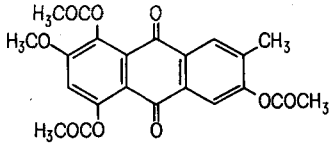
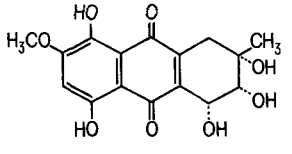
The pigment in ethanol showed an absorption spectrum characteristic for a naphthazarine (5,8-dihydroxynaphthoquinone)-type compound. The absorption of the CO groups in the IR-spectrum (1600 cm^{-1}) was in agreement with this finding.

Acetylation of the pigment with acetic anhydride and a trace of concentrated H_2SO_4 yielded several acetates. The main product obtained in a pure state was not characterized as a naphthoquinone derivative but as an anthraquinone triacetate by means of mass spectroscopic and spectrophotometric analysis. The substance with molecular formula $C_{22}H_{18}O_9$ showed physico-chemical properties similar to the so-called compound VII (I) described by NODA et al.[1] The identity was confirmed by exact comparison of the IR-spectrum of our product with that of compound VII[2]. Compound VII is one of the products obtained by NODA and his co-workers as a derivative of bostrycin, a tetrahydroanthraquinone pigment (II) produced by the fungus *Bostrychonema alpestre* Ces. and it was thought that the *Arthrinium* pigment might be identical with bostrycin. The identity was established by direct comparison with authentic bostrycin. The *Arthrinium* pigment showed complete agreement in all respects (MS, UV-vis., IR, TLC). The molecular structure of bostrycin shows that it can be dehydrated easily. Conc. H_2SO_4 was the dehydrating agent in the acetylation reaction indicated above.



I



II

The fungus forms some minor yellow and red pigments, which were not investigated. Some other metabolites isolated and identified by comparison with authentic samples were ergosterol and succinic acid. A metabolic compound detected on silica gel F_{254} thin layers as a dark spot in short wave UV-light and giving coloured spots by spraying with phenolic reagents[3] was also formed. The amount isolated was too small for complete characterization. Mass spectrometry revealed a molecular formula of $C_{18}H_{20}O_5$. Other physico-chemical data are shown below.

The strain labelled as *Bostrychonema alpestre*[4] did not produce conidia. The original specimen appeared too scanty for recognition. The systematic position of the genus *Bostrychonema* Ces. is still problematic. Since no type material is available and the original description is too vague, the genus should be regarded as doubtful. It was not possible to compare the relationship of *A. phaeospermum* with *B. alpestre*[5].

Physico-chemical data[6]. Anthraquinone derivative (I): m.p. 239–240 °C. mol. wt. 426.09700, calc. for $C_{22}H_{18}O_9$ 426.09507[7]. ν_{max} (KBr) 1775, 1765, 1672, 1660, 1595, 1580, 1370, 1350, 1290, 1210, 1192, 1180, 1090, 1018, 975, 892, 786 cm^{-1}. Bostrycin, (II): subl. at 200 °C, no exact m.p. due to sintering. MS: *m/e* 336 (M^+, $C_{16}H_{16}O_8$). λ_{max} (EtOH): 228 (log ε 4.42), 301 (3.87), 480 sh (3.74), 506 (3.81), 541 (3.64). ν_{max} (KBr): 3490, 3390, 1600 cm^{-1}. Phenolic compound: mol.wt. 316.1299, calc. for $C_{18}H_{20}O_5$ 316.1310. M–C_7H_{11}: 221.0451, calc. for $C_{11}H_9O_5$ 221.0450. M* 186.46: transition $221^+ \rightarrow 203^+ + 18$ (H_2O). λ_{max} (Ether): 224, 241, 295, 336 nm; λ_{max} (EtOH): 224 sh, 242 sh, 301, 330 sh nm; λ_{max} (EtOH/KOH): 253, 366 nm; ν_{max} (KBr) 3390 br, 3010, 2960, 2930, 2880, 2860, 1712, 1620, 1592, 1580 sh, 1571 sh, 1562 sh, 1438, 1290, 1195, 1180, 1158, 1142, 1082, 1040, 980, 938, 872, 858, 840, 765, 708 cm^{-1}.

Zusammenfassung. Als Hauptfarbstoffkomponente im Mycelium und Nährmedium eines Stammes des Schimmelpilzes *Arthrinium phaeospermum* wurde Bostrycin, ein Tetrahydroanthrachinon-Pigment isoliert und durch Vergleich mit der authentischen Substanz identifiziert. Dieser Pilz produziert auch Ergosterol, Bernsteinsäure und eine phenolische Verbindung $C_{18}H_{20}O_5$.

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[5] We are grateful to Dr. W. GAMS and Dr. R. A. SAMSON of our institute for examining the fungi.

