# Chapter 8

# Allelopathic Properties of Alkaloids and Other Natural Products

## Possible Modes of Action

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Out of a selection of 30 alkaloids and 26 other compounds 19 natural products were found with allelopathic properties and *in vitro* assays were carried out to elucidate their modes of action. Most compounds affect more than one molecular target: 8 compounds interact with DNA, 10 inhibit DNA polymerase I, reverse transcriptase, and protein biosynthesis and 3 lead to membrane leakage. It is suggested that the allelopathy observed is (at least) partly due to interaction of the compounds tested with these basic targets such as DNA and related processes, protein biosynthesis and membrane stability.

Plants compete with other plants for light, water and nutrients and have evolved complex strategies during evolution to cope with this problem. The production and accumulation of secondary compounds, which inhibit the germination or the development of other plants, is one way to enhance the fitness of a plant producing them. A number of plants and plant products with allelopathic properties have been reported (*1-3*), including many phenolics, terpenes and alkaloids (*1-4*).

Many secondary compounds are also toxic to animals and in many instances their modes of action have been elucidated already by biochemists, pharmacologists and toxicologists. This sort of knowledge and understanding is most often missing for natural products with allelopathic properties (*2*). Molecular targets which might be modulated in allelopathic interactions include membrane stability, enzymes, electron transport chains, photosynthesis, signal transduction, respiration, replication, transcription, protein biosynthesis, transport processes or hormone metabolism (*1-4*).

We have started to establish *in vitro* assays to elucidate possible interactions of a natural product with basic molecular targets whose intergrity is essential for survival and growth of plants (*4-7*) in order to understand the mechanisms of allelopathic activities. Here we have tested 30 alkaloids and 26 other compounds for allelopathic properties. For 19 active substances we have studied whether they interact with DNA, inhibit DNA polymerase I, reverse transcriptase, protein biosynthesis or destabilize biomembranes.

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## Allelopathic Properties of Alkaloids and Other Natural Products

Using germinating seeds of *Lepidium sativum* we have assayed a selection of alkaloids and other natural products for their allelopathic properties (Table I). Since the biological variation was substantial we consider only deviations of 20% from untreated controls (which were run with each set of experiments under identical environmental conditions) to be meaningful. As can be seen from Table I radicle length was usually a more sensitive measure than hypocotyl growth.

If 1% solutions were applied we observed allelopathic effects for many compounds including inorganic salts, amino acids, and organic acids which are not considered to be specific plant growth inhibitors. To obtain more specific information we evaluated only the results of our natural products seen in 0.01 and 0.1% solutions.

Considering the inhibition of radicle elongation, allelopathic compounds (growth < 80% as compared to control; inhibitor concentration of 0.01%) are the alkaloids tryptamine, aconitine, colchicine, ergometrine, gramine, harmaline, papaverine, quinidine, quinine, salsoline, and the metabolic inhibitors cycloheximide and ethidiumbromide (Table I). For an evaluation of possible targets we have additionally included compounds which showed a 50% growth reduction in a concentration of 0.1%.: tannin, caffeine, cinchonine, lobeline, sanguinarine, theophylline, and essential oils of *Chamomilla, Mentha* and *Thymus*.

## Interaction of Alkaloids with Molecular Targets

In a first approach we have chosen some basic targets whose integrity and function is essential for growth and development of all plants. These processes include interactions of allelochemicals with DNA and RNA, protein biosynthesis and membrane stability.

**Replication and Interaction with DNA and RNA.** We have established a number of assays to measure the potential interaction of a natural product with DNA. The measurement of the melting temperature of DNA (*8*) gave the most reproducible results (Latz-Brüning and Wink, in prep.). For the intercalating compounds mentioned in Table II, the melting temperature of DNA was augmented by more than 5 °C (Figure 1). A strong association between DNA and these natural products was also evident from assays with methyl green (*9*) or when DNA-alkaloid complexes were separated by gel filtration or agarose gel electrophoresis (Latz-Brüning and Wink, in prep.).

Compounds which strongly intercalate in DNA are usually inhibitors of DNA repair. As an experimental system we employed a modified "nick translation" assay (*10*) using DNA polymerase I (Figure 2). As can be seen from Table II, the intercalating compounds are indeed those which were inhibitory in this assay.

