

the amounts isolated from active insects indicated a natural content of about one part in four million. However, with hibernating termites, the pheromone titre fell considerably.

The molecular weight of the pheromone was determined by mass spectrometry as 272, thereby confirming a diterpenoid hydrocarbon constitution. The ultra-violet spectrum (in cyclohexane) showed only end absorption above 220 m μ and the infra-red spectrum (liquid film) included strong bands at 3,070, 1,640 and 880 cm⁻¹, which suggested one or more α -substituted vinyl groups. On catalytic hydrogenation, the pheromone gave an inactive octahydro-derivative (molecular weight 280, by mass spectrometry) and it presumably has a monocyclic structure, with four isolated double bonds.

An apparently identical pheromone has been isolated in small amounts from the arboreal termites, *Nasutitermes walkeri* (Hill) and *N. graveolus* (Hill), using *N. exitiosus* as the test animal. However, the pure pheromone shows no activity with the more distantly related *Coptotermes lacteus* (Froggatt) and it may therefore prove to be genus specific. Screening tests (with *exitiosus*) of various essential oils have also revealed activity in one plant species, namely the West Australian sandalwood, *Eucarya spicata* (R. Br.) Sprag. and Summ. Preliminary isolation experiments with this material indicate that activity is definitely associated with the small diterpenoid hydrocarbon fraction of the oil, and probably with a specific component which parallels closely, in thin-layer and gas chromatography, the behaviour of the true pheromone. Attempts are now being made to obtain this plant constituent in a state of purity.

Final confirmation of the nature of the pheromone must await an unambiguous synthesis. However, the possibility, always present in this type of work, that the substance isolated merely represents a carrier for an even more potent pheromone seems in the present instance rather remote.

I thank Dr. J. S. Shannon for the mass spectrum of the pheromone and Dr. J. D. Morrison for the mass spectrum of its perhydro-derivative.

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¹ Lüscher, M., and Müller, B., *Naturwissenschaften*, **47**, 503 (1960).

² Stuart, A. M., *Physiol. Zool.*, **36**, 39 (1963).

³ Stuart, A. M., *Proc. Zool. Soc. Lond.*, **143**, 43 (1964).

A Biochemical Factor in the Zonation of Marine Molluscs

THE trochids *Monodonta turbinata* and *Gibbula divaricata* are midlittoral and sublittoral animals respectively. On the rocky shores of Malta, *M. turbinata* is found semi-exposed in the very narrow "intertidal" zone. *G. divaricata* is usually found submerged in shallow water. It was considered that *G. divaricata* might be adapted for prolonged submersion by having among other factors a higher capacity to incur an oxygen debt than *M. turbinata*. The experiments described here were carried out to test this hypothesis.

The snails were removed from the shells, weighed and finely minced with scissors. The operculum was discarded. The minced tissue was homogenized with sea water containing 10 mM sodium fluoride. The homogenate contained 10 per cent snail tissue. 1 ml. homogenate was incubated for 30 min at 25° C with 0.8 μ moles pyruvate and 1.3 μ moles nicotinamide-adenine dinucleotide (NADH) in a final volume of 2 ml. In one set of experiments the amount of pyruvate consumed by the reaction mixture was determined. The pyruvate was estimated as the dinitrophenylhydrazone. The oxygen consumption of the reaction mixture was measured with a Warburg respiro-

meter in another set of experiments. The gas phase was air. The results were expressed in μ moles pyruvate or oxygen consumed per minute per gram dry weight of homogenate. The dry weight determinations were made on the material precipitated from the homogenate by trichloroacetic acid; this material was washed twice with distilled water and dried at 110° C for 24 h.

The mean rates of pyruvate and oxygen consumption observed are given in Table 1.

Table 1

	Pyruvate consumption (μ moles/min/g dry wt.) (Mean \pm S.E.)
<i>M. turbinata</i>	1.54 \pm 0.08 (n = 5)
<i>G. divaricata</i>	1.94 \pm 0.06 (n = 5)
	Oxygen consumption (μ moles/min/g dry wt.) (Mean \pm S.E.)
<i>M. turbinata</i>	3.04 \pm 0.10 (n = 3)
<i>G. divaricata</i>	2.26 \pm 0.04 (n = 3)

The difference between the mean rates of pyruvate consumption is significant (0.002 < P < 0.01). The mean rates of oxygen consumption also differ significantly (P = 0.002). Because 1 mole of pyruvate requires 2.5 moles of oxygen for its complete oxidation, it will be seen that the pyruvate consumed by the homogenates was not completely oxidized. The pyruvate which was consumed but not oxidized may be regarded as an oxygen debt as NADH was added to the reaction mixtures to favour the reduction of pyruvate to lactate. The homogenized tissues of *G. divaricata* consumed pyruvate at a higher rate and oxygen at a lower rate, and showed a greater oxygen debt as defined here, than the homogenized tissues of *M. turbinata*. This property may enable *G. divaricata* to withstand longer periods of submersion than *M. turbinata*.

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Paralytic Shellfish Poison: Serological Assay by Passive Haemagglutination and Bentonite Flocculations

PARALYTIC shellfish poison (PSP) is a potent toxin, of small molecular weight (C₁₀H₁₇N₂O₄·2HCl), which is produced by the dinoflagellate *Gonyaulax catenella* and which is concentrated, with no ill effects, in bivalves that use the *Gonyaulax* as a food source¹. Human ingestion of PSP results in paralytic poisoning and occasionally death². Mouse bioassay is nowadays used for the detection of PSP in contaminated bivalves. This test, however, is not specific, as other marine toxins, such as puffer fish poison (tetradotoxin), produce similar paralytic symptoms in mice³. Recently, PSP was shown to be haptenic when conjugated to proteins by formaldehyde condensation⁴.

This report compares passive haemagglutination and bentonite flocculation with mouse bioassay for the specific detection of PSP in contaminated shellfish. The approach used here for the sensitive and specific detection of PSP in bivalves should be applicable to the serological study of other marine toxins.

Details of the source of purified PSP, preparation of PSP-protein conjugates, production of antisera to PSP in rabbits, passive haemagglutination (HA), and haemagglutination-inhibition (HI) have previously been described⁴. Non-toxic butter clams (*Saxidomus giganteus*)