Hyphodontal, a New Antifungal Inhibitor of Reverse Transcriptases from *Hyphodontia* sp. (Corticiaceae, Basidiomycetes)

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*Hyphodontia* sp., Basidiomycetes, Sesquiterpenes, Isolactaranes, Reverse Transcriptases

In a search for inhibitors of RNA-directed DNA polymerases a new isolactarane sesquiterpenoid, hyphodontal (1), was isolated from fermentations of a Canadian *Hyphodontia* species. Its structure was elucidated by spectroscopic methods. Hyphodontal strongly inhibits the growth of several yeasts and is a non-competitive inhibitor of avian myeloblastosis virus (Kj 346 μM) and Moloney murine leukemia virus (Ki 112 μM) reverse transcriptases. In addition, cytotoxic and antifungal activities were observed.

**Introduction**

In a screening of basidiomycetes for the production of inhibitors of avian myeloblastosis virus (AMV) RT (Erkel, 1990) we detected that mycelial cultures of a Canadian *Hyphodontia* species produced a compound strongly inhibitory towards this enzyme and in addition to the yeast *Nematospora coryli*. In the following we describe the fermentation, isolation, structural elucidation, and biological characterization of this new metabolite from *Hyphodontia* sp. strain 87229. Previously we have reported on the isolation of podoscyphic acid (Erkel et al., 1991) from a Tasmanian *Podoscypha* species, and clavicoronic acid from *Clavicorona pyxidata* (Erkel et al., 1992).

**Materials and Methods**

**General**

Spectral data were recorded on the following instruments: 1H and 13C NMR, Bruker AC-200 and AM-400; EI-MS, A.E.I. MS-50; FAB-MS, Kratos Concept H-System; IR, Perkin-Elmer 1420; UV, Perkin-Elmer Lambda 16 and Varian Cary 17; CD, Jobin Yvon CNRS Roussel-Jouan Dichrographe III. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter. The m.p.'s were determined with a Reichert hot plate microscope and are uncorrected. For TLC aluminium foils coated with silica gel Merck 60 F254 were used. PTLC was carried out on glass plates precoated with silica gel (Merck 60 F254, 2 mm). Merck silica gel 60 (230–400 mesh) and Pharmacia Sephadex LH-20 were used for column chromatography. All solvents were distilled prior to use.

*Hyphodontia* sp. strain 87229

Mycelial cultures were obtained from spore prints of a fruiting body growing on wood in Port Alberni, B.C., Canada. The specimen shows the characteristics of the genus as described by Ryvarden and Eriksson (1976). The species, however, could not be identified. Voucher specimen and cultures are deposited in the collection of the Lehrbereich Biotechnologie, University of Kaiserslautern.

**Fermentation**

For maintenance the fungus was cultivated in YMG medium composed of: yeast extract 0.4%, malt extract 1%, glucose 0.4% and agar 1.5% for...
solid media. For the production of hyphodontal a corn meal-glucose medium (M3) was used containing (g/l): corn meal (20), glucose (10), KH₂PO₄ (1.5), KCl (0.5), NaNO₃ (0.5), MgSO₄·7H₂O (0.5), pH 5.0. A well-grown seed culture of *Hyphodontia* sp. 87229 (200 ml) in YM G was used to inoculate 20 l of medium M3 in a Biolafitte C6 fermentation apparatus. The fermenter was incubated at 22 °C with an aeration rate of 31 air/min and agitation (130 rpm). The production of hyphodontal was followed by a plate diffusion assay using *Nematospora coryli* as test organism or by estimating the inhibitory effect of 2.5 µl of a crude extract (centrifuged 100 times as compared to the culture fluid) in the standard assay of AMV reverse transcriptase.