We did not employ a true transcription system but used a reverse transcription assay with poly $A^+$ mRNA and reverse transcriptase instead (Figure 3). All compounds which were found to intercalate in DNA and to block nick translation also inhibited reverse transcription (Table II). In addition, papaverine is an active compound in this context.

**Inhibition of Protein Biosynthesis.** As an experimental system we employed

**Table I. Modulation of Radicle and Hypocotyl Growth of *Lepidium sativum* by Natural Products**

| | Effect (control = 100%)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Hypocotyl Elongation | | | Radicle Growth | | |
| Compound | 0.01% | 0.1% | 1.0% | 0.01% | 0.1% | 1.0% |
| *Salts* | | | | | | |
| KCl | 100 | 129 | 41 | 93 | 109 | 18 |
| $Na_2SO_4$ | 94 | 94 | 44 | 108 | 81 | 11 |
| NaCl | 100 | 123 | 54 | 123 | 158 | 27 |
| $NaH_2PO_4$ | 100 | 111 | 78 | 126 | 147 | 50 |
| $NaNO_3$ | 94 | 113 | 50 | 72 | 62 | 19 |
| *Amino acids/amines* | | | | | | |
| Asparagine | 89 | 95 | 84 | 106 | 91 | 28 |
| Glycine | 93 | 100 | 33 | 77 | 77 | 9 |
| Lysine | 111 | 100 | 28 | 122 | 83 | 22 |
| Tryptamine | 94 | 47 | 0 | 35 | 8 | 0 |
| *Sugars* | | | | | | |
| Sucrose | 94 | 94 | 88 | 94 | 123 | 87 |
| *Phenolics* | | | | | | |
| Naringinin | 100 | 100 | 100 | 114 | 116 | 108 |
| Salicine | 100 | 105 | 78 | 92 | 56 | 56 |
| Tannin | 107 | 60 | 27 | 89 | 27 | 9 |
| *Organic acids* | | | | | | |
| Ascorbic acid | 94 | 106 | 75 | 103 | 103 | 52 |
| Citric acid | 100 | 100 | 64 | 109 | 147 | 22 |
| Gibberellic acid | 126 | 137 | 121 | 100 | 93 | 74 |
| Tropic acid | 105 | 113 | 0 | 100 | 49 | 0 |
| *Alkaloids* | | | | | | |
| Aconitine | 125 | 0 | . | 75 | 10 | . |
| Ajmalicine | 116 | 104 | . | 104 | 110 | . |
| Atropine | 100 | 100 | 20 | 90 | 70 | 13 |
| Berberine | 101 | 38 | . | 79 | 16 | . |
| Caffeine | 92 | 31 | 0 | 72 | 17 | 0 |
| Canadine | 118 | 111 | . | 111 | 93 | . |
| Chelidonine | 119 | 117 | . | 114 | 71 | . |
| Cinchonine | 110 | 16 | . | 121 | 6 | . |
| Colchicine | 35 | 31 | . | 10 | 8 | . |
| Cytisine | 100 | 77 | . | 79 | 46 | . |
| D-Ephedrine | 115 | 105 | . | 87 | 64 | . |
| L-Ephedrine | 100 | 125 | 112 | 93 | 93 | 58 |
| Ergometrine | 115 | 65 | . | 68 | 29 | . |
| Ergotamine | 125 | 130 | . | 83 | 75 | . |
| Gramine | 94 | 70 | . | 70 | 44 | . |
| Harmaline | 70 | 1 | . | 19 | 8 | . |
| Hyoscyamine | 100 | 100 | 28 | 108 | 114 | 9 |

