In earlier experiments water had proved to be a good solvent for pheromones of the sting glands. Results: own marked bridges: 217 tested workers, none refused; control unmarked bridge: 34 workers tested, 30 refused; legs: 42 workers tested, 39 refused; sting glands and sting sclerites: 62 workers tested, 59 refused; remaining sclerites of the gaster: 43 workers tested, 37 refused; rectum: 35 workers tested, 30 refused.

This stands in contrast to Lane's results on *Leptothorax unifasciatus*[3] where the poison gland secretion is said to release trail following. According to our investigation[2] the poison gland secretion functions as releaser for tandem running and as a following signal for conspecifics. So far we lack any hint as to where the trail secretion might originate from. During tandem running the sting chamber is open with the sting sometimes visibly protruding. No comparable behavior was detected in trail laying individuals. Trail laying individuals pressed their gasters upon the substrate. On smoked glass plates they thereby generated tracks in which the imprints of gastral hairs but no sting marks could be recognized.

We interpret our finding that *Leptothorax affinis* lays individual trails as follows: the colonies of this ant organize their foraging and other outside activities mainly individually. Therefore it is important for the workers to be able to find their own trails quickly and securely and to be able to distinguish them from the trails of other colony members. Recruited individuals come to know their routes directly by tandem running or social carrying without the need to use the trails of nestmates. Our investigations will be continued.

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Teratogenic effects of cadmium on *Bufo arenarum* during gastrulation[1]

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Summary. Developing *Bufo arenarum* embryos were treated during gastrulation with cadmium chloride in concentrations ranging from 6×10^{-7} to 1.5×10^{-5} M Cd^{++} at 20 and 30 °C. Initial failures at gastrulation result mainly in axial incurvations, microcephaly, hydropsy and abnormal tail formation. The higher temperature has a dual effect: at high concentrations of Cd early malformations are significantly increased, whereas at low concentrations the higher temperature prevents alterations.
Key words. Teratogenesis; cadmium; temperature dependence; amphibian; gastrulae.

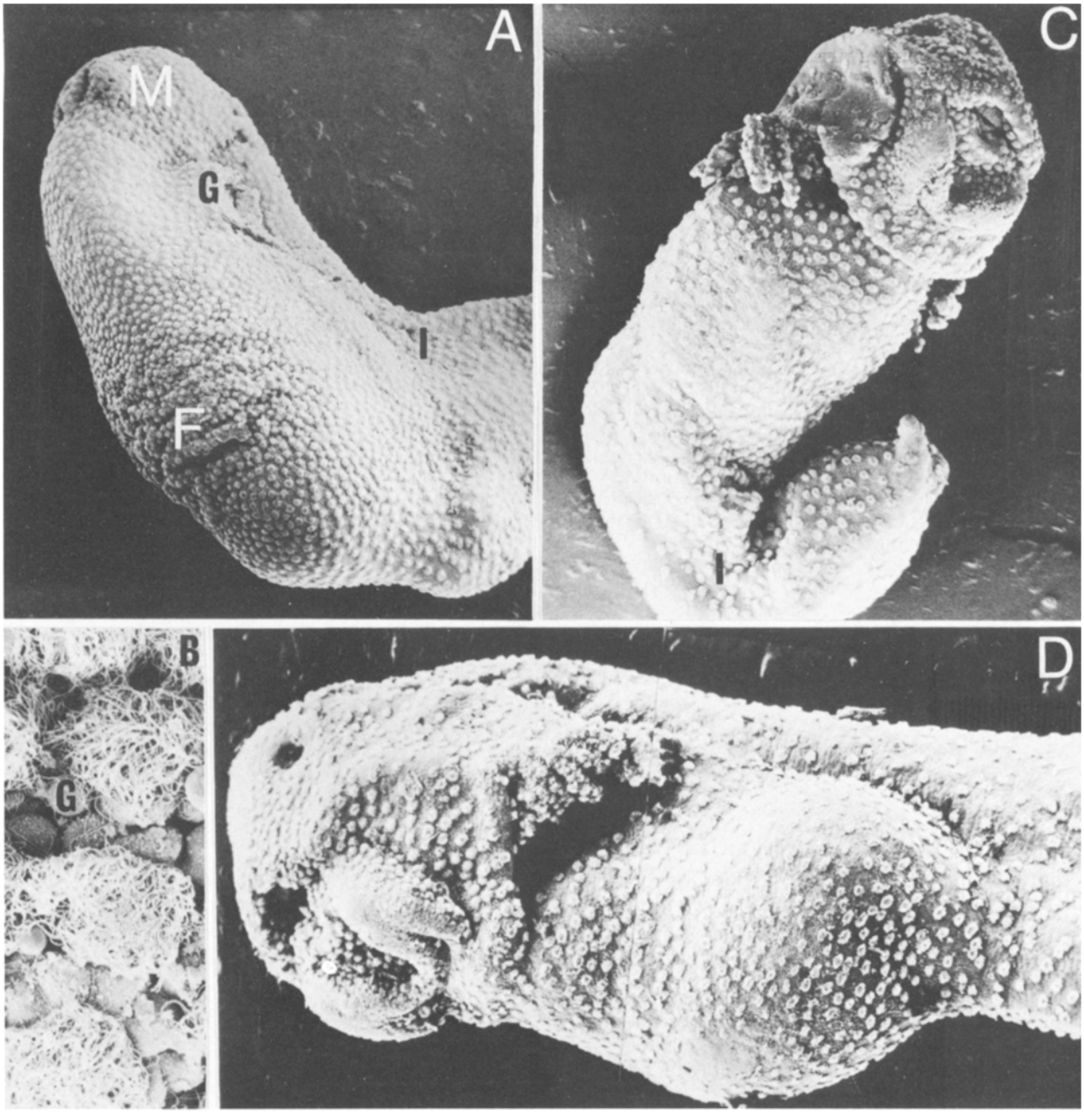
The increasing incorporation of cadmium into ecosystems as a result of its negligent use in industry and agriculture has been repeatedly reported[4,5]. Its teratogenic effects on vertebrates include skeletal malformations, behavioral disorders, delayed development and reduced body size[6]. Particularly in amphibians, Cd applied continuously from the 2-cell stage onwards produces cellular dissociation, alterations in ectodermal tissues and in the formation of gills and fins[7,8]. In order to establish whether any of these effects are related to the particular stage of development at which Cd is applied, we studied the alterations caused by Cd in *Bufo arenarum* embryos, exposing them at the gastrula stage and analyzing embryocidal and teratogenic effects. Gastrulation involves displacements of cells, changes in cellular adhesiveness and inductions which establish the basic pattern of the organism[9].

