

that isoelectric focusing alone is not the most useful tool to distinguish between actins of various sources, they also corroborate recent findings by our group¹⁵ and by others¹⁷, that actins may well be distinguished by their antigenic differences. It is hoped that combined studies on the chemistry and the immunology of vertebrate actins will further elucidate the phylogenetic origin of this conservative ubiquitous structural protein.

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Effects of araC and aphidicolin on DNA chain elongation rate in HeLa S₃ cells

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Summary. The effects of araC and aphidicolin on DNA chain elongation rate were tested. The rate was markedly reduced at low concentrations. Total DNA synthesis was more inhibited, indicating a role of DNA polymerase α in replicon initiation.

1- β -D-Arabinofuranosylcytosine (araC) is known to be a potent inhibitor of DNA synthesis¹⁻³ probably acting through its triphosphate araCTP. The antibiotic aphidicolin produced by the mold *Cephalosporidium aphidicola* Petch is also a very effective inhibitor of semiconservative DNA synthesis^{4,5}. Both araCTP and aphidicolin inhibit DNA synthesis in an isolated HeLa S₃ nuclear system^{3,6,7}. Moreover araCTP and aphidicolin have a marked inhibitory action on DNA polymerase α partially purified from isolated HeLa S₃ nuclei. The activities of the DNA polymerases β and γ are not affected^{6,7}. These findings indicate a major role of DNA polymerase α in DNA replication. This is supported by other reports^{8,9}.

To link the results of our in vitro experiments with the in vivo situation we have examined the effects of araC and aphidicolin on the DNA chain elongation rate in whole HeLa cells.

The method of Painter and Schaefer¹⁰ was used. HeLa cells in suspension culture were synchronized and reversed by addition of thymidine as described earlier³. 2.5 h after

reversal, araC or aphidicolin was added to give the final concentrations shown in the table. 30 min later, cell suspensions of 20 ml ($2.0\text{--}2.5 \times 10^5$ cells/ml) were pulse labelled with [³H]thymidine (19.5 Ci/mmole, final concentration 5 μ Ci/ml) for the time periods given in the table. The cells were then pelleted, cooled on ice and washed free of soluble radioactivity by 3 washes with ice-cold medium containing amethopterin (1 μ M), adenosine (50 μ M) and bromodeoxyuridine (16 μ M). The cells were resuspended in 20 ml medium containing bromodeoxyuridine (16 μ M) and incubated at 37°C for 3 h in the dark. The cells were then pelleted ($500 \times g$ for 5 min), resuspended in 2 ml SSC and mixed with [¹⁴C]thymidine-labelled cells (as marker for DNA of normal density). DNA was isolated as described by Painter and Schaefer¹⁰ and 1 M NaOH added to a final concentration of 0.1 M NaOH. DNA was sheared by 4 passages through a 23-G needle or by ultrasonication and analyzed in alkaline sucrose gradients and alkaline CsCl/Cs₂SO₄ gradients¹¹ thereby allowing estimation of the size of the DNA (B) and the fraction of radioactivity in bromo-

Effects of araC and aphidicolin on the rate of DNA chain elongation per growing point and overall DNA synthesis

Inhibitor	Inhibitor concentration (M)	Size of DNA after shearing (daltons)	[³ H]thymidine labelling time (min)	Fraction of radioactivity in BrdUrd-labelled molecules after shearing ultrasonication			Overall DNA synthesis (% of control)	Rate of DNA chain elongation	
				f_{sh}	f_u	$f = f_{sh} - f_u$		daltons/min	% of control
AraC(I)	0	1.59×10^7	33	0.186	0.024	0.162	100	1.49×10^6	100
	10^{-8}	1.46×10^7	33	0.231	0.039	0.192	59	1.15×10^6	77
	10^{-7}	1.24×10^7	33	0.340	0.099	0.241	45	0.78×10^6	52
AraC(II)	0	2.06×10^7	30	0.283	0.026	0.257	100	1.33×10^6	100
	10^{-7}	1.58×10^7	30	0.398	0.084	0.314	43	0.84×10^6	65
	5×10^{-7}	1.81×10^{-7}	30	0.546	0.040	0.516	15	0.60×10^6	45
Aphidicolin	0	0.72×10^{-7}	28	0.155	0.060	0.095	100	1.35×10^6	100
	2.4×10^{-7}	1.08×10^{-7}	28	0.290	0.080	0.210	55	0.92×10^6	68
	4.8×10^{-7}	0.82×10^{-7}	28	0.440	0.120	0.320	23	0.46×10^6	34

deoxyuridine-labelled molecules (f). The rate of chain elongation (L) can be estimated according to the equation $L = B/2f$. To avoid error due to [^3H]-thymidine remaining in the precursor pool after addition of bromodeoxyuridine the value of f after ultrasonication (f_u) was subtracted from the value observed after shearing (f_{sh}).