*Continued on next page*

**Table I. Continued.**

| Compound | Effect (control = 100%)[a] Hypocotyl Elongation 0.01% | 0.1% | 1.0% | Radicle Growth 0.01% | 0.1% | 1.0% |
|---|---|---|---|---|---|---|
| Lobeline | 95 | 9 | . | 83 | 10 | . |
| Narcotine | 113 | 94 | 25 | 118 | 112 | 3 |
| Nicotine | 104 | 93 | . | 80 | 60 | . |
| Papaverine | 105 | 28 | 6 | 51 | 2 | 2 |
| Quinidine | 111 | 8 | . | 76 | 11 | . |
| Quinine | 100 | 18 | 0 | 73 | 7 | 0 |
| Salsoline | 89 | 64 | . | 50 | 13 | . |
| Sanguinarine | 86 | 57 | . | 82 | 15 | . |
| Scopolamine | 105 | 103 | . | 132 | 131 | . |
| Sparteine | 100 | 100 | 25 | 92 | 94 | 16 |
| Strychnine | 114 | 76 | . | 146 | 73 | . |
| Theophylline | 100 | 37 | 0 | 84 | 27 | 0 |
| Tropine | 100 | 131 | 61 | 94 | 100 | 33 |
| *Metabolic inhibitors* | | | | | | |
| Cycloheximide | 0 | 0 | 0 | 0 | 0 | 0 |
| Ethidiumbromide | 42 | 0 | 0 | 11 | 0 | 0 |
| *Terpenes/Essential oils* | | | | | | |
| Balm mint | 87 | 73 | . | 70 | 71 | . |
| *Chamomilla* | 89 | 0 | . | 106 | 0 | . |
| *Citrus* | 95 | 71 | 0 | 93 | 71 | 0 |
| *Eucalyptus* | 115 | 87 | . | 108 | 98 | . |
| *Foeniculum* | 104 | 89 | . | 89 | 85 | . |
| *Mentha* | 86 | 0 | . | 87 | 0 | . |
| *Picea* | 112 | 90 | . | 93 | 77 | . |
| Saponin | 105 | 94 | 35 | 89 | 31 | 9 |
| *Thymus* | 83 | 0 | . | 81 | 0 | . |

[a]50 seeds were employed in each assay and all experiments were performed in dublicates; values represent means; growth of controls, which were run for each compound, was set 100% (*5*); · = not determined.

**Table II Molecular Targets Affected by Allelopathic Compounds[a] (of Table I)**

| Compound | DNA Inter-actions[b] | DNA Pol I[c] | RT[d] | Protein bio-synthesis[e] | Membrane leakage[f] |
|---|---|---|---|---|---|
| *Phenolics* | | | | | |
| Tannin | | * | * | * | |
| *Alkaloids* | | | | | |
| Aconitine | | | | | |
| Berberine | * | * | * | * | |
| Caffeine | | | | | |
| Cinchonine | * | * | * | * | |
| Colchicine | | | | | |
| Cytisine | | | | | |
| Gramine | | | | | |
| Harmaline | * | * | * | * | |
| Lobeline | * | * | * | * | |
| Papaverine | | | | * | * |
| Quinidine | * | * | * | * | |
| Quinine | * | * | * | * | |
| Salsoline | | | | * | |
| Sanguinarine | * | * | * | | * |
| *Metabolic inhibitors* | | | | | |
| Cycloheximide | | | | * | |
| Ethidiumbromide | * | * | * | | |
| *Terpenes/Essential oils* | | | | | |
| Saponin | | | | | * |
| *Chamomilla* | | | | | * |

[a]Compounds which inhibited radicle or hypocotyl growth by more than 50% at a concentration of 0.1% were considered to be allelopathic.

[b]DNA-binding was assessed by measuring the melting temperature of DNA (*8*) and displacement of methylgreen (*9*).

[c]DNA polymerase I was tested in a "Nick translation" assay (*10*).

[d]Reverse transcription (RT) was tested in a "cDNA-assay" (*10*).

[e]For protein biosynthesis a reticulocyte lysate was employed (*10*).

[f]Membrane stability was determined in erythrocytes: released hemoglobin was measured photometrically.