### Isolation of hyphodontal (1)

During purification hyphodontal was detected using *Nematospora coryli* as test organism and by the standard assay for AMV reverse transcriptase. After removal of the mycelia by filtration, hyphodontal was extracted from the culture filtrate (19 l) with ethyl acetate (two times 5 l). Evaporation of the organic phase yielded a crude extract (3.5 g), which was further purified by chromatography on Sephadex LH-20 (elution with methanol) resulting in 830 mg of an enriched product. This was further purified by chromatography on silica S 200 (Merck) and finally preparative HPLC (LiChrogel PS 1, column 2.5×25 cm, elution with 2-propanol) to yield 110 mg of hyphodontal (1).

### Physicochemical properties of hyphodontal (1)

Colorless microcrystals, m.p. 165–170 °C, Rₙ 0.31 [toluene–HCO₂Et–HCO₂H (100:100:1)]; [α]D²₀ −85 °C (c 0.75, CHCl₃); UV (MeOH) λmax 260 nm (log ε 3.63); CD (MeCN) λmax 223 nm (Δεrel −1), 242 (0), 257 (+0.24), 283 (0), 332 (−0.20), 390 (0); IR (KBr) cm⁻¹ 3315, 2942, 2923, 2860, 1745, 1652, 1455, 1089, 1058, 958, 896; ¹H and ¹³C NMR spectra see Table I; HREI-MS (70 eV; DI 180 °C) m/z (relative intensity %) 262.1201 (26, M⁺, calced for C₁₅H₁₉O₄ 262.1197), 247 (18, C₁₄H₁₁O₄), 244 (64, C₁₅H₁₀O₃), 229 (100, C₁₄H₁₃O₃), 216 (21, C₁₄H₁₉O₂), 201 (41, C₁₃H₁₃O₂), 187 (24), 159 (22), 145 (25), 105 (30), 91 (31), 41 (24). O-Acetyl-4-deoxydihydrohyphodontal (4a)

Sodium borohydride (10.0 mg) was added to a solution of 1 (4.0 mg) in methanol (3 ml). After stirring for 2 h at 20 °C, chloroform (25 ml) was added and the mixture acidified to pH 3 with 0.1 N HCl. The aqueous layer was extracted with chloroform (3×10 ml) and EtOAc (2×10 ml) and the dried organic phases (Na₂SO₄) were evaporated in vacuo. The resulting oil was treated with acetic anhydride (0.05 ml) and pyridine (1.0 ml) for 3 h at 20 °C. Evaporation of the solvents and purification of the residue by PTLC [silica gel, petroleum ether₄₀–₆₀–Et₂O (1:1)] afforded 4a (3.8 mg) as colorless oil; Rₙ 0.39 [petroleum ether₄₀–₆₀–Et₂O (1:1)]; [α]D²₀ +50 (c0.22, CHCl₃); UV (MeCN) λmax 220 nm (Δεrel 0), 242 (−1), 252 (0), 265 (+0.23), 292 (0); IR (CHCl₃) cm⁻¹ 2945, 1760, 1734, 1271, 1243, 1228, 1094, 1076, 1017; ¹H NMR (400 MHz, CDCl₃) δ 0.98 (3H, s), 1.14 (3H, s), 1.15 (1H, d, J = 5.0 Hz), 1.17 (1H, dd, J = 12.0 and 11.0 Hz), 1.33 (1H, m), 1.55 (1H, d, J = 5.0 Hz), 1.75 (1H, ddd, J = 12.0, 7.0 and 1.4 Hz), 2.08 (3H, s), 2.10 (1H, dm, J = 17.0 Hz), 2.21 (1H, dm, J = 17.0 Hz), 2.43 (1H, m), 2.45 (1H, dd, J = 14.9 and 7.4 Hz), 4.29 (1H, dd, J = 9.1 and 1.0 Hz), 4.34 (1H, dd, J = 9.1 and 0.5 Hz), 4.64 (2H, m); ¹³C NMR (100.6 MHz, CDCl₃) δ 20.94, 20.98, 22.51, 28.49, 29.51, 29.68, 30.46, 37.19, 37.67, 43.49, 46.99, 62.54, 70.31, 121.14, 143.75, 170.71, 177.20; EI-MS (70 eV; DI 180 °C) m/z (relative intensity %) M⁺ 481 not observed, 233 (100), 230 (41, M⁺–HOAc), 215 (38), 187 (28), 185 (20), 171 (20), 43 (57).