[6] The technical assistance of Mr. H. J. ROEYMANS is gratefully acknowledged.

[7] The author is indebted to Dr. W. HEERMA and Mr. C. VERSLUYS, Analytical Laboratory, State University of Utrecht, for measuring the mass spectra.

Hairless Mice, Human Leprosy and Thymus-derived-Lymphocytes

It is generally believed that lepromatous leprosy patients have a nonspecific impairment of cell-mediated immunity. The mediators of cellular immune response are the thymus-derived (T) lymphocytes, as opposed to bone marrow-derived (B) lymphocytes, which are the mediators of humoral immunity. Recently two groups of workers reported a significant decrease of T-cell populations in lepromatous patients and a concomitant increase in B-lymphocytes[1,2]. Other workers were unable to corroborate these observations[3]. In 'normal' mice, multiplication of *Mycobacterium leprae* is restricted to the footpads[4]. The infection which develops approximately 6 months after inoculating the mouse footpads with *M. leprae*, does not spread to other tissues in the animal. In neonatally thymectomized X-irradiated mice, i.v. inoculation with *M. leprae* has been reported to result in generalized

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Multiplication of *Mycobacterium leprae* in hairless mice

Strain of mouse	Route of inoculation	No. of bacilli inoculated	No. of bacilli harvested
Hairless	Footpad	1.0×10^4	$7.0 \pm 1.0 \times 10^6$/footpad
Hairless	Subcutaneous	1.0×10^7	Skin: negative Footpad: negative Spleen: negative
Hairless	Intravenous	1.0×10^7	Skin: negative Spleen: negative Footpad: negative
NIH	Footpad	1.0×10^4	$5.3 \pm 0.93 \times 10^6$/footpad

infection[5]. This has been attributed to the induced deficiency of immune response in the host animals. The nine-banded armadillo is the only species in which at least some of the animals have been shown to be naturally susceptible to the systemic form of leprosy[6]. Hairless (nude) mice are characterized by hypoplastic thymus glands and a consequent depletion of T-lymphocytes[7,8]. Congenitally athymic (nude) mice have been found to accept for their life-time skin grafts from distantly related animals species including man[9], demonstrating an impairment of thymus-dependent immunity in these mice.

Methods. To study whether there would be increased multiplication of *M. leprae* in hairless mice, the animals were inoculated in the footpads as well as i.v. and s.c. with suspensions of viable bacilli. The nude mice were offspring heterozygotes (brown, black and nonpigmented strains), obtained from the Jackson Laboratory, Bar Harbor, Maine, USA. The suspension of *M. leprae* was prepared from skin biopsies of lepromatous patients. To test the viability of the organisms, the bacilli were inoculated into the footpads of an NIH strain of Swiss mice. 2 experiments were completed using 30 hairless mice and 20 NIH mice. 6 months after inoculation, the animals were sacrificed and their footpads as well as skin and spleen tissues were examined, and the organisms present were enumerated. (In autopsies of lepromatous patients, we have detected greater numbers of leprosy bacilli in the spleen than in other internal organs.) Several of the hairless mice died in the course of the experiment; however, a number of animals survived for more than 6 months. The life span of the nude mouse is approximately 7 months[10]. The mice that died were also examined for any evidence of bacterial multiplication.

Results and discussion. Typical results presented in the Table show that there is no significant increase in proliferation of *M. leprae* in hairless mice as compared to 'normal' mice. Despite their proven T-cell deficiency[8], the nude mice do not promote generalized infection with *M. leprae*. Immune deficiency disorders are usually accompanied by high incidence of malignancies. However, RYGAARD and POVLSEN[10] found that hairless mice do not develop spontaneous tumors. These authors propose a third (as yet unknown) expression of immunological surveillance in nude mice, separate from cell-mediated and humoral immunity. Our observations suggest that deficient T-lymphocyte function alone might not provide a satisfactory explanation for excessive susceptibility to leprosy.

Zusammenfassung. Die Vermehrung und Ausbreitung von *Mycobacterium leprae* in einem haarlosen Mäusestamm wurde untersucht. Trotz Fehlen der T-Lymphocyten (zelluläre Immunität) wurden gleiche Resultate wie beim Mäusestamm mit T-Lymphocyten-Aktivität erzielt.

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Does 2,4-D Induce Mitotic Irregularities in Plant Tissue Cultures?

An exogenous growth regulator is required for initiation and maintenance of plant tissue cultures; 2,4-D is, perhaps, the most commonly used chemical for this purpose. It induces both mitotic and meiotic irregularities in vivo in a number of plant species[1-3]. Earlier reports suggested that exogenous 2,4-D induces mitotic irregularities in plant tissue cultures[4,5]. Recently BAYLISS[6] concluded that 2,4-D induces anaphase anomalies in carrot

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