

Bryozoa und Brachiopoda schliesslich spezielle Larvenformen ohne morphologische Beziehungen zur *Trochophora* aufweisen.

Summary. The confusing term '*Trochophora*' is elucidated and restricted to a developmental stage of the Annelida and the protannelid Echiurida, derived convergently with the molluscan *Pseudo-Trochophora* from

the common *Pericalymma*-larva. All other so-called *Trochophorae* cannot be termed as such, the name being morphologically misleading as in Sipunculida and other.

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Growth Stimulating Effects of the Bromophenol, Lanosol, on Red Algae in Axenic Culture

Brominated compounds in red algae have attracted attention since the thirties from chemical and pharmacological point of view¹. The most frequently occurring compound is lanosol, 2,3-dibromo-4,5-dihydroxybenzyl alcohol, isolated from *Polysiphonia lanosa*². Lanosol has been known as a highly toxic substance for bacteria and algae. McLACHLAN and CRAIGIE³ give a LD₅₀ of 0.03 mM for unicellular marine algae. However, in nature lanosol seems to appear in the red algae mostly as a disulphate and this form does not inhibit growth of bacteria or fungi^{4,5} nor growth of unicellular algae³. PROVASOLI⁶ found lanosol influencing the morphology of *Ulva*. As many simple phenols, like 3-methylcatechol, 4-methylcatechol, and 3,4-dihydroxybenzoic acid, stimulate growth of axenically cultivated *Goniotrichum alsidii* (Zanard.) HOWE⁷ lanosol and its sulphuric ester were tested on this alga as well as on *Polysiphonia urceolata* (Dillw.) Grev.

Lanosol⁸ was isolated from *Polysiphonia brodiaei* (Dillw.) Grev. and its disulphate² from *Brongniartella byssoides* (Good et Wood.) Schmitz. The chemical preparations were made by Dr. P. SAENGER, Melbourne University, at his stay in our institute. The lanosol preparation contained a few percent of the corresponding aldehyde, and during storage it changed colour to dark brown and lost its activity. The methods for cultivation of the red algae and the arrangements for the experiments are described earlier^{7,9}. The influence of the bromophenols on algal growth was tested in a range from 4 to 200 µmole/l. The growth of the algae on different concentrations is given in the Table as mean dry weight of algae from 6 parallel flasks, each flask containing 25 ml of the artificial seawater, ASP 6⁹.

In *Polysiphonia urceolata*, lanosol inhibited growth in a concentration of 0.2 mM/l, a result corresponding very well with the observation made by McLACHLAN and CRAIGIE³ on the red alga *Porphyridium sp.* Lower con-

centrations, however, strongly stimulated growth, giving an increase near to 90% at a concentration of 4×10^{-6} M. In *Goniotrichum alsidii* no inhibition was noted but the growth stimulation reached only 50%. The disulphate of lanosol was not only nontoxic but showed a pronounced growth stimulation in both species at the two highest concentrations tested.

At separation on a Sephadex column of seawater collected in the *Fucus-Ascophyllum* zone some fractions containing phenolic compounds were obtained which influenced growth and morphology of *Goniotrichum*¹⁰. The active substances in that experiment were not identified, but later it has been shown that *Polysiphonia brodiaei* exudes bromophenols, mainly lanosol in some form soluble in sea-water¹¹. CHAN and McMANUS¹² followed the bacterial flora on *Polysiphonia lanosa* and found the same richness from May to September, which could indicate that the nontoxic disulphate was exuded.

Analyses of bromophenols in Swedish red algae have shown that, whereas *Polysiphonia urceolata* contains

¹ H. BASLOW, Marine Pharmac., Baltimore 14, 286 (1969).

² J. H. HODGKIN, J. S. CRAIGIE and A. G. McINNES, Can. J. Chem 44, 74 (1966).

³ J. McLACHLAN and J. S. CRAIGIE, J. Phycol. 2, 133 (1966).

⁴ M. PEGUY, C.R. hebdom. Séanc. Acad. Sci., Paris 252, 2131 (1961).

⁵ K.-W. GLOMBITZ and H. STOFFELN, Planta med. 22, 391 (1972).