O-(4-Bromobenzoyl)-4-deoxydihydrohyphodontal (4b)

Hyphodontal (1) (7.0 mg) was reduced with sodium borohydride as described above. The resulting oil was dissolved in THF (3 ml) and treated with pyridine (16 µl), 4-(dimethylamino)-pyridine (3.0 mg) and 4-bromobenzoyl chloride (42 mg) in THF (1 ml) for 24 h at 20 °C. The mixture was diluted with Et₂O (40 ml) and washed successively with saturated aqueous NaHCO₃ (2×20 ml), saturated aqueous NaHCO₃ (2×20 ml) and brine (2×20 ml). The organic phase was dried over Na₂SO₄ and evaporated to dryness. Purification of the residue by PTLC on silica gel using petroleum ether₄₀–₆₀–EtOAc (5:1) afforded 4b
(8.5 mg) as colorless oil; Rf 0.43 [petroleum ether, EtOAc (5:1)]; [α]D0 +37 (c 0.45, CHCl3); UV (MeCN) λmax 199 nm (εrel 1), 243 (0.66); IR (CHCl3) cm⁻¹ 2950, 1760, 1713, 1583, 1363, 1263, 1109, 1096, 1011; 1H NMR (200 MHz, CDCl3) δ 1.01 (3H, s), 1.07 (3H, s), 1.18 (1H, d, J = 5.0 Hz), 1.20 (1H, dd, J = 12.2 and 10.8 Hz), 1.36 (1H, dd, J = 15.2 and 14.4 Hz), 1.57 (1H, d, J = 5.0 Hz), 1.79 (1H, ddd, J = 12.2, 7.0 and 1.3 Hz), 2.17 (1H, dm, J = 17.2 Hz), 2.31 (1H, d, J = 17.2 Hz), 2.46 (1H, m), 2.48 (1H, dd, J = 15.2 and 7.4 Hz), 4.35 (1H, d, J = 9.0 Hz), 4.49 (1H, d, J = 9.0 Hz), 4.90 (2H, m), 7.60, 7.87 (each 2H, ‘d’, AA’BB’ system), J = 8.6 Hz); EI-MS (70 eV; DI 180°C) m/z (relative intensity %) M⁺ not observed, 231 (32), 230 (100, M⁺-C6H4BrCO2H), 215 (60), 187 (48), 186 (48), 185 (54), 183 (39), 171 (46).

Preparation of compound 5

To a solution of 1 (4.0 mg) in DMF (1 ml) were added 2,4-dinitrophenylhydrazine (50 mg) in DMF (0.5 ml) and one drop of conc. HCl. After stirring at 20 °C for 3 h, the solution was diluted with EtOAc (20 ml) and washed successively with saturated aqueous NaHCO3 (2×10 ml) and brine (2×10 ml). The organic layer was dried over Na2SO4 and evaporated to dryness. The resulting oil afforded on column chromatography [silica gel, CH2Cl2-MeOH (10:1)] the crude bishydrazone (2.5 mg), Rf 0.38 [dichloromethane–methanol (10:1)].

