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STRUCTURE/ACTIVITY RELATIONSHIP OF SOME NATURAL MONOTERPENES AS ACARICIDES AGAINST *PSOROPTES CUNICULI*

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ABSTRACT.—The pharmacological activity of many essential oils on a large number of human and animal pathogens, as used in folk medicine, has been confirmed world-wide by several laboratory investigations. Unfortunately, the biological properties of essential oils can be extremely inconsistent because of the variability of their chemical composition. The acaricidal activities of some natural terpenoids, which are the main constituents of several essential oils, were evaluated in vitro against the mange mite (*Psoroptes cuniculi*) of the rabbit , by direct contact and by inhalation. Because the test components represent different chemical classes (hydrocarbons, alcohols, and phenols, with free and esterified or etherified functional groups), it was also possible to discern in a preliminary fashion a correlation between chemical structure and acaricidal activity. The results obtained suggest that molecules possessing free alcoholic or phenolic groups showed the most potent acaricidal activity.

The biological activity of essential oils toward several microorganisms (mainly bacteria and fungi), but also toward arthropods, has been confirmed by many reports (1–7). Data also show great inconsistency, however, among these materials; the reasons for the variability of their composition are due to many environmental (i.e., climate, soil type, height above sea level, etc.) and genetic factors influencing the biosynthetic pathways of the secondary metabolites. Therefore, the identification of the main constituents responsible for the activity of a crude essential oil is of interest. A comparative study of the activity of each compound, even if it does not permit assessment of the potential synergy and antagonism among the components of an essential oil, could enable a determination of the necessary structures for their pharmacological action. This information should also allow the prediction of the biological activity of other structurally related chemical substances, and the assessment of their possible modes of action.

In the present study we have evaluated in vitro miticidal properties against *Psoroptes cuniculi* (Delafond, 1859), by direct contact and by inhalation, of some monoterpenes which are the main constituents of several essential oils. In order to ascertain the possible structure-activity relationships, simple hydrocarbons (limonene, myrcene, γ-terpinene), alcohols (linalool, geraniol, nerol, terpinen-4-ol, α-terpineol, menthol), and phenols (thymol, eugenol), with free ester (linalyl acetate) or ether (estragole) functional groups and open or closed chains, were chosen for testing. In a few cases, namely nerol and geraniol, the effect of stereochemistry of the molecules was investigated in order to further define the structure-activity relationships.

Although other investigators have attempted to define the main components responsible for the antibacterial, antifungal, and insecticidal activity of essential oils (7–14), there appear to have been no studies to date that examine systematically the acaricidal activity of these substances with reference to their chemical structures.

The results obtained are summarized in Tables 1 and 2. Table 1 shows the lethality of the test compounds and Table

TABLE 1. Acaricidal Activity (Percent Lethality) of Terpenes Against *Psoroptes cuniculi*. Mean Values of Six Replications±Standard Deviation.

Compound	Direct Contact			Inhalation		
	1.0%	0.25%	0.125%	6.0 µl	3.0 µl	1.0 µl
Limonene	0.0	0.0	0.0	0.0	0.0	0.0
Myrcene	0.0	0.0	0.0	6.7±	0.0	0.0
					5.2	
γ-Terpinene	0.0	0.0	0.0	0.0	0.0	0.0
Linalool	100.0	100.0	97.0±	100.0	75.0±	36.7±
			5.2		8.4	8.2
Geraniol	100.0	100.0	100.0	96.7±	93.3±	95.0±
				5.2	8.2	5.5
Nerol	100.0	98.3±	100.0	100.0	83.3±	36.7±
		4.1			10.3	10.3
Terpinen-4-ol	100.0	100.0	100.0	100.0	41.7±	5.0±
					11.7	8.4
α-Terpineol	100.0	100.0	100.0	98.3±	98.3±	73.3±
				4.1	4.1	5.2
Menthol[a]	100.0	100.0	100.0	100.0	100.0	100.0
Linalyl acetate	40.0±	35.0±	5.0±	20.0±	13.3±	0.0
	12.6	12.2	8.4	20.0	5.2	
Thymol[a]	100.0	100.0	100.0	100.0	100.0	100.0
Estragole	100.0	63.3±	0.0	5.0±	6.7±	0.0
		10.3		8.4	8.2	
Eugenol	100.0	100.0	100.0	100.0	96.7±	86.7±
					5.2	8.2

[a]Substances, in inhalation assays, diluted 1:6 (w/w) in vaseline oil and used at 36, 18, 6 µl.