⁶ L. PROVASOLI, Publ. 1700 Natn. Acad. Sci. Washington (1969).

⁷ L. FRIES, J. exp. mar. Biol. Ecol., in print (1973).

⁸ J. S. CRAIGIE and D. E. GRUENIG, Science 157, 1058 (1967).

⁹ L. FRIES, Physiologia Pl. 16, 695 (1963).

¹⁰ L. FRIES, Proc. VII int. Seaweed Symp. Sapporo 1971, p. 575. (Ed. K. NISIZAWA; Univ. of Tokyo Press, Tokyo 1972).

¹¹ M. PEDERSEN, P. SAENGER and L. FRIES, Phytochemistry, in print (1974).

¹² E. C. S. CHAN and E. A. McMANUS, Can. J. Microbiol. 15, 409 (1969).

Effects of lanosol and sulfonated lanosol on growth of the 2 axenically cultivated red algae, *Goniotrichum alsidii* and *Polysiphonia urceolata*. Sporelings of *Goniotrichum* and pieces of *Polysiphonia* threads used as inocula

Species investigated	Substances added	Mean dry weight of algae per 25 ml nutrient medium (mg)			
		Addition per l/µmole			
		0	200	40	4
<i>Goniotrichum alsidii</i>	2,3-dibromo-4,5-dihydroxy-benzyl alcohol (lanosol)	4.9 ± 0.9	5.6 ± 0.5 ^a	7.5 ± 0.6 ^a	7.2 ± 0.4 ^a
	2,3-dibromo-4,5-dihydroxy-benzyl alcohol disulphate	4.9 ± 0.9	6.3 ± 0.2 ^a	7.1 ± 0.6 ^a	6.5 ± 1.0
<i>Polysiphonia urceolata</i>	2,3-dibromo-4,5-dihydroxy-benzyl alcohol	0.8 ± 0.2	0.2 ± 0	1.1 ± 0.1 ^a	1.5 ± 0.2 ^b
	2,3-dibromo-4,5-dihydroxy-benzyl alcohol disulphate	0.8 ± 0.2	1.5 ± 0.2 ^a	1.1 ± 0.1 ^a	0.8 ± 0.2

^a Significantly different from control at $p < 0.05$. ^b Significantly different from control at $p < 0.01$, indicating growth stimulation. Inoculation time 28 days. 6 parallels in each series.

abundance of them, they are completely absent in *Goniotrichum*¹¹. The further addition of lanosol in the highest concentration, $2 \times 10^{-4} M$, could thus produce an inhibiting surplus in *Polysiphonia*, while *Goniotrichum*, having no pool of bromophenols, was stimulated by all concentrations tested.

As some simple phenolic compounds influence growth and/or morphology in *Goniotrichum*, they are presumed to play a fundamental role in metabolism, either as such or as lower steps in the formation of some growth regulators. In many other red algae, bromophenols are stored either as sugar derivatives¹³ or as sulphates. It might be possible that aromatic compounds are mobilized from this pool when required in the metabolism of these species, while *Goniotrichum* has to be supplied by exudates from other organisms. Lanosol is as yet the only naturally occurring bromophenol tested, and since the role of bromine in this connection is completely unknown, no

further conclusions can be drawn. Further work is now in progress to elucidate these problems.

Zusammenfassung. Wachstumstimulation von 2,3-Dibromo-4,5-Dihydroxybenzylalkohol (Lanosol) in Konzentrationen von 4×10^{-5} – 4×10^{-6} wurde in axenischen Kulturen der Rotalgen *Goniotrichum alsidii* und *Polysiphonia urceolata* festgestellt. Während das Disulfatdikaliumsalz nicht toxisch und von niedrigerer Aktivität war, hatte $2 \times 10^{-4} M$ Lanosol eine Hemmwirkung auf *Polysiphonia*.

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¹³ S. RÖNNERSTRAND Bot. Marina 11, 107 (1968).