Considering that alterations of temperature might change the susceptibility of embryos to Cd[10], we also studied the effects of exposing the embryos to different concentrations of this heavy metal at 20 and 30 °C.

Material and methods. Ovulation was induced by injecting an adult female toad with a suspension of homologous hypophysis. Oocytes were fertilized in vitro and jelly coats were removed with 2% thioglycolic acid pH 7.2. Batches of 30 embryos were treated at the onset of gastrulation (stage 10, S. 10)[11] with 10% Holtfreter's solution containing 6×10^{-7}, 3×10^{-6} and 1.5×10^{-5} M Cd^{++} at 20 and 30 °C until controls reached the late gastrula stage (S. 12) and then maintained in Holtfreter's solution at 20 °C. Controls were untreated embryos maintained at the two temperatures between S. 10 and S. 12 and then at 20 °C until they completed their development. Teratogenic effects were observed

Effects of cadmium (chloride) at 20 and 30 °C of *Bufo arenarum* gastrulation. Survival (A), intense malformations (B) and slight malformations (C); data collected at the neural fold stage (S. 14), cardiac beating stage (S. 19) and complete operculum stage (S. 25) of *Bufo arenarum* embryos exposed to cadmium (chloride) during gastrulation. Data are expressed in percentages

Cd^{++} concentration (M)	°C	Stages 14			19			25		
		A	B	C	A	B	C	A	B	C
Control	20	100	0	0	100	0	0	96.7	0	0
6×10^{-7}		100	0	0	90	3.3	86.7	90	3.3	86.7
3×10^{-6}		100	0	0	90	3.3	86.7	90	3.3	86.7
1.5×10^{-5}		100	0	0	100	6.7	93.3	90	0	90
Control	30	100	0	0	100	6.7	3.3	100	6.7	3.3
6×10^{-7}		100	0	0	96.7	3.3	10	93.3	3.3	6.7
3×10^{-6}		100	0	0	93.3	6.7	13.3	93.3	6.7	13.3
1.5×10^{-5}		100	30	0	96.7	33.3	63.3	96.7	40	56.7



A Partial view of a *Bufo arenarum* embryo treated with 1.5×10^{-5} M Cd^{++} at 20 °C, × 50. Notice the folds in the ventral ectoderm (F), microcephaly (M), underdevelopment of gills (G) and the axial incurvation (I). *B* Higher magnification of the folds region, × 800. Notice the voluted surface of the glandular cells (G). *C* Partial view of a *Bufo arenarum* embryo treated with 1.5×10^{-5} M Cd^{++} at 30 °C, × 40. Notice the severe incurvation of the axis (I) and the abnormal tail. *D* Partial view of a control embryo, × 40.

with a Wild stereoscopic microscope and individuals of each group were prepared for scanning electron microscopy (SEM)[12] and observed in a JEOL JSM-U3 operated at 5–10 kW.

