2, c), particularly on the 4th and 5th podomeres of the pereiopods. The same types of pappose setae are often found in association with cuticular spines as on the dorsal surface of the uropods. Here a row of cuticular spines possesses a frings of pappose setae just proximal to them, and also groups of setae set between adjacent spines. A similar condition is seen on the chelae and carapace of older animals where setae tend to be replaced by cuticular spines.

Cuspidate setae are strongly developed on the distal edge of the dactyl of the 2nd maxillipeds where they are associated with bundles of rod-like setae (figure 2, e). These 2 setal types are associated at the tips of the 3rd and 4th pairs of walking legs. Both types are innervated and it now requires electrophysiological studies to ascertain their precise functions.

On the margins of the uropods and telson the plumose setae are subtended dorsally by a fringe of alternating long and short slim acuminate setae. This association is also seen on the pleopod margins. Both these setal types play a part in the expansion of the 'tail-fan' surface during the escape movements of the crayfish. Both are innervated, whether these setae act as mechanoreceptors or chemoreceptors remains unanswered.

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Comparison of inhibitory effects of royal jelly acid and myrmicacin on germination of *Camellia sinensis* pollens

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Summary. Royal jelly acid (10-hydroxy-2-decenoic acid) secreted by honeybees and ant-origin myrmicacin (3-hydroxydecanoic acid) inhibited germination of *Camellia sinensis* pollens, and the latter was stronger in the inhibition. Their inhibiting activities were stronger at lower pH, and their inhibitions were reversible.

Royal jelly acid was isolated from royal jelly[1] produced by honeybees *(Apis mellifera)* and identified as 10-hydroxy-2-decenoic acid[2], and the acid was proved to be an essential constituent for antibacterial activity of royal jelly by Blum and co-workers[3].

Myrmicacin (l-3-hydroxydecanoic acid), found in the secretions of South American leaf-cutting ants *(Atta sexdens)* by Schildknecht and Koob[4], is assumed to prevent germination of collected seeds and spores in the ants' nest during storage. With respect to biological activity of myrmicacin, Iwanami and Iwadare[5,6] have reported its inhibiting effect on germination of pollens of several higher plants, and Iwanami[7] has described its peculiar ability to stop mitotic division of generative nuclei of pollens even after metaphase.

Since both the compounds were normal fatty acids with 10 carbon atoms, their structural analogy led us to study further and compare effects of the compounds on pollen germination.

The pollens of *Camellia sinensis* used in this study were collected from the freshly opened flowers. Sugar-agar plates (sucrose 10% and agar 1%) were employed for culture of the pollens. Royal jelly acid (m.p. 63–63.5 °C) was purchased from Nihon Shoji Co. dl-Myrmicacin (m.p. 58–58.5 °C) was prepared according to Meyers' synthesis[8]. Capric acid (m.p. 31 °C), the chemically parent compound of both acids, was supplied by Tokyo Kasei Co. and used as one of the controls.

It has been reported[5,6,9,10] that the activities of royal jelly acid and myrmicacin are stronger at lower pH. Thus pH dependency of activities of the acids was first examined. There pH was adjusted by titration with diluted sodium hydroxide solution. The top chart of figure 2 shows germination of pollens treated with various concentration of the agents at pH 4, 5 and 6. Germination, expressed in percentage, was measured 1.5 h after sowing. The chart reveals strong inhibiting activity of royal jelly acid on pollen germination, similar to that of myrmicacin, and that the latter is stronger than the former over all the measured range of pH. The result that royal jelly acid loses most of the activity at pH 6 is in good agreement with Townsent and colleagues' reports[10,11] that strong antitumor activity of royal jelly acid at low pH is lost at a pH higher than 6.

As the conditions, under which royal jelly acid and myrmicacin exerted the inhibitory activities, were revealed through the above experiments, then release from the inhibition was investigated. The *Camellia* pollens were cultured on the separate culture media containing royal jelly acid (100 ppm) or myrmicacin (50 ppm) or capric acid (50 ppm) at pH 4.5. After 1, 2, 3 and 4 h ungerminated pollens were transfered to the agent-free cultures and growth of the pollens was observed.

As seen in the last chart of figure 2, the pollens treated with the insect-origin inhibitors restored germination after transfer to the inhibitor-free medium, whereas treatment of pollens with capric acid resulted in complete loss of germinative ability. Although growing ability was retained during culture on the natural inhibitors, the longer treatment caused the less restoration of growth after release from the

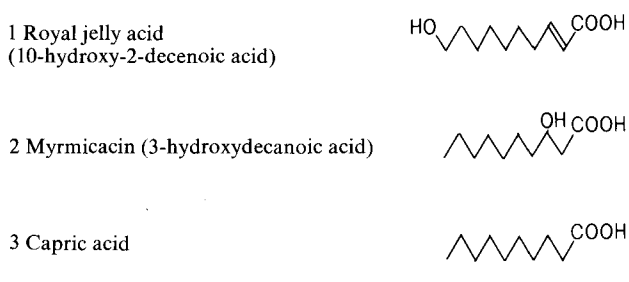

Fig. 1. The agents used in the experiments and their chemical structures.