To a solution of the bishydrazone (2.5 mg) in THF (1 ml) was added N-methylmorpholine (0.1 ml) and sec-butyli chloroformate (0.13 ml) at −15 °C. After stirring at 20 °C for 20 min, the mixture was diluted with EtOAc (20 ml) and washed successively with saturated aqueous NH4Cl (10 ml) and brine (10 ml). The organic layer was dried over Na2SO4 and concentrated under reduced pressure to give an oil, which was chromatographed on a silica gel column (eluent: CH2Cl2). Further purification on Sephadex LH-20 [eluent: CH2Cl2-MeOH (5:1)] afforded pure 5 (0.6 mg), red oil; Rf 0.59 [petroleum ether, EtOAc (2:1)]; UV/VIS (MeCN) λmax 191 nm (εrel 1), 262 (0.29, sh), 298 (0.19, sh), 380 (0.34); IR (CHCl3) cm⁻¹ 2955, 1615, 1339, 1261, 1090, 1010, 808; 1H NMR (200 MHz, CDCl3) δ 1.08 (3H, s), 1.16 (3H, s), 1.28 (1H, t, J = 12.0 Hz), 1.87 (1H, d, J = 5.5 Hz), 1.62 (1H, m), 1.92 (1H, dd, J = 12.0 and 7.5 Hz), 2.27 (1H, d, J = 5.5 Hz), 2.49 (2H, m), 2.61 (1H, s), 2.88 (1H, dd, J = 13.7 and 6.8 Hz), 7.87, 7.96 (each 1H, d, J = 8.6 Hz), 8.07 (1H, s), 8.38, 8.51 (each 1H, dd, J = 8.6 and 3.0 Hz), 8.83 (1H, d, J = 3.0 Hz), 8.88 (1H, s), 9.18 (1H, d, J = 3.0 Hz). 11.24 (1H, s, NH); (+)-FAB-MS (mNBA) m/z 605 (M+H)+.

Biological assays

Antimicrobial spectra, cytotoxicity and macromolecular syntheses in whole L 1210 cells were measured as described previously by Weber et al. (1990). The effect of hyphodontal on cell growth was measured according to Mirabelli et al. (1985) with slight modifications (Erkel, 1990). L 1210 cells (ATCC CCL 219), HeLa cells (ATCC CCL 2.2) and Ehrlich ascites carcinoma cells (H. Probst, University of Tübingen) were grown in Ham’s F 12 medium, BHK 21 (ATCC CCL 10) in G-MEM, Balb 3T3/MMSV cells (moloney murine sarcoma virus transformed, ATCC CCL 163.2) and HUT 78 cells (ATCC TIB-161) in RPMI 1640 supplemented with 10% fetal calf serum and 65 μg/ml penicillin G and 100 μg/ml streptomycin sulfate in a humidified atmosphere containing 5% of CO₂ at 37 °C.

Nucleic acid syntheses in permeabilized L 1210 cells were performed according to Berger (1978). RNA syntheses in isolated nuclei were measured as described by Marzluff and Huang (1984).

Assay for RNA-directed RNA polymerase of vesicular stomatitis virus (VSV) was carried out as described previously (Erkel, 1992).

Assay for avian myeloblastosis virus (AMV) RT: The method reported by Hanajima et al. (1985) was modified: a reaction mixture (50 μl) consisting of 80 mM Tris-HCl (pH 8.3), 6 mM dithiothreitol (DTT), 5 mM MgCl₂, 60 mM KCl, 200 μg/ml bovine serum albumin (BSA), 10 μM dTTP containing 0.01 μCi [2-14C]dTTP (44 cpm/ pml), 5 μg/ml poly(A)-(dT)₅ and 20 U/ml AMV RT (Boehringer, Mannheim) were incubated at 37 °C for 60 min. The reaction was terminated by adding 1 ml of cold 20% trichloroacetic acid (TCA) containing 20 μM pyrophosphate. The acid-insoluble fractions were collected on cellulose nitrate filters presoaked with 20 μM pyrophos-
phate solution. The filter papers were washed three times with cold 5% TCA solution and the remaining radioactivity was measured in a liquid scintillation counter.

Assay for Moloney murine leukemia virus (MMuLV) RT: The reaction mixture (50 μl) contained 80 mM Tris-HCl (pH 8.3), 10 mM DTT, 8 mM MgCl₂, 30 mM KCl, 200 μg/ml BSA, 5 μg/ml poly(A)-(dT)₁₅, 14 μM dTTP containing 0.01 μCi [2-¹⁴C]dTTP (40 cpm/pmol) and 20 U/ml MMuLV RT (Pharmacia, Uppsala). Unless otherwise specified the reaction mixture was incubated for 60 min at 37 °C and the radioactivity of the acid-insoluble fractions were determined as described above.