Results. In embryos kept at 20 °C and exposed to the different concentrations of Cd, there was a delay in the rate of development during gastrulation (controls: S. 12, experimentals: S. 11) but the development was similar from the stage of neural folds (S. 14) onwards. In all concentrations studied, malformations were not observed before the cardiac beating stage (S. 19). At this time, almost all embryos exhibited mild axial incurvations, microcephaly and underdevelopment of gills (all considered to be slight malformations) (fig. A). However, the frequency of alterations did not increase (table). The surface of experimental embryos observed with the SEM showed folds in the ectoderm (fig. B) and an increased number of ciliated cells. The development of embryos maintained at 30 °C was not delayed. However, in 33% of the embryos treated with 1.5×10^{-5} M Cd^{++}, the blastopore failed to close and severe axial incurvations, hydropsy and abnormal formation of the tail (all considered to be severe malformations) were present (fig. C). The latter were so severe that the swimming behavior of the embryos was markedly affected. The SEM showed ciliated cells atypically distributed in cords or clusters. In the two lowest concentrations a few cases of hydropsy and abnormal formation of the tail (severe malforma-

tions) were observed (table). Mortality of embryos was similar in all groups and independent of both Cd concentration and incubation temperature (table).

Discussion. These observations indicate that under the experimental conditions explored, the survival of embryos does not seem to be noticeably affected by Cd. In contrast, the experimental embryos exhibit malformations, which are dose and temperature related. The fact that Cd toxicity may be influenced by temperature has been reported for other organisms[10, 13, 14]. In the case of *Bufo arenarum* embryos, high temperature may protect them against the teratogenic effects of Cd at lower concentrations, but at high concentrations of Cd the early malformations increase. These observations may be interesting because from an ecotoxicological point of view it is more probable that embryos will be exposed to low concentrations of Cd than to higher ones. In addition, as embryo development progresses, the initial protective effect of high temperature disappears, probably owing to a 'catch-up' phenomenon that occurs in the embryos treated at 20 °C. Considering hydropsy; this malformation may be related to a disturbance of the osmoregulatory mechanism by Cd[15]. Axial incurvations might be interpreted as being due to a displacement of calcium, as has been reported for fishes[16] and mammals[17]. The existence of cords and clusters of ciliated cells may be due to a disturbance in the differentiation of glandular cells which are intercalated between those with cilia.

The interference that Cd exerts on embryonic development seems to be related to its multiple effects upon enzymatic and structural proteins[18], and nucleic acids[18], as well as on the availability of essential elements[18,19] and energy-rich molecules[18, 19], with a consequent reduction of the performance of the embryo.

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A novel antagonist of serotonergic receptors, hymenidin, isolated from the Okinawan marine sponge *Hymeniacidon* sp.

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Summary. A novel bromine-containing pyrrole compound, hymenidin, has been isolated from the Okinawan marine sponge *Hymeniacidon* sp. as a potent antagonist of serotonergic receptors and its structure elucidated using spectral data.
Key words. Sponge; hymenidin; *Hymeniacidon* sp.; bromopyrrole; antiserotonergic action.

In our studies on bioactive metabolites of marine organisms[2–5], extracts of numerous sponges collected in Okinawa were screened on the isolated rabbit aorta. The bioassay-directed purification resulted in isolation of a novel α-adrenoceptor blocking agent from the marine sponge *Hymeniacidon* sp.[6]. More recently, another fraction from an extract of the same sponge was found to inhibit markedly the contraction of the aorta induced by serotonin, but did not affect that induced by KCl or norepinephrine (NE). In this communication, we report the isolation and structure determination of **1**, a novel antagonist of serotonergic receptors, from the marine sponge *Hymeniacidon* sp.

Male albino rabbits (2–3 kg) were used. The procedure for preparing the isolated rabbit aorta and the technique of measurement of contractions were as previously described[7]. The sponge *Hymeniacidon* sp., collected at Ishigaki Island, Okinawa, in June 1984, was stored at −20 °C until used. The methanol-toluene (3:1) extract of the sponge was partitioned between toluene and water. The aqueous phase was then extracted with chloroform, ethyl acetate and n-butanol, successively. The butanol-soluble portion was subjected to a silica gel column with chloroform-n-butanol-acetic acid-water (3:12:2:2) to afford an active fraction. This fraction was chromatographed on a C_{18} reversed phase HPLC column (Develosil ODS, 5 μ, 10 × 250 mm) with methanol-water (2:3) containing 0.05 M acetic acid to yield hymenidin **1** (0.003% wet weight of the sponge) as an amorphous colorless solid.

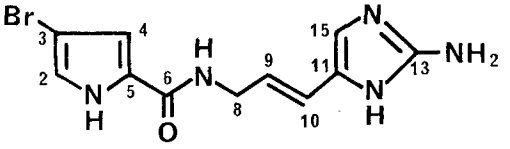

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Figure 1. Chemical structure of hymenidin **1**.