*= significant activity

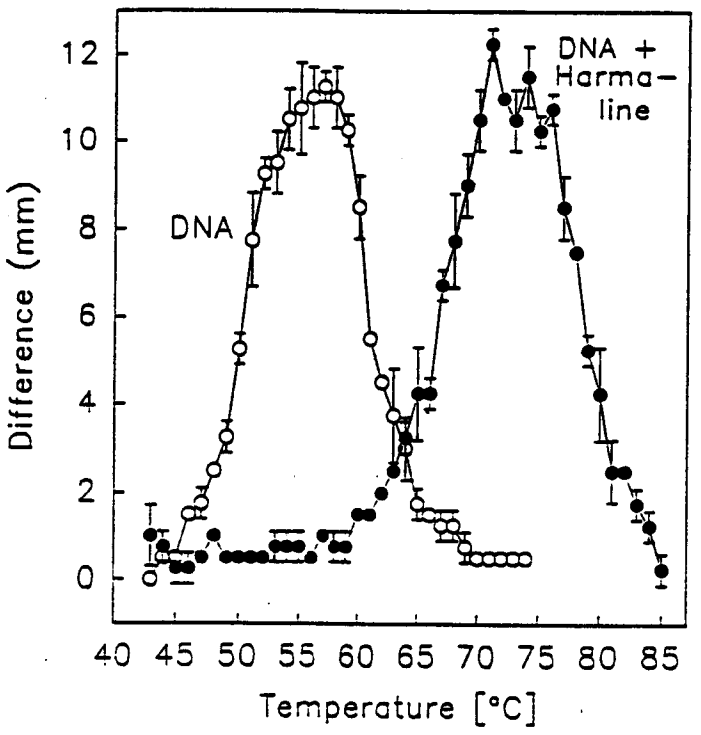


**Figure 1 Influence of a DNA intercalating alkaloid (Harmaline) on melting temperature.**

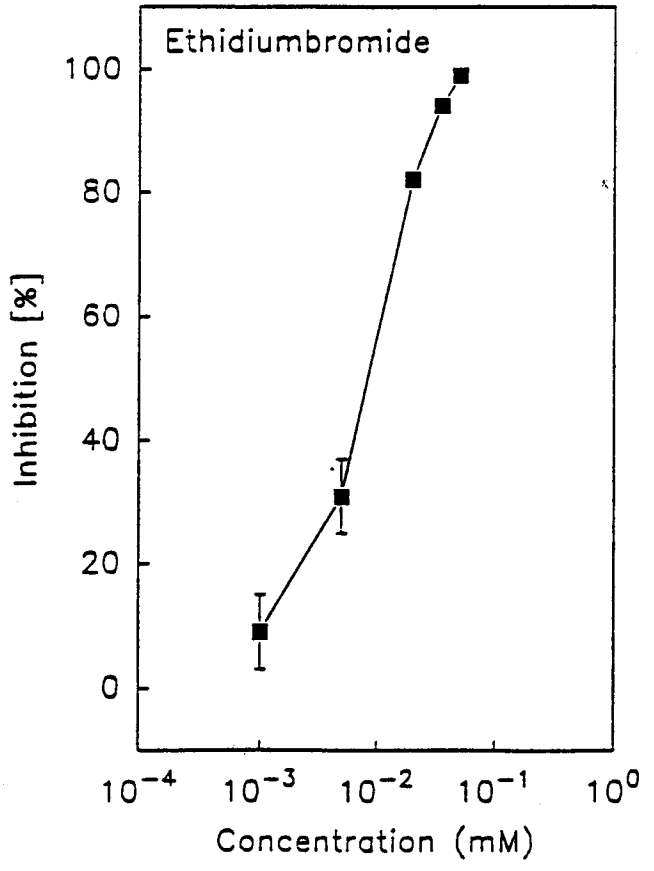


**Figure 2 Inhibition of the "Nick Translation Assay" by ethidiumbromide.**

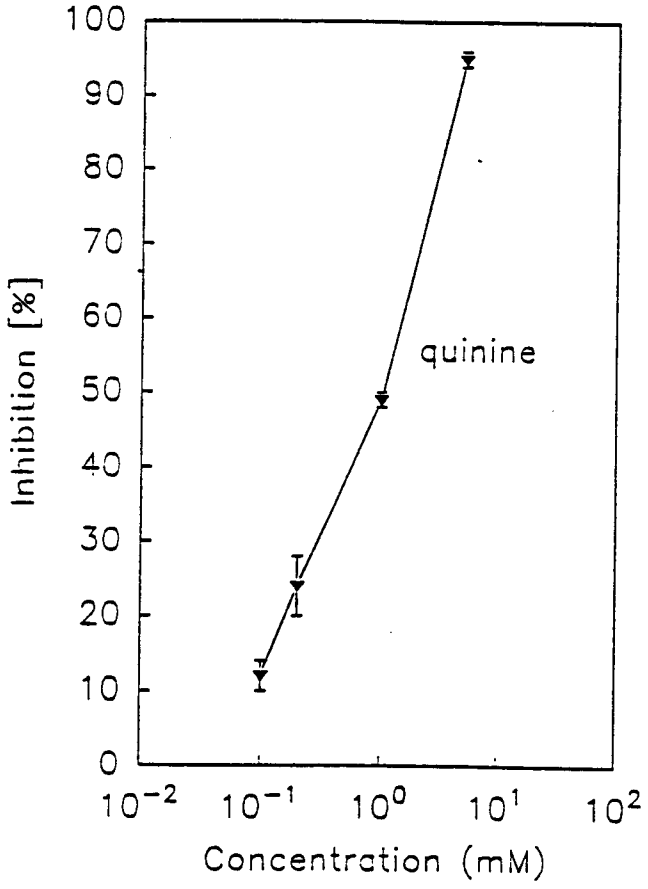


**Figure 3 Inhibition of reverse transcription by the alkaloid quinine.**

a reticulocyte lysate to which mRNA was added (Figure 4). As can be seen from Table II nearly all compounds which interfere with DNA and reverse transcription also inhibit protein biosynthesis substantially. Sanguinarine and ethidiumbromide which were active in the former assays are inactive in translation assays (but sanguinarine was tested at a low concentration only). Cycloheximide and to a minor degree salsoline are specific translation inhibitors. Tannin is a potent protein complexing agent which inhibits most enzymes; its activity in the DNA polymerase, reverse transcriptase and translation assay is therefore not surprising.

**Induction of Membrane Leakage.** We have chosen erythrocytes as an assay system and measured hemolysis (as an indicator for membrane leakage) photometrically (Figure 5). Only sanguinarine, saponin and essential oil from *Chamomilla* proved to be membrane destabilizing (Table II).

## How to Explain These Allelopathic properties?

The allelopathic compounds berberine, cinchonine, harmaline, lobeline, papaverine, quinidine, quinine, sanguinarine and ethidiumbromide affect several molecular targets at the same time. Since these targets are basic for the functioning of a cell, it is likely that these interactions are responsible for the allelopathic effects observed. This does not rule out that additional targets are also envolved.