Assay of HIV-1 RT: HIV-1 RT (United States Biochemicals) activity (20 U/ml) with poly(A)-(dT)₁₅ as template primer was assayed using the reaction conditions described for MMuLV RT.

The RT assay with a 1080 b.p. LTR template (kindly provided by Dr. S. Weiss (Weiss et al., 1992), Boehringer, Mannheim) and a 18mer complementary primer was performed as follows: 2.8 μM LTR template and 20 μM 18mer primer were combined after annealing at 66 °C and slowly cooling to room temperature with 80 mM Tris-HCl (pH 8.3), 8 mM MgCl₂, 30 mM KCl, 10 mM DTT, 200 μg/ml BSA, 10 μM dTTP, dATP, dCTP, dGTP, 1 μCi [²H]dTTP (3.34 pmol), and 20 U/ml HIV-1 RT. The reaction mixture (50 μl) was incubated for 60 min at 37 °C and the radioactivity in the acid-insoluble fractions was determined as described above.

Test for mutagenicity: Mutagenicity was tested according to the method of Ames et al. (1975). Mutants of Salmonella typhimurium strain TA 98 and TA 100 were used in the pour plate assay as described by Venitt et al. (1984).

Results and Discussion

Production of hyphodontal

In 201 fermentations the production of hyphodontal, as measured by the inhibition of AMV reverse transcriptase and by a plate diffusion assay with N. coryli, starts approximately 10 days after inoculation. The highest concentration of the inhibitor is reached after 22 days (Fig. 1).

Structural elucidation

Hyphodontal, m.p. 165–170 °C, has the molecular formula C₁₅H₂₀O₄ as determined by high resolution mass spectrometry. The base peak at m/z 262 arises from loss of water and a methyl group from the molecular ion. At room temperature the ¹H and ¹³C NMR spectra of 1 show strong line broadening which leads to the disappearance of some of the signals. However, at −33 °C well resolved spectra are observed which allow the assignment of all hydrogen and carbon atoms (Table I).

The presence of an α,β-unsaturated aldehyde is indicated by a strong conjugated carbonyl absorption at 1657 cm⁻¹ in the IR spectrum, a singlet at δＨ 9.72 in the ¹H NMR spectrum and signals at δC 189.87, 165.01, and 129.88 in the ¹³C NMR spectrum. Lactone and lactol groups are characterized by signals at δC 176.20 and 98.55, respectively, in the ¹³C NMR spectrum and absorptions at 1745 and 3315 cm⁻¹ in the IR spectrum. The lactol protons give rise to doublets at δＨ 5.90 and 6.99, the latter disappearing on addition of D₂O. Further signals in the NMR spectra can be assigned to a C(CH₃)₂
Table I. *H and 13C NMR data of hyphodontal (1) (400 MHz and 100.6 MHz, respectively; δ-values; in d8-THF at -33 °C).

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<th>Proton</th>
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<th>Carbon</th>
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a Assignments confirmed by H,H and C,H correlation experiments.
b Signals show strong line broadening at 20 °C.
c Signals may be interchanged.

unit, an isolated cyclopropane methylene group (δH 1.15 and 1.44, 1JH,H = 4.9 Hz, δC 20.29, 1JCH = 168 Hz) and a (C=CH2–CH(C–CH2–C) moiety. The methine proton of this unit is connected through long-range coupling (1JH,H = 2.6 Hz) with the A part of an AB system at δH 2.64 and 2.77 (J = 19.0 Hz). From this evidence and the 1H–13C long-range couplings (Fig. 2), formula 1 can be assigned to hyphodontal.

Formula 1 explains the large 4J coupling between the pseudoaxial allylic proton 9-H and one of the methylene protons at C-1 across the β-carbon of the unsaturated aldehyde unit. Ample precedence for this kind of coupling is found in the spectrum of merulidial (2) (Gianetti et al., 1986).

