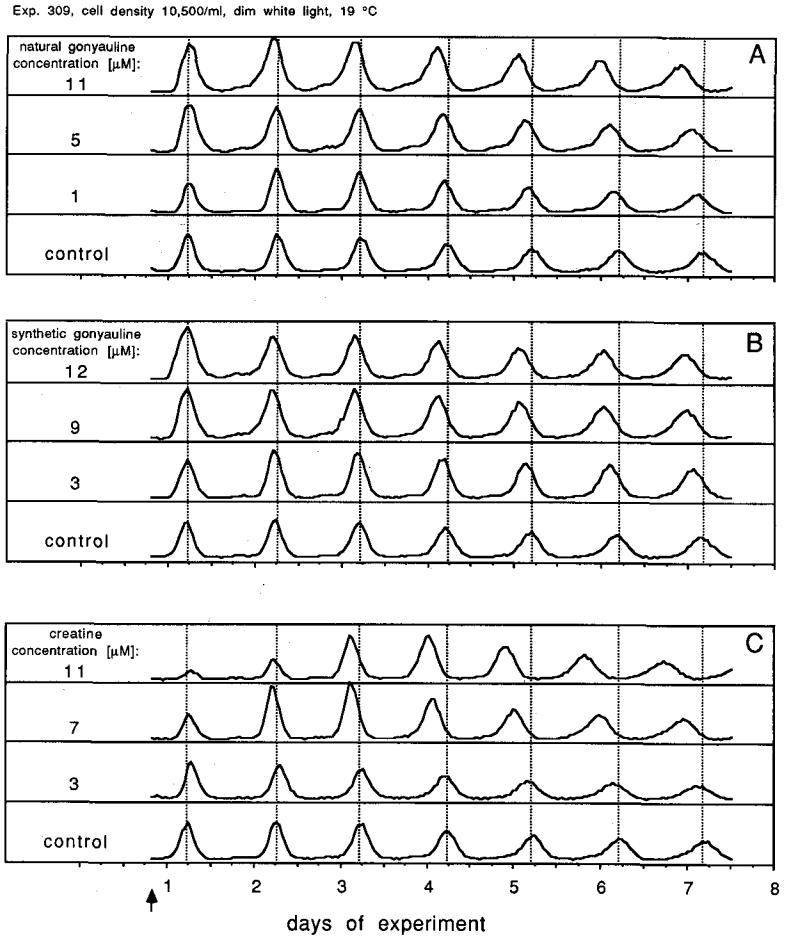

Figure 1. The effects of different amounts of natural gonyauline (A) and of synthetic gonyauline (B) as well as of authentic creatine (C) on the bioluminescent glow rhythm of the alga, *Gonyaulax polyedra*. Cells were grown under a light-dark cycle (LD 12:12). Aliquots of 10 ml were dispensed in scintillation vials and transferred to constant conditions of dim light (30 $\mu E\,s^{-1}\,m^{-2}$) and temperature. Substances were added at the beginning of the experiment (arrow). The bioluminescence of each culture was measured with a photomultiplier for 40 s every 45 min[9,10]. To compare the circadian period of the experimental cultures and controls, vertical lines are drawn at the times of the glow peaks of the control cultures. The controls, shown in fig. 1 A-C, represent three different cultures measured in the same experiment. Note the accuracy of the period length.

cus in *Drosophila*[8], gonyauline is an example of an endogenous substance that affects the period length of the circadian clock.

The circadian rhythm of bioluminescence was used as an assay[7] for the isolation of the active compound. A detailed description of the method for recording the circadian rhythm of bioluminescence is given elsewhere[9,10].

The dose-dependent τ-shortening activity of the purified substance is shown in figure 1A. The activity was found in the aqueous phase and in fractions containing molecules of low molecular weight (judged by elution volume on a G-10 column). The active substance, termed gonyauline, was isolated by a method similar to the isolation of creatine[7]. Unialgal cultures of *G. polyedra* (strain E-5) were grown at 20 ± 2 °C in modified sea water[11] to a density of 10^4 cells/ml (under LD 12:12 approximately 3 weeks). Harvested cells were homogenized in 2-propanol and centrifuged (29,000 × g). The supernatant was diluted with water and extracted with hexanes. The aqueous layer was passed through disposable ODS-columns (J. T. Baker) and concentrated. The fractions from a Sephadex G-10 column (Pharmacia) containing τ-shortening activity were pooled and purified further by HPL-chromatography, using double distilled water as the mobile phase (TSK 3000 PW column, [Toyosoda] and an ODS column [Altex]). 200 mg of pure active material were used for physico-chemical analysis.

