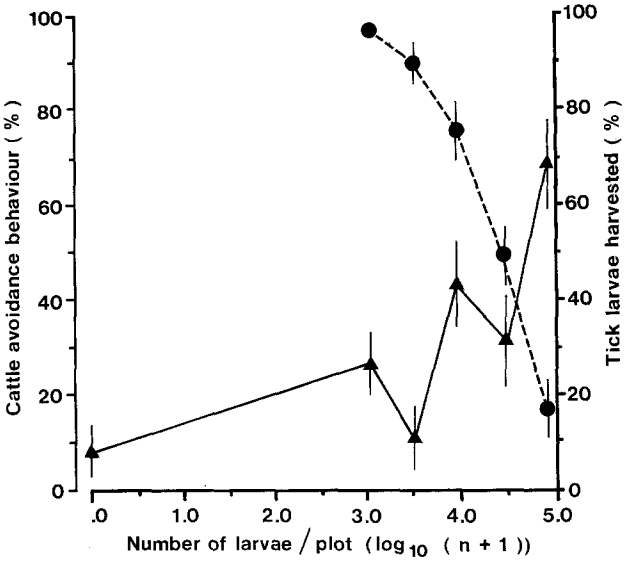

Figure 3. The effect of larval tick density on the incidence of avoidance behavior of cattle (△—△) and on the consequent percentages of larvae which were harvested (●—●). Vertical lines show ± 1 standard error.

first 3 h each day, the response of cattle encountering circles was recorded as 'obvious avoidance' when they changed direction abruptly on entering a circle, or 'no response' when they did not do so. An animal was considered to have entered a circle if its nose had entered. The observers were not told which circles contained ticks. After the 48 h of grazing, the tick larvae were recovered by clipping grass tips, using a vacuum cleaner and sweeping with flannelette bats. The procedure is known to recover about 90% of all larvae present[8]. The numbers of larvae were then estimated volumetrically in the laboratory.

Avoidance by the cattle was obvious and highly predictable when large numbers of tick larvae were present (fig. 2). Responses varied with the temperament of the individual animals and ranged from turning away or reversing to subtle bypassing movements. First awareness of the ticks was indicated by various signs of increased alertness. Vision appeared to be important in detecting the dark brown larvae, as they became clearly visible when they moved onto the upper surface of grass blades in response to the presence of a host (fig. 2).

Both the proportion of avoidance reactions of cattle and the percentage of larvae harvested by the cattle were strongly density dependent (fig. 3). Under normal grazing conditions, high concentrations of tick larvae can occur on pastures, because most mature female ticks usually drop off their host in the vicinity of overnight camp sites where cattle awaken at first light[9]. Whilst cattle appear unable to detect the larval progeny of a single female of *B. microplus* (2000 larvae), they are capable of detecting heavily contaminated foci of tick larvae. Similar avoidance of dense concentrations of ticks by nesting birds[10] and of mosquitoes by intolerant hosts of various species[11] has been reported previously. However our observations differ in that the cattle avoided the ticks before they came into physical contact with them.

We conclude that our results reveal a third regulating mechanism for tick populations. The three mechanisms in sequence are 1) avoidance of larvae on pastures by the host, 2) reduced survival and potential fecundity of ticks on the host and, as a last resort, 3) mortality of the most susceptible hosts. The current findings enable refinement of tick population models[3] as well as highlighting a population regulating mechanism that may have wider relevance in parasitology.

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Anthraquinone pigments from a conidiating mutant of *Trichoderma viride**

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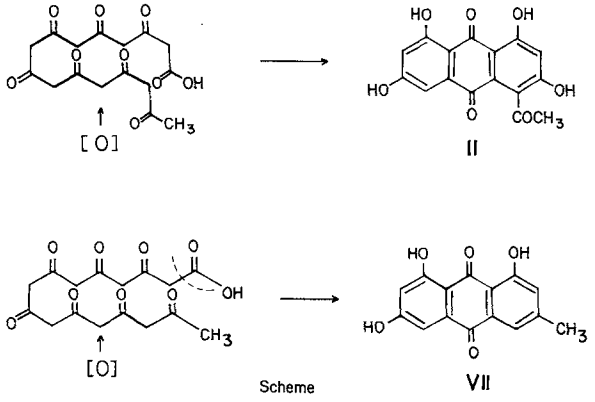
Summary. Two pigments responsible for the yellow-orange color of a brown conidiating mutant of the deuteromycete *Trichoderma viride* were isolated and spectroscopically identified as 1,3,6,8-tetrahydroxyanthraquinone (**I**) and 1-acetyl-2,4,5,7-tetrahydroxy-9,10-anthracenedione (**II**). Both compounds are known substances but were not yet reported as metabolites of this fungal species. Their relationship to other anthraquinones produced by *T. viride* is discussed.

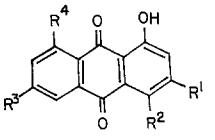
Key words. Trichoderma viride; conidiating mutant; anthraquinones.

The wild type of the deuteromycete *Trichoderma viride* produces dark-green conidia. Their formation is induced by light pulses[1]. Using UV radiation, a series of mutants were prepared, belonging either to the non-conidiating or to the conidiating types. The conidia of the latter type mutants are white, yelow or brown[2–4]. One mutant of the last type was found to accumulate indicator-like pigments both during and after conidiation.