The results show that both the inhibitors exert markedly inhibitory effects on the DNA chain elongation rate (table). The total DNA synthesis seems to be somewhat more

inhibited than the DNA chain elongation rate. This can be explained by an inhibition of the initiation of new replicons. Similar results have been obtained with low concentrations of araC in HeLa cells by Friedland¹².

As both araCTP and aphidicolin inhibits DNA polymerase α rather specifically, our results seem to indicate a role of DNA polymerase α in the initiation process at the replicon level. Further evidence is needed, however, to draw definite conclusions.

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Occurrence in mushrooms (Homobasidiomycetes) of cis- and trans-octa-1,5-dien-3-ol, attractants to the cheese mite *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae)

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Summary. Cis- and trans-octa-1,5-dien-3-ol were identified in 15 Homobasidiomycetes; these compounds exhibited a significant attraction for the cheese mite *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae).

The volatile compounds emitted by *Trichothecium roseum* (Fungi Imperfecti) at a very low concentration have been found strong attractants for *Tyrophagus putrescentiae* (Schrank, 1781) (Acarina, Acaridae). The main attractant constituents of these volatile materials have been identified as cis- and trans-octa-1,5-dien-3-ol^{1,2}. Cis-octa-1,5-dien-3-ol has been also isolated from a Rhodophyta³. This paper describes the identification of cis- and trans-octa-1,5-dien-3-ol in 15 Homobasidiomycetes.

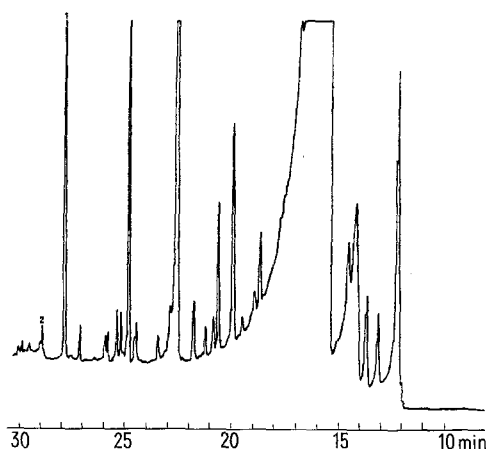
After collection, the mushrooms were crushed with 1 part of anhydrous sulfate; the volatile materials were removed from this mixture at room temperature, for 72 h, by a nitrogen flow and preconcentrated on Tenax GC®. In these conditions of trapping, the isolated volatiles are qualitatively and quantitatively representative of the original odor produced by the mushrooms⁴.

After the preconcentration step, the volatile materials trapped on Tenax were directly injected on to the head of the gas-liquid chromatography (GLC) columns by means of an external flash vaporizer inlet and separated either on an FFAP 100 m \times 0.5 mm inner diameter glass capillary column (analytical studies) or on a packed column (10% carbowax 20 M on chromosorb W HP 80-100 mesh (preparative studies)).

Cis- and trans-octa-1,5-dien-3-ol were identified (co-injections with authentic samples, analytical data from GLC/MS studies) and their relative concentrations were further expressed as a fraction of the total volatile components emitted by the mushrooms (table).

There was no relationship between the concentration of the octadienols and oct-1-en-3-ol which was also identified in the chromatograms (table); thus the higher concentration of octadienols was found in *Lepiota seminuda* and the lower

in *Gymnopilus spectabilis*. An example of chromatogram of the GLC separation of a mushroom crude extract is given in the figure. As previously reported for 7 other mushroom species⁵, the main volatiles found in the investigated species have 8 carbon atoms. Octadienols present a strong 'mushroom-like' odor and thus contribute to the general odor produced by the mushrooms.



Gas-liquid chromatogram of the crude volatile materials isolated from *Clitocybe nebularis*. Chromatographic conditions: liquid phase FFAP 100 m \times 0.5 mm inner diameter glass capillary column; helium flow rate: 3 ml/min; oven temperature: 65–190°C at 2.5°C/min for 6 min then 5°C/min and finally isothermal; injector and detector temperature: 295°C. 1. Oct-1-en-3-ol; 2. cis- and trans-octa-1,5-dien-3-ol.