Cycloheximide and salsoline are translation inhibitors; at least for cycloheximide this target seems to be sufficient to explain the toxicity observed. The allelopathic effect of saponins and essential oils is probably due to interference with biomembranes, as measured *in vitro*.

Whereas tannin is an extremely potent protein inhibitor *in vitro*, its *in vivo* activity is only moderate. Tannin is a polar compound which cannot be resorbed easily by plant cells. Thus it cannot exort comparable detrimental effects *in vivo*.

For a few compounds, such as aconitine, caffeine, colchicine, cytisine, and gramine no active target was found in our assays. A few pharmacological properties have been reported for these compounds (*4*): Aconitine modulates $Na^+$ channels, caffeine inhibits phosphodiesterase, colchicine is a microtubuli blocker and thus prevents cell division, and cytisine binds to nicotinic acetylcholine receptors. It needs to be studied whether these targets are also relevant for allelopathy. Gramine might be an auxine modulator, because of some structural similarities.

In order to be effective in nature these compounds must be produced in high amounts by a plant and released to the soil either by active secretion from the rhizosphere or by leaching from leaves, stems, seeds or roots. Concentrations must be high enough in soil to reach inhibitory levels, but this depends on the type of soil, microbial degradation, thermal effects and drainage, only to mention a few variables which may affect allelopathy.

Our study shows that molecular targets can be identified for most compounds which plausibly explain their allelopathic activities. For most compounds more than one target seems to exist which is a common strategy for defence compounds of plants which have to protect against a wide variety of organisms ranging from microorganisms, other plants to arthropods and vertebrates (*3,4*). "Evolutionary molecular modelling" as we might describe this process obviously used several tar-