A stationary culture of *T. viride* mutant CCM F-742 was grown



Scheme



	R^1	R^2	R^3	R^4
I	OH	H	OH	OH
II	OH	$COCH_3$	OH	OH
III	OH	COC_3H_7	OCH_3	OH
IV	OH	COC_3H_7	OCH_3	OCH_3
V	CH_3	H	H	H
VI	CH_3	H	H	OH
VII	CH_3	H	OH	OH

on a modified Czapek-Dox medium containing 4% of sucrose and 0.5% of yeast extract under a day-night regime for 21 days. Broth liquor and mycelium were extracted by ethyl acetate. The extracts were combined, solvent was evaporated, the residue washed by hexane, and separated by column chromatography on silica gel in the system benzene-acetone (75:25). Two main components were purified by preparative thin-layer chromatography on precoated Silufol plates (benzene-acetone 75:25). The detection was performed at 366 nm and using ammonia vapors. Compound F-742-B (**I**): yellow powder, m.p. 277–279 °C (hot acetone). UV/VIS spectrum λ_{max} (log ε), nm, CH_3OH: 226 (4.60), 250 (4.32), 275 (4.41), 368 (3.68), 410 (3.57). IR spectrum (Nujol, cm^{-1}): 1580, 1640, 1665. Mass spectrum (EI, 70 eV, direct inlet at 180 °C): 272 (100%, $C_{14}H_8O_6$, M^+), 255 (4%, $C_{14}H_7O_5$), 244 (19%, $C_{13}H_8O_5$), 227 (5%, $C_{13}H_7O_4$), 216 (23%, $C_{12}H_8O_4$), 202 (5%, $C_{11}H_6O_4$), 187 (13%, $C_{11}H_7O_3$). ^1H NMR (d_6-DMSO): 6.57 d (J = 2.4 Hz), 7.10 d (J = 2.4 Hz), 12.18 s (exchangeable proton). ^{13}C NMR (d_6-DMSO): 108.1 d (2C), 108.6 s (2C), 108.8 d (2C), 135.0 s (2C), 164.3 s (2C), 181.3 s, 188.6 s. Comparison of ^{13}C NMR spectra with published data[5] using the similarity index[6] (S = 0.9312) confirms the identification.

Compound F-742-C (**II**): orange crystals, m.p. 224–226 °C (decompn.) (diethyl ether – ethyl acetate 1:2). UV/VIS spectrum λ_{max} (log ε), nm, CH_3OH: 224 (4.71), 255 (4.62), 263 (4.64), 294 (4.32), 316 (3.90), 365 (3.72), 457 (3.61). IR spectrum (Nujol, cm^{-1}): 1318, 1495, 1660, 1670, 1695. Mass spectrum (EI, 70 eV, direct inlet at 180 °C): 315 (5.7%), 314 (31%, $C_{16}H_{10}O_7$, M^+), 300 (18.4%), 299 (100%, $C_{15}H_7O_7$), 149.5 (2.9%), 122 (2.9%), 69 (9.2%), 43 (11.5%). ^1H NMR spectrum (CD_3OD): 2.51 s (3H), 6.51 d (J = 2.4 Hz), 6.59 s, 7.06 d (J = 2.4 Hz); (d_6-DMSO, room temperature): 2.40 s (3H), 5.52 br s (2H), 6.53 d (J = 2.4 Hz), 6.63 s, 7.00 d (J = 2.4 Hz), 12.04 s, 12.36 s. Four hydrogen atoms are exchangeable for deuterium (MS, ^1H NMR). ^{13}C NMR ** (d_6-DMSO, 80 °C). 30.2 Q (127.9), 107.9 d (6.8), 108.0 D (163.1), 108.0 Dd (162.2, 4.2), 108.5 Dd (167.1, 5.0), 125.3 d (4.0), 126.7 d (2.0), 130.7 mt 134.3 s, 160.8 d (2.9), 163.3 d (2.9), 164.9 dd (3.9, 2.0), 181.5 d (4.9), 188.3 s, 200.0 q (5.8). The UV/VIS, IR, and mass spectra closely resemble those of rhodolamprometrin (**II**), found in crinoids *Heterometra savingii* and *Lamprometra kluzingeri*[7]. Because of rather large differences in some proton chemical shifts (0.17 and 0.11 ppm) and the relatively small amount of the compound for the chemical correlation, we resorted to the extensive use of ^{13}C NMR spectroscopy. The deduced functional groups (chelated and unchelated quinone carbonyl, four phenolic hydroxyls, and one C-acetyl group) fit neatly into structure **II**. There are also three aromatic protons in the molecule: two meta-oriented and one isolated. The relative positions of the functional groups with respect to the aromatic and hydroxyl protons can be inferred from the proton-coupled ^{13}C NMR spectra. The chelated quinone carbonyl exhibits no splitting (i.e., has no peri-standing protons), the non-chelated one is a doublet (i.e, one such a proton)[8]. The group occupying the second peri-position can only be the acetyl group, otherwise the quinone carbonyl would be involved in hydrogen bonding. Three enolic carbons have each one geminal coupling, one (165.2 ppm) has two such couplings. There is only one quaternary carbon (134.3 ppm) that is a pure singlet. All these observations are consistent with structure **II** only.

Both **I** and **II** are related to the rhodocomatulin derivatives **III** and **IV** isolated from the sea lilies[9,10]. The other anthraquinones found so far in *T. viride* – pachybasin (**V**), chrysophanol (**VI**), and emodin (**VII**)[11] though also biosynthetized through the acetate-malonate pathway, are formed by different folding patterns of the polyketide chain (scheme)[12].

* Dedicated to Dr Z. Vanek, on the occasion of his 60th birthday.

** Upper case letters denote multiplicity arising from the directly bonded protons, lower case letters that from the geminal and vicinal couplings.

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