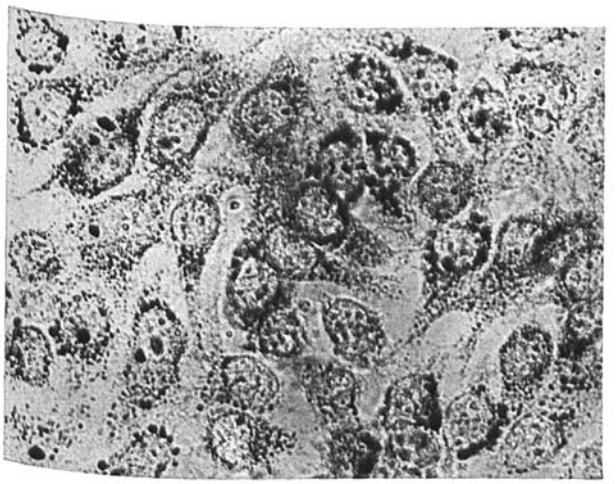

Fig. 1. Kontrollkultur mit sehr ausgeprägter SDH-Aktivität, × 700.

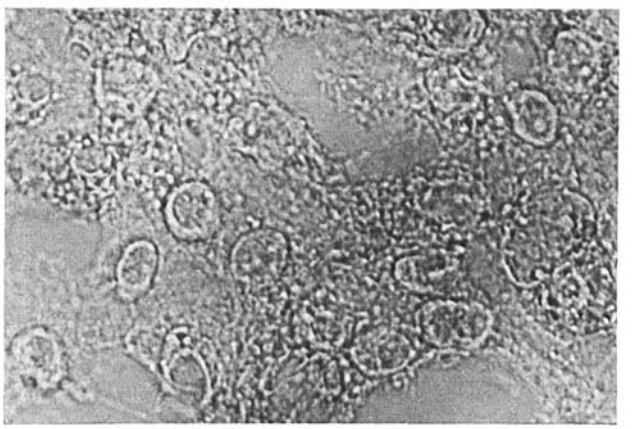

Fig. 2. Mit *Hepatitis-infectiosa*-Virus infizierte Kultur, die keine SDH-Aktivität aufweist, × 700.

beobachtet, während sich die vollkommene Inhibierung erst am Ende des 3. Tages nach der Infizierung zeigte.

Auf Grund dieser Ergebnisse kann man annehmen, dass die Hemmung der SDH-Aktivität nicht in direktem Zusammenhang mit der Biosynthese des Virus steht. Anderseits haben die elektronenoptischen Untersuchungen erwiesen[4], dass in den von Viren infizierten Zellen die Mitochondrien tiefgehende Veränderungen erleiden. Es ist also wahrscheinlich, dass die Enzyminaktivierung den Veränderungen im Mitochondrialapparat zuzuschreiben ist. Da die mikroskopischen zytopathogenen Effekte der Virusinfektion erst 6 Tage nach der Infizierung festgestellt werden können, ist es für die frühzeitige Erkennung der Virusinfektion von besonderem Interesse, das Verhalten der SDH-Aktivität zu untersuchen.

Summary. The inhibitory action of *Hepatitis infectiosa* virus on the SDH activity of Detroit-6 (VA) cell lines was investigated. The full inhibition of the SDH activity took place at the end of the third day after the infection. As this phenomenon precedes the cytopathogenetic effect of viral infection, it may be of some help in the early detection of the infection.

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A Polypeptide Antibacterial Agent Isolated from *Trichoderma viride*

Antibiotic U-22324 was discovered in the culture broth of the fungus *Trichoderma viride*. Fermentations were conducted under submerged culture conditions in a medium containing black strap molasses, 20 g/l; dextrin, 30 g/l; fish meal, 15 g/l; Pharmamedia (Traders Oil Mill Co., Fort Worth, Texas), 15 g/l; and tap water to make 1 l. The final pH of this medium was adjusted to 6.8 prior to sterilization. Antibiotic titers were measured by a microbiological disc-plate assay similar to the one described by Hanka et al.[1], using *Sarcina lutea* as the test organism. The log dose-response curve was linear over a concentration range from 1–5 mg U-22324/ml. Peak titers were usually obtained after 4–5 days' incubation at 25 °C and amounted to 8–10 mg/ml. This represents an unusually high yield for a single peptide to be synthesized by a microorganism.

Antibiotic U-22324 was isolated from a 1600 ml aliquot of filtered fermentation broth by precipitation of the active material at pH 4.6. The precipitate was collected with the aid of 1% (w/v) of Celite and the filter cake extracted with 400 ml of acetone. An oil, recovered by removal of the acetone at 30 °C, crystallized upon refrigeration. This crude material (2.7 g) was purified by dissolving it in a minimum amount of absolute ethanol at room temperature, treatment with 200 mg of charcoal (Darco G60), concentration to 25 ml, and the slow addition of

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25 ml diethyl ether to incipient crystallization. After filtration and drying the white crystals weighed 1.7 g.

The antibiotic exhibits a single pKa' value at 5.5, titrating as an acid, which, together with the fact that it does not react with ninhydrin, indicates it is a cyclic peptide. It exhibits no absorption above 220 nm and contains no sulfur.