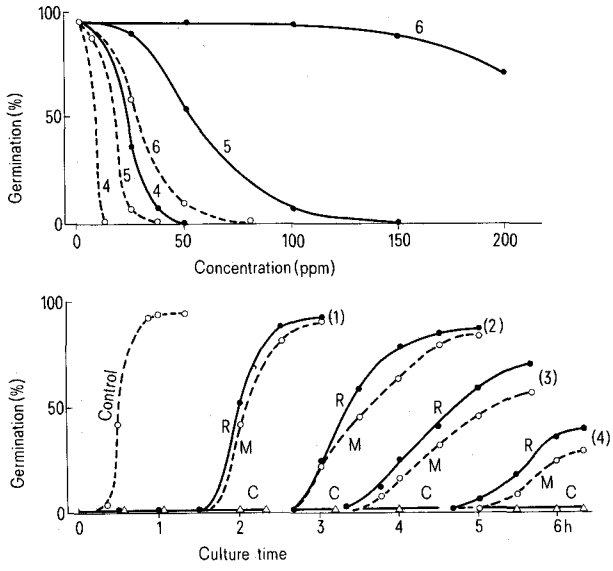

Fig. 2. Effects of royal jelly acid and myrmicacin on germination of *Camellia sinensis* pollens (–●– royal jelly acid, –○– myrmicacin); top chart: relations of agent concentration to germination percentage (numbers indicate culture pH); bottom chart: change of germination percentage of inhibited pollens after transfer to agent-free cultures; R: royal jelly acid (100 ppm), M: myrmicacin (50 ppm), C: capric acid (50 ppm), Control: agent-free medium (pH 5.5), numbers in parantheses indicate period of inhibition in h.

inhibition, and culture on the agents over 6 h brought no germination.

In our previous paper[6], we pointed out the essential structure of a terminal carboxyl group and of normal chain with 8 through 10 carbon atoms for the inhibitory activities of myrmicacin and analogous compounds. From a consideration of our previous and present experimental results, it seems reasonable to assume that release from the inhibition yielded with the agents is concerned with the presence and position of hydroxyl group in the agent molecules. These natural inhibitors used by the insects should also be able to be employed as 'soft inhibitors' in various biological researches.

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Globin gene expression in MSV-transformed fibroblasts

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Summary. The activation of globin gene expression on viral transformation of 3T3 cells was investigated. Globin mRNA was determined using a radioactive complementary DNA probe. No difference was found between 3T3 and transformed 3T3 cells. There does not therefore appear to be a random activation of extensive regions of the cellular genome.

Transformation of 3T3 mouse embryo fibroblasts with mouse sarcoma virus (MSV/3T3) results in loss of contact inhibition, and a rapid increase in cell growth rate as well as cell density, without the production of active virus, type C particles or MSV gene products[2]. The MSV genome has been shown to remain in a stable heritable form for more than 100 generations[3].

Groudine and Weintraub[4] detected the presence of 100–500 copies of fetal globin mRNA sequences per cell in Rous sarcoma virus transformed chicken embryo fibroblasts (RSV/CEF), whereas in cells infected with a mutant carrying a deletion of the 'onc' gene (believed to be responsible for the phenotypic changes that occur on viral transformation), transcription of globin genes did not occur[4]. In 3T3 cells, very low levels of globin mRNA sequences are present in polysomes, but no haemoglobin is produced[5].

An investigation of globin mRNA metabolism in 3T3 and MSV/3T3 cells may help towards understanding the basic differences in the molecular events between normal and transformed cells, especially with regard to random versus specific gene activation. In our experiments, we found no increased levels of globin mRNA sequences in either the nuclear or cytoplasmic compartments of MSV/3T3 cells.

Materials and methods. AMV reverse transcriptase was supplied by Dr J.W. Beard (Life Sciences Inc., Florida, USA); (^{3}H)-dCTP was obtained from Radiochemical Centre, Amersham; Oligo $d(T)_{12-18}$, Oligo d(T) cellulose, S_1 nuclease and DNase from Miles Laboratories, deoxyribonucleoside triphosphates from Boehringer, Mannheim (Federal Republic of Germany); and 3T3 cells from Flow Laboratories. MSV/3T3 cells were a gift from Dr E.L. Wilson (Clinical Science and Immunology, University of Cape Town).

Isolation of globin mRNA. ICR/HA mice were made anemic by s.c. injections of 0.6 ml per 100 g body mass of a 0.25% solution of phenylhydrazine chloride in saline. Blood was collected by cardiac puncture, and RNA isolated from washed red blood cells as described by Lanyon et al.[6]. The poly A containing RNA (mRNA) was separated by Oligo d(T) cellulose chromatography[7]. Such RNA preparations have been shown to stimulate the synthesis of globin in an ascites cell free extract (D.E. Woods, unpublished).

Polyacrylamide gel-electrophoresis. Electrophoresis of RNA on 2.6% polyacrylamide gels was as described by Loening[8]. RNA samples were dissolved in electrophoresis buffer containing 0.5% SDS, heated to 60 °C for 10 min, chilled rapidly and applied to the gels. Electrophoresis was for 90 min at 5 mA per gel. The markers used were 4S yeast tRNA and 5S, 18S and 28S ribosomal RNA.

cDNA synthesis. (^{3}H)-cDNA complementary to mouse glo-