

Reactions of Oxygen-Containing Terpenes with Peptides and Proteins

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Introduction

Eucalypts produce large amounts of volatile organic compounds (VOCs) ranging from common organic acids, aldehydes, ketones and alcohols, to plant-specific terpenes. The types of compounds and their rates of emission are influenced by ecosystem and environmental factors. Eucalypts grow widely in Australia and release terpenes into the environment at tera-gram ($T_g = 10^{12}$ g) levels each year [1]. Terpenes are involved in a number of plant functions such as defense against herbivores and pathogens, and as anti-oxidants. Terpenes react strongly in the atmosphere and are also produced by soil organisms and in large amounts during bushfires.

Naturally occurring terpenes such as 1,8-cineole (eucalyptol) and α -terpineol (Fig. 1) are extensively used as fragrance and flavoring agents in cosmetics or pharmaceutical preparations of nasal spray, cold medications and to enhance the skin's permeability to lipid soluble compounds. Terpenes are the main water-soluble components of tea tree oil – widely reported as having insecticidal, antimicrobial and anti-inflammatory properties [2, 3].

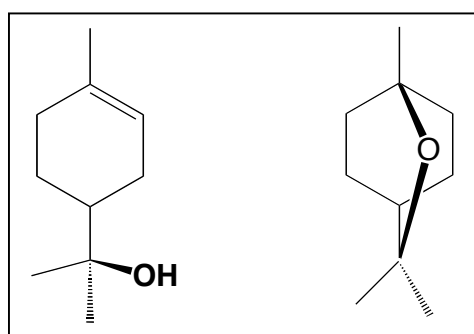


Fig. 1. Structures of α -terpineol and 1,8-cineole

Biotransformation studies of some terpenes have been investigated for several well-characterized metabolic compounds [4]. The high affinity interactions of cytochrome P450 enzymes with substrates such as 1,8-cineole (μ M dissociation constant of enzyme-substrate) have resulted in their specific biotransformation and well characterized by-products. While reactions of many reactive oxygen species such as hydroxyl radicals with proteins have been widely investigated [5, 6], far less information is available regarding interactions of proteins with other oxygen-containing compounds.

We are investigating reactions of oxygen-containing terpenes with peptides and proteins in order to identify if these reactions could have deleterious effects on protein structures and their activity.

Results and Discussion

A series of commercially available peptides with diverse sequences (Table 1) were selected to study their reactions with several terpenes. Aqueous peptide solutions containing various terpenes were incubated at room temperature for several hours at peptide to terpene ratios of 1:1, 1:10 and 1:100. The reaction mixtures were analyzed by electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS).

Table 1. Reactions of peptides with terpenes

peptide	sequence	oxidation with	
		1,8-cineole	α -terpineol
methionine enkephalin	Y G G F M	✓	✓
methionine enkephalin -Arg-Gly-Leu	Y G G F M R G L	✓	✓
substance P	R P K P Q Q F F G L M	✓	✓
amyloid β -protein fragment 1-40	D A E F R H D S G Y E V H H Q K L V F F A E D V G S N K G A I I G L M V G G V V	✓	✓
homatopoietic cell adhesion	V T C G	x	x
α_1 -mating factor fragment 1-6	W H W L Q L	x	x
bradykinin fragment 2-7	P P G F S P	x	x

These studies revealed that 1,8-cineole and α -terpineol oxidize methionine side chains with high specificity. Oxidation of the methionine residue was preferred over the aromatic or heterocyclic amino

acid side chains (i.e. tryptophan, phenylalanine or tyrosine). Methionine was exclusively oxidized in all peptides ranging from 5 to 40 amino acids.

Mass spectra for substance P (Fig. 2) after reactions with 1,8-cineole and α -terpineol show doubly protonated molecular ions of the unmodified peptide at m/z 674.3 and the mono-oxidized peptide at m/z 682.3. Ratios of ion abundances of oxidized to unoxidized peptides after reactions with 1,8-cineole are twice those of reactions with α -terpineol, suggesting 1,8-cineole is the more reactive under similar conditions. Greater reactivity of 1,8-cineole was consistent for other peptides. The epoxide oxygen atom (i.e. strain between two carbons) of 1,8-cineole is more prone to reactions with sulfur in comparison to the hydroxyl group of α -terpineol.

Other oxygen-containing terpenes such as linalool (acyclic hydrocarbon structure containing a hydroxyl group) were un-reactive toward peptides. Interestingly, the cysteine residue of tetrapeptide VTCC did not oxidize under similar reaction conditions. Tandem mass spectrometry confirmed the exclusive oxidation of methionine residues for oxidative products of two methionine enkephalin peptides and substance P.