Analysis of an hydrolysate of the antibiotic in a Beckman Spinco automatic amino acid analyzer[2] showed it to have the following composition: $(Glu)_3(Pro)_2(Gly)_1(Ala)_2$-$(Val)_2(Leu)_1$ and an unknown amino acid which emerged at the position ordinarily occupied by cysteine. Amide nitrogen analysis indicated that 2 of the glutamic acid residues were present in their amide form. Sufficient material (2.5 g) was then hydrolyzed to permit isolation of the individual amino acids in larger quantities. The procedure of HIRS et al.[3] was used for this purpose. Each of the amino acids was obtained in crystalline form and in approximately 70% yield based upon the total quantitative ninhydrin method used to locate the various fractions[4].

The unknown constituent amino acid was identified by IR- and NMR-spectroscopy as 2-methylalanine (or α-amino isobutyric acid). $8M$ are present per M of polypeptide. To our knowledge this amino acid has been isolated only once before[5], and from an antibiotic. Positive identification of the other amino acids was obtained by comparison of their IR-spectra with authentic samples. The optical activities of the amino acids were determined and all the active ones proved to have the L-configuration. A cyclic polypeptide with the composition $(GluN)_2(Glu)_1$-$(Pro)_2(Gly)_1(Ala)_2(Dimethyl\ ala)_8)(Val)_2(Leu)_1$ has the theoretical elementary composition for $C_{81}H_{135}N_{21}O_{23}$ of C, 54.90; H, 7.68; N, 16.6. Found: C, 54.89; H, 7.74; N, 15.7. While the observed nitrogen value is more in accord with N_{20}, which would indicate a single glutamine residue, direct amide nitrogen analysis yielded 1.43%; theory for 2 glutamine residues, 1.58%.

The in vitro antibacterial spectrum of antibiotic U-22324 (Table I) indicates that the compound inhibits G+ bacteria exclusively when tested in the 2-fold broth dilution assay[6].

The maximum tolerated dose of U-22324 in mice was 40 mg/kg/day when administered s.c. and 80 mg/kg/day orally. The antibiotic was inactive in mice experimentally infected with *S. hemolyticus* when administered s.c. at the maximum tolerated dose. No blood levels were demonstrated in mice dosed s.c. with 80 mg/kg as determined by the method of LEWIS et al.[6].

Of 14 pathogenic fungi tested in vitro, the antibiotic inhibited *Blastomyces dermatitis* at a concentration of 100 µg/ml and *Nocardia asteroides, Hormodendrum compactum, Histoplasma capsulatum, Trichophyton mentagrophytes* UC-4800 and *Coccidioides immitis* at concentrations of 1000 µg/ml as shown in Table II.

Antibiotic U-22324 decreases the surface tension of water extensively and might, therefore, have some utility as a detergent. A solution containing 100 µg/ml of the antibiotic in deionized water had a surface tension of 41.6 dynes/cm at 25 °C as compared to 71.8 dynes/cm for deionized water and 52.7 dynes/cm for a 100 µg/ml solution of sodium lauryl sulfate (Duponol) respectively[7].

Table I. In vitro antibacterial activity of antibiotic U-22324 in brain heart infusion broth

Test organism		Minimal inhibitory concentration µg/ml
E. coli	ATCC-26	> 1000
K. pneumoniae	PCI-602	> 1000
P. vulgaris	ATCC-8427	> 1000
Ps. aeruginosa	ATCC-9027	> 1000
S. paratyphi	UC-263	> 1000
S. pullorum	MSDH-75	> 1000
S. typhosa	UC-215	> 1000
S. aureus	UC-76	62.5
S. aureus	UC-70	125
S. faecalis	ATCC-6057	31
S. hemolyticus	C-203	31
S. viridans	UC-155	31

Table II. In vitro antifungal activity of antibiotic U-22324 in Agar dilution test

Test organisms	Minimal inhibitory concentration µg/ml
Nocardia asteroides	1000
Blastomyces dermatitidis	100
Coccidioides immitis	1000
Geotrichum sp.	> 1000
Hormodendrum compactum	1000
Cryptococcus neoformans	> 1000
Histoplasma capsulatum	1000
Sporotrichum schenckii	> 1000
Monosporium apiospermum	> 1000
Trichophyton rubrum	> 1000
Trichophyton interdigitale	> 1000
Candida albicans	> 1000
Trichophyton violaceum	> 1000
Trichophyton asteroides	> 1000
Trichophyton mentagrophytes UC-4797	> 1000
Trichophyton mentagrophytes UC-4860	1000

The antifungal tests were done on agar plates. Different concentrations (1000, 100, 10 or 1 µg/ml) of the antibiotic were dissolved in the agar and the plates were inoculated by the cross-streak technique. Results are expressed as the minimal inhibitory concentration of antibiotic yielding total inhibition of fungal growth. Endomycin inhibits these organisms at 1 or 10 µg/ml.

Zusammenfassung. Antibiotikum U-22324 ist ein zyklisches Peptid, das aus Kulturflüssigkeit von *Trichoderma viride* isoliert wurde. Das Peptid hat die Aminosäurenzusammensetzung $(GluN)_2(Glu)_1(Pro)_2(Gly)_1(Ala)_2(Dimethyl\ ala)_8(Val)_2(Leu)_1$.

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