The active substance was identified as a novel molecule, a cyclopropanecarboxylic acid (fig. 2A), by the following physico-chemical measurements.

1) The molecular formula was determined by an electron impact high resolution mass spectrum (observed : 146.03993; calculated for $C_6H_{10}O_2S$: 146.04015).
2) The 1,2-disubstituted cyclopropane ring was deduced on the basis of proton and carbon-13 nuclear magnetic resonance (NMR) spectra; small geminal proton-proton couplings and large proton-carbon-13 couplings characteristic to cyclopropyl groups were observed (see table)[12].

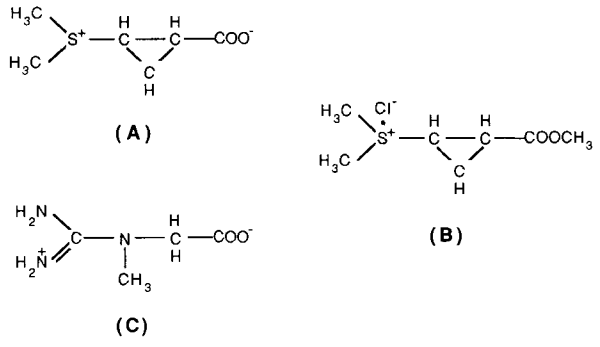

Figure 2. The chemical structures of gonyauline (A) and of its methyl ester (B), as well as of creatine (C).

Proton (500 MHz) and carbon-13 (125 MHz) nuclear magnetic resonance (NMR) data for gonyauline in D_2O with t-butanol and 1,4-dioxane as internal standards, respectively ($\delta_H = 1.22$, $\delta_C = 67.3$)[a-c]

Carbon	δ_H	m	J_{H-H}	δ_C	m	J_{C-H}
C-1	2.23	q	6.5	23.4	d	171
C-2	2.76	d, t	8, 6.5	29.1	d	177
C-3	1.49	q	6.5	14.6	t	170
	1.66	d, t	8, 6.5			
COO-				176.8	s	
SMe_2	2.89, 2.98	s		27.6	q	145

[a] Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplicity; J, coupling constant (Hz).
[b] Assignments were made by proton homo-decoupling and proton selective hetero-decoupling experiments.
[c] Carbon-13 multiplicity and proton-carbon-13 coupling constants were determined by Distortionless Enhancement through Polarization Transfer (DEPT) experiments.

3) The relative stereochemistry of the 1,2-disubstituted cyclopropane was established from a nuclear Overhauser effect experiment; 8% signal enhancement of the C1 methine proton was caused by irradiation on the C2 methine proton.
4) The presence of a dimethyl sulfonium group was deduced primarily on the basis of the proton and carbon-13 NMR spectra (see table)[13].
5) The carboxyl group was characterized by carbon-13 NMR (δ 176.8) and confirmed by the formation of a methyl ester (fig. 2B) upon treatment with HCl-methanol. The spectral features of the methyl ester (chloride form, fig. 2B) were recorded as follows: $[\alpha]_D$ + 134° (c 0.270, H_2O); fast atom bombardment mass spectrum; m/z 161 $(M\text{-}Cl)^+$; ^1H NMR (D_2O) d 1.71 (1H, q, J = 6.5 Hz), 1.89 (1H, dt, J = 8, 6.5 Hz), 2.60 (1H, dt, J = 8, 6.5 Hz), 2.94 (3H, s), 3.03 (1H, q, J = 6.5 Hz), 3.04 (3H, s), 3.78 (3H, s).

Gonyauline is an optically active molecule ($[\alpha]_D$ + 214°; c 0.830, MeOH). Its biogenesis might be derived from methionine or derivatives such as vitamin U (*S*-methylmethionine) by a deamination-cyclization reaction. Although the absolute configuration of gonyauline is still to be determined, it might be related to that of methionine on the basis of its mode of reaction. Cyclopropane amino acids are already known as constituents of marine algae[14,15], and the biogenesis of ethylene, the aging hormone of plants, has been shown to derive from methionine via 1-amino-2-cyclopropanecarboxylic acid (ACC)[16]. Synthetic gonyauline[17] is not optically active, but it also shortens the circadian period (fig. 1B). It is, however, less effective at comparable concentrations (fig. 3), which may suggest that only one of the optically active forms of gonyauline has a τ-shortening activity.

The effects of natural and synthetic gonyauline of the circadian clock are slightly weaker than, but generally comparable with those of creatine (figs 1C and 3). The magnitude of the effect is dependent on the concentration of the substance in the medium as well as on cell density (the higher the cell density, the smaller the effect), suggesting that the activity depends on the uptake of the molecule by the cells. Both creatine[7] and gonyauline exhibit their activity only in constant blue or fluorescent white light; under constant red light, significant period changes were not observed (fig. 3).