Reversal of Spinning Behaviour in Last Instar Larvae of *Pieris brassicae* Treated with Juvenile Hormone Derivatives¹

More than 20 years ago PIEPHO² demonstrated that the presence or absence of active corpora allata influences the spinning behaviour of last instar larvae of *Galleria mellonella*. When NOVAK³ discussed this work, he claimed that the juvenile hormone (JH) would influence the instinct of spinning indirectly by inhibiting the morphogenesis of the pupal or imaginal brain. Since morphogenesis in insects is supposed to be a one-way process, the theory of NOVAK would imply that no reversal of behaviour is possible. However, reversible JH-dependent behaviour has been demonstrated in the adult Colorado potato beetle⁴. The following observations on last instar

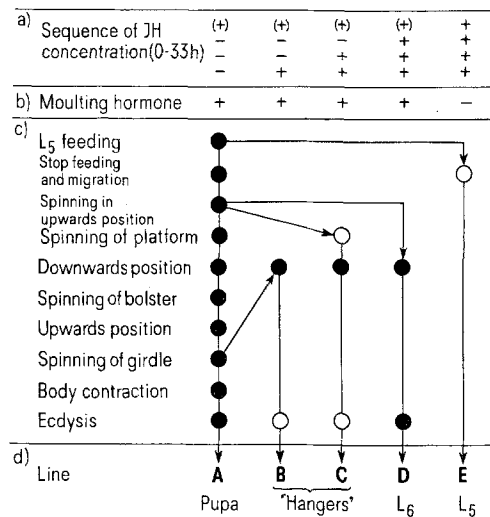
larvae of the cabbage butterfly *Pieris brassicae* treated with JH derivatives (JHD) show that reversal of JH dependent behaviour is also possible in an insect which has not finished development.

The JHDs used were mixtures of isomeres of either methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate or 6,7-epoxy-3,7-dimethyl-1-[3,4-(methylenedioxy)-phenoxy]-2-nonene. Last instar larvae of *P. brassicae* of different age were treated topically with 2 times 1 µl of acetone for controls or acetone solutions with different concentrations or one of the JHDs, applied dorsally on the thorax and the last third of the abdomen. Time of treatment is indicated as hours after the L₄/L₅ moult.

Our observations show that spinning behaviour of normal premoulting larvae of *P. brassicae* proceeds along one of two lines, depending on whether (at a relatively high level of JH) a larval/larval or (at a very low level of JH) a pupal moult is induced.

Untreated young larvae of *P. brassicae*, when ready for a larval moult, spin a net of silk threads and take on a position with the head pointing downwards. They keep this position until the old skin ruptures on the back of the thorax and is shed. After a rest of about 2 h the larva resumes feeding. This behaviour corresponds approximately with line D in the Figure.

Untreated or acetone treated last instar larvae ready for a pupal moult leave the food and, as a rule, move to a vertical wall where they construct a dense silk platform with a bolster of loose silk threads at the lower end of it. Each larva then positions itself on the platform with the head pointing upwards and the hind end lying on the bolster. It bends the head backwards and, moving it from one side of the platform over the back of the second abdominal segment to the other side of the platform, spins a girdle of silk threads which later on will fix the anterior part of the pupa to the platform. After some time the larva contracts to form the prepupa or pharate pupa. Within 12 h the larval skin ruptures on the back of the thorax and the old skin is shed. The hooks of the last



Influence of juvenile hormone (JH) on pattern of behaviour of last instar larvae of *P. brassicae*: a) Hypothetical sequence of JH concentrations in larvae at 0-33 h after the L₄/L₅ moult (JHD present = +, absent = -; hypothetical presence of small amounts of natural JH = (+)). b) Production of moulting hormone. c) Sequences of actions, i.e. patterns of behaviour according to pattern of JH concentrations and presence or absence of moulting hormone; the sequence of line A represents the natural pattern of behaviour leading to the pupal moult; all degenerations from this line have been induced by treatment with JHDs. Full circles, action completed; open circles only part of action exhibited. d) Developmental result (L₆ = supernumerary larval stage; L₅ = 'dauer-larva').

¹ The author thanks Hoffmann-La Roche Ltd., Basel for samples of the substances.

² H. PIEPHO, Z. Tierpsychol. 7, 424 (1950).

³ V. J. A. NOVAK, *Insect Hormones* (Methuen, London 1966), p. 104.

⁴ J. DE WILDE and J. A. DE BOER, J. Insect Physiol. 15, 661 (1969).