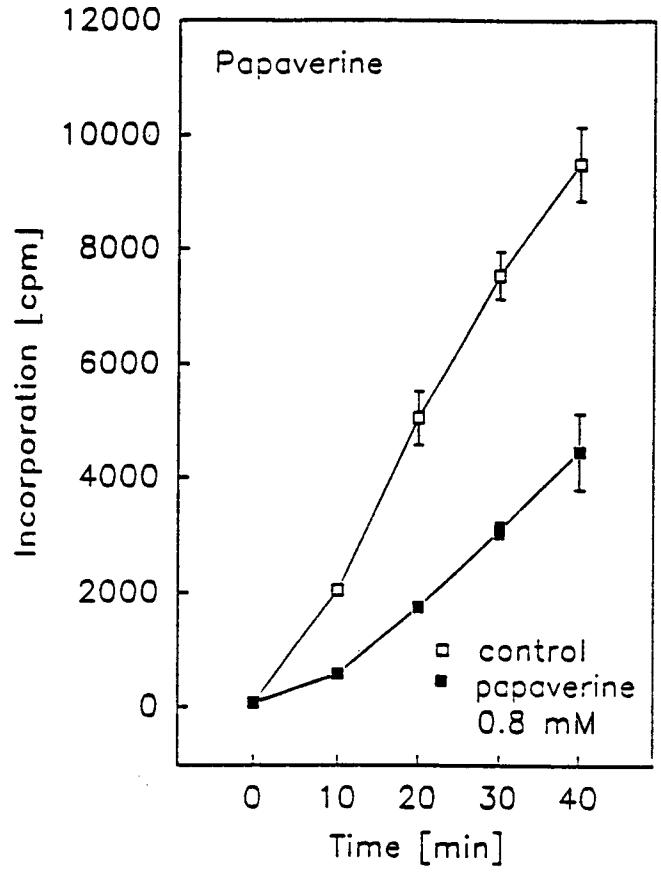


**Figure 4 Inhibition of protein biosynthesis ("In Vitro-Translation") by the alkaloid papaverine.**

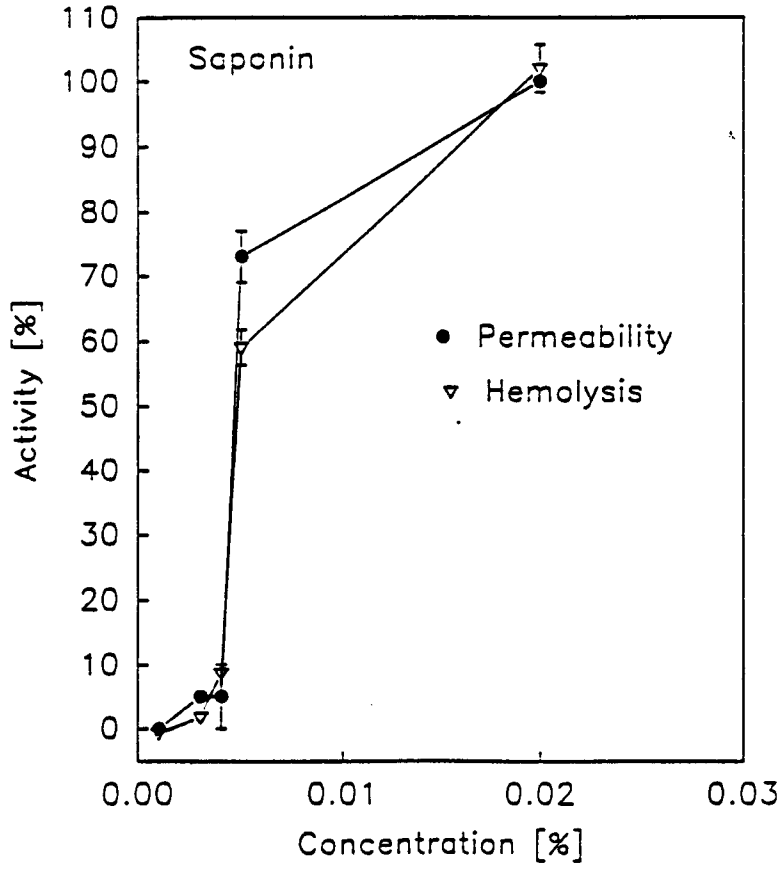


**Figure 5 Induction of membrane instability (permeability and hemolysis) by the triterpen saponin.**

gets to obtimize the structures of defence chemicals which is in contrast to modern chemical approaches where "molecular modelling" is orientated towards a single target.

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