It is evident from molecular models that the large chemical shift difference of the methylene protons at C-8 in the 1H NMR spectrum of 1 is caused by the anisotropic effect of the neighbouring lactone carbonyl group. Only the pseudoequatorial proton at δH 2.44 cis to the lactone ring is deshielded whereas the pseudoaxial proton at δH 1.39 is unaffected. Since the coupling constant between this proton and 9-H is large (12.5 Hz), the two hydrogens must be in a trans diaxial arrangement and, thus, 9-H is in a cis arrangement to the cyclopropane ring.

The trans relationship of the lactol hydroxy group and the cyclopropane ring in 1 follows from the close agreement of the chemical shift of the exo cyclopropane proton with that of marasmic acid and 5α-O-butylnarasmic acid (Dugan et al., 1966; Greenlee and Woodward, 1980; Anke et al., 1989).

The absolute configuration of hyphodontal given in formula 1 can be determined by comparison of its CD spectrum (Fig. 3) with that of marasmic acid (3) of known absolute stereochemistry (Cradwick and Sim, 1971). Since isomers 1 and 3 contain enantimorphic chromophores CD curves of opposite sign are observed. The absolute stereochemistry of 1 is in accord with that of other isolactaranes from Basidiomycetes (Gianetti et al., 1986; Konitz et al., 1977; Sterner et al., 1990; Trost and Hipkinson, 1992).

Reduction of hyphodontal (1) with sodium borohydride followed by acetylation or 4-bromo-benzoylation provided the esters 4a and 4b, respectively. On reaction of 1 with 2,4-dinitrophenylhydrazine and cyclization of the resulting bishydrazone with sec-butyl chloroformate a 4,5-dihydro-3-oxopyridazine 5 was obtained.

Neither of these compounds showed any line broadening in the NMR spectra. This points to a rapid opening and reclosing of the hemiacetal group in 1 as explanation for the coalescence phenomena observed in its 1H and 13C NMR spectra. As indicated in Table I the signals of the lactone and cyclohexene ring of 1 are most heavily

![Fig. 2. Important 1H–13C long-range couplings (COLOC experiments) of hyphodontal (1).](image-url)
involved whereas the signals of the dimethylcyclopentane unit, the aldehyde, and the methylene group of the cyclopropane ring remain nearly unaffected.

**Biological properties**

The inhibitory effect of hyphodontal on the reverse transcriptases of AMV and MMuLV is shown in Fig. 4.

The activity of MMuLV reverse transcriptase was reduced to 50% at a concentration of 100 µM (26 µg/ml; IC_{50}) whereas the IC_{50} for AMV reverse transcriptase was 423 µM (110 µg/ml) without preincubation and 96 µM (25 µg/ml; IC_{50}) after preincubation of the enzyme with the inhibitor for 10 min. Preincubation of MMuLV reverse transcriptase with 77 µM (20 µg/ml) resulted in a complete inhibition of enzyme activity (data not shown). The initial rates of incorporation of [\textsuperscript{14}C]dTTP were amounts of hyphodontal. As shown in Fig. 5 and 6 the Lineweaver-Burk plots indicate a non-competitive inhibition of AMV and MMuLV RTs with respect to dTTP. The K_i values for hyphodontal were calculated to 346 µM for the AMV and 112 µM for the MMuLV RT.

A tenfold increase of the concentration of primer template poly(A)-(dT)_{15} decreased the inhibitory effect of hyphodontal on the MMuLV RT to 50% (Table II), whereas no influence on the inhibitory activity on AMV RT could be observed.

The activities of hyphodontal on the RNA-directed RNA polymerase of VSV and the HIV-1 RT are compared in Table III. The IC_{50} for the
RNA-directed RNA polymerase of VSV was 385 μM (100 μg/ml). Hyphodontal exhibits only weak inhibitory activity on HIV-1 RT with the synthetic heteropolymeric template poly(A)-(dT)_{15}. The inhibition of HIV-1 RT was 30% at 577 μM (150 μg/ml) as shown in Table III. With the natural primer template the IC_{50} was determined to 77 μM (20 μg/ml).