However, the effects of creatine and gonyauline differ in some respects. At higher concentrations, creatine initially depresses the amplitude of the bioluminescence rhythm (top trace in fig. 1C) but, at the same time, increases the motility of the cells[7]; cultures generally do not survive concentrations above 20 µM. At higher concentrations of gonyauline, the amplitude is generally increased somewhat (figs 1A and B), but the concentration threshold for killing is lower.

The polar groups of gonyauline are dimethylsulfonium and carboxylate, while those of creatine are methyl guanidinium and carboxylate. In gonyauline, the cyclo-

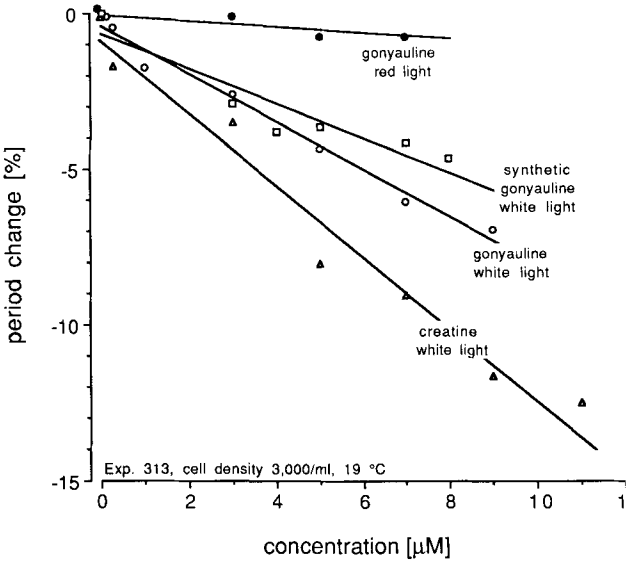

Figure 3. The period shortening effects (expressed as difference from the control period in %) of different concentrations of natural and synthetic gonyauline as well as creatine (open symbols: constant dim white light; dots: natural gonyauline in constant red light, > 600 nm [spectral composition[5]]). The calculation of the respective period lengths is described elsewhere[7]. The effects of all three substances as well as their lethal concentrations depend on cell density (see text). Note that the cell densities in the two experiments shown here and in figure 1 differ.

propane ring might have an important role in stabilizing the three-dimensional structure of the molecule, and thus favor its binding to a target molecule and/or generating a reactive species[18]. Creatine itself could not be detected in *Gonyaulax* extracts (for method of detection see[19]); however, judged by its τ-shortening capacities (fig. 3), creatine might be a potent and non-metabolizable analog of gonyauline.

Since gonyauline is related to methionine, a possible mechanism for the τ-effect might involve the regulation of proteins and/or nucleic acids by methylation[20] and, because of the spectral dependence of the effects, these regulations might be found in cellular pathways of light transduction. However, it is too early to speculate whether gonyauline is produced specifically for the purpose of modulating the frequency of the circadian clock or whether it also serves other functions; further studies are required to elucidate the biochemical mechanisms underlying the effects of both gonyauline and creatine on the circadian period.

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The postpharyngeal glands and the cuticle of Formicidae contain the same characteristic hydrocarbons

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Summary. Comparison of the contents of the postpharyngeal gland and cuticular hydrocarbons of five species of ant have shown them to contain the same compounds and to be characteristic of the species. For four species (*Formica selysi, Camponotus lateralis, Camponotus vagus* and *Manica rubida*), quantitative similarity was very close, but more divergent in the fifth (*Myrmica rubra*). Glands and cuticle of *M. rubra* queens were shown to be closely similar to those of workers, except the glands of queens are larger, but the cuticle of larvae was different from that of adult cuticle and postpharyngeal glands.
Key words. Formicidae; ants; postpharyngeal gland; cuticle; hydrocarbons; larvae; queens; workers.

The postpharyngeal gland, found in all castes of all species of the Formicidae, occupies an important part of the cephalic capsule. It is usually described as having finger-like projections and is filled with a clear or pale yellow fluid. The structure and contents of this unique organ have been little studied, and its function remains unknown today. Peregrine et al.[1,2] and Delage-Darchen[3] have described the gland and considered various suggestions for its function. Partly because it is connected directly to the pharynx, but also from the results of some labelling experiments, most authors attribute to it an alimentary role. The known chemistry of the gland was recently reviewed[4].

Brian and Blum[5] demonstrated the importance of the head of *Myrmica rubra* queens for the control of growth of their larvae and suggested that fatty acids, possibly