This investigation revealed *in-vitro* reactions of terpenes could lead to exclusive oxidation of methionine residues for peptides with diverse sequences. The reactivity of cysteine will be further evaluated in other peptides and proteins. These studies are also being expanded to proteins containing methionine residues with various degrees of solvent accessibility.

References

1. Maleknia S.D., Bell, T.L., Adams, M.A. (2007) *Int. J. Mass Spectrom.* **262**, 203-210.
2. Hawkes, D.B., Adams, G.W., Burlingame, A.L., Ortiz de Montellano, P.R., De Voss, J.J. (2002) *J. Biol. Chem.* **277**(31), 27725-27732.
3. Brand, C., Ferrante, A., Prager, R.H., Riley, T.V., Carson, C.F., Finlay-Jones, J.J., Hart, P.H. (2001) *Inflamm. Res.* **50**, 213-219.
4. Duisken M, Sandner F, Blomeke B, Hollender J (2005) *Biochim. Biophys. Acta* **1722**:304-311.
5. Maleknia, S.D., Downard, K.M. (2001) *Mass Spectrom. Rev.* **20**, 388-401.
6. Maleknia, S.D., Reixach N., Buxbaum, J.N. (2006) *FEBS J.* **273**, 5400-5406.

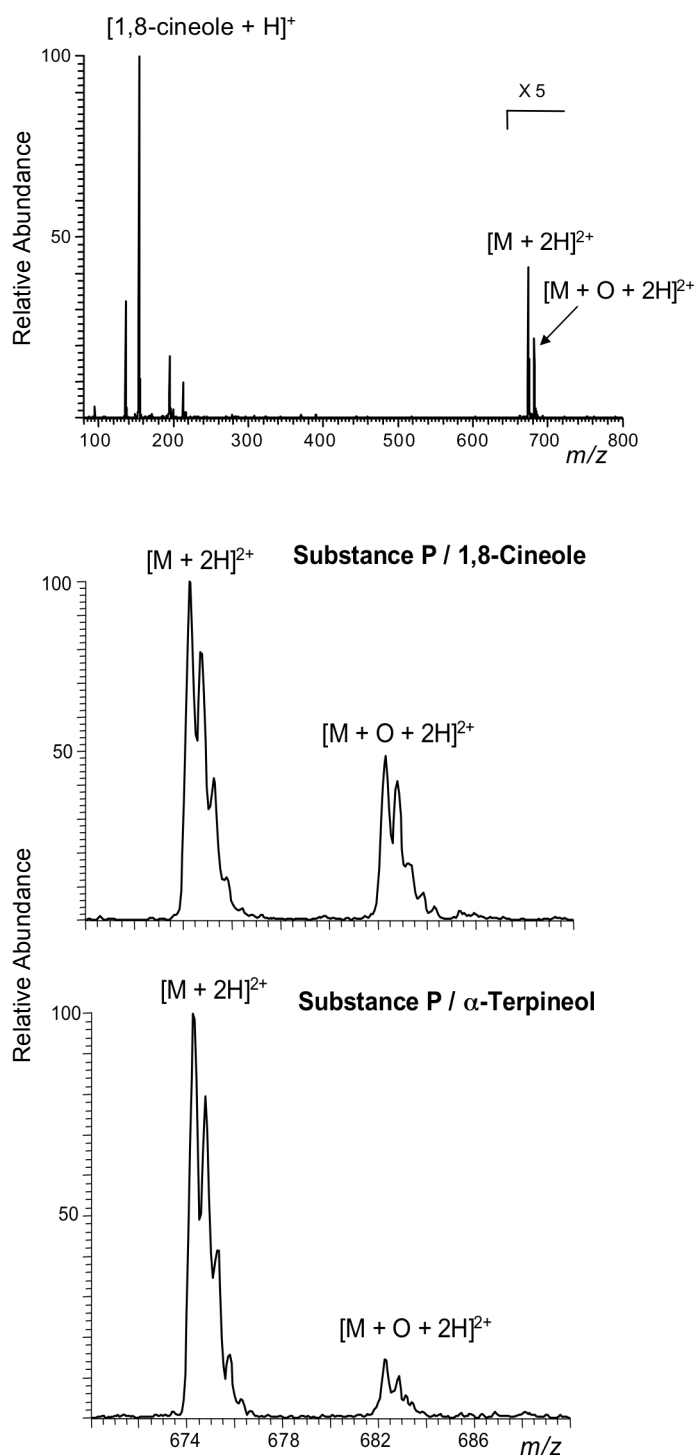


Fig. 2. ESI-MS of substance P from reactions with terpenes after 12 hours.