RNA and DNA syntheses starting from the corresponding nucleoside triphosphates were tested in permeabilized L 1210 cells and isolated nuclei. In permeabilized cells 189 μM (49 μg/ml) of hyphodontal inhibited the incorporation of UTP into RNA 50% (Fig. 7). The IC_{50} for the incorporation of TTP into DNA was 108 μM (28 μg/ml).

In isolated nuclei of L 1210 cells 193 μM (50 μg/ml) inhibited the RNA polymerase I by 85%, the RNA polymerase II by 83% and the RNA polymerase III by 75% (Table IV). In contrast to results obtained with permeabilized L 1210 cells the RNA syntheses in isolated nuclei (distinguished by the fungal toxin α-amanitin) were more sensitive to the inhibition by hyphodontal. This might be due to the loss of factors required

<table>
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<th>Hyphodontal [μg/ml]</th>
<th>HIV-1 RT poly(A)-(dT)_{15} Inhibition</th>
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<th>VSV RNA polymerase</th>
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<td>150</td>
<td>30</td>
<td>n.t.</td>
<td>n.t.</td>
</tr>
</tbody>
</table>

n.t., not tested. Controls without antibiotic (100%): VSV RNA-directed RNA polymerase: 260 pmol/120 min per assay. HIV reverse transcriptase (poly(A)-(dT)_{15}): 290 pmol/60 min per assay. HIV reverse transcriptase (LTR template): 2.57 pmol/60 min per assay.
Table IV. Effect of hyphodontal on RNA syntheses in isolated nuclei of L 1210 cells. 1–2×10⁷ nuclei were incubated in the presence of varying amounts of α-amanitin (control) or with α-amanitin and addition of 50 μg/ml hyphodontal as described by Marzluff and Huang (1984).

<table>
<thead>
<tr>
<th>Incorporation of [¹⁴C]UMP [pmol]</th>
<th>Control</th>
<th>Hyphodontal 50 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA polymerase I</td>
<td>39.44</td>
<td>5.85</td>
</tr>
<tr>
<td>RNA polymerase II</td>
<td>44.11</td>
<td>7.38</td>
</tr>
<tr>
<td>RNA polymerase III</td>
<td>6.56</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Control:

<table>
<thead>
<tr>
<th>Incorporation of [¹⁴C]UMP [pmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amanitin</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>0.5 μg/ml</td>
</tr>
<tr>
<td>100 μg/ml</td>
</tr>
</tbody>
</table>

\[ a \] RNA polymerase I, II, III
\[ b \] RNA polymerase I, II
\[ c \] RNA polymerase I.

for correct transcription during nuclear isolation as discussed by Gilroy et al. (1984).

Similar sesquiterpenoid dialdehydes have been isolated from a number of natural sources (Anke et al., 1989; Heim et al., 1988) and many of them exhibit antibacterial, antifungal, cytotoxic and mutagenic activities. In order to compare the inhibitory activities on reverse transcriptases, several structural related dialdehydes to hyphodontal were tested including 9-hydroxymarasmic acid (6), isovelleral (7) and acetylmerulidial (8). As shown in Fig. 8 and 9 derivatives with the marasmane skeleton showed the highest activities on AMV and MMuLV reverse transcriptase.

Hyphodontal is a cytotoxic compound. BHK 21, HeLa S3, and 3T3/MMSV cells were completely lysed at concentrations of 10 μg/ml. Growth of L 1210, ECA, and HUT 78 cells was inhibited at concentrations between 10 and 100 μg/ml.
In the agar diffusion assay hyphodontal exhibits modest antibacterial (*Bacillus brevis, B. subtilis*) and antifungal (*Nematospora coryli, Saccharomyces cerevisiae, and Nadsonia fulvescens*) activities at concentrations starting from 10 µg/disc (Erkel, 1990).

In the test for mutagenicity according to Ames et al. (1975) and Venitt et al. (1984) no induction of revertants of *S. typhimurium* TA 98 and TA 100 could be observed with 100 µg of hyphodontal plate (pour plate assay with and without addition of rat liver microsomes).

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