2 the lethality percentage of the controls.

In the direct contact tests all monoterpene hydrocarbons, either alicyclic (i.e., myrcene) or cyclic (i.e., limonene and γ-terpinene) did not show any miticidal activity at the doses tested. The double-bond position and/or number seems to be unimportant for this kind of biological activity.

In contrast, the terpene alcohols, such as linalool, geraniol, nerol, menthol, terpinen-4-ol, and α-terpineol, are able to kill by direct contact nearly 100% of mites at the doses tested. Therefore, the the oxygenated functional groups potentiate the acaricidal properties among these compounds. Neither the alicyclic (i.e., linalool, geraniol, nerol) nor cyclic (i.e., menthol, terpinen-4-ol, α-terpineol) nature of the compound appeared to influence miticidal activity. Similarly, neither the sites of linkage to the ring or to a side-chain, nor the nature of the hydroxyl group (primary, secondary, or tertiary), influenced the activity. The cis/trans isomerism represented by nerol and

TABLE 2. Percent Lethality of Mites in Control Studies. Mean Values±Standard Deviations of Six Replications.

Compound	Direct Contact	Inhalation
Acacerulen®[a]	100.0	0.0
Vaseline oil	5.0±8.4	6.7±5.2
Physiological saline	1.7±4.1	5.0±5.5
Untreated controls	8.3±7.5	9.7±0.8

[a]Acacerulen® active principles: 1% dichlorophen and 1% of a pyrethrum extract containing 0.25% of pyrethrins.

geraniol also seems to be unimportant.

Thymol and eugenol killed nearly 100% of the parasites at all dosages used in the direct contact tests, indicating that a phenolic function can enhance the miticidal characteristics of terpenes.

The low susceptibility of mites to linalyl acetate, particularly at the lowest doses, could be related to the esterification of the oxygenated function.

Estragole, structurally close to eugenol, but with a methylated phenolic function exhibited, at a concentration of 1%, an activity comparable with the same dose of eugenol, but at 0.25% this action decreased (63%) and fell to zero at 0.125%. These results indicate that the best miticidal activity of the monoterpenes examined, in direct contact tests, can be related to compounds with free alcoholic or phenolic functional groups.

Experiments with the mites subjected to inhalation of the volatile fraction showed that, at 6 µl, the active compounds demonstrated results comparable to the direct contact tests; thus, while simple hydrocarbons were ineffective, alcohols and phenols maintained almost 100% miticidal activity. Lowering the dose to 3 µl, all the alcohols represented possessed nearly the same acaricidal action, except nerol (83.3%) and terpinen-4-ol (41.7%). At 1 µl, geraniol, menthol, and thymol maintained their acaricidal properties unchanged, while the activity of linalool, eugenol, α-terpineol, terpinen-4-ol, and nerol was diminished. Linalyl acetate and estragole, like the hydrocarbons, were partially or completely ineffective at all doses tested.

The identification of novel active natural compounds could increase the number of available chemotherapeutic agents, thereby reducing the frequency of resistance phenomena of pathogen arthropods and providing alternative drugs with greater acceptance, especially in terms of environmental safety. Thus, the acaricidal activity showed in these assays by some natural monoterpenes suggests that some of these compounds might find use as alternative miticidal agents.

EXPERIMENTAL

TEST COMPOUNDS.—All monoterpenoids used in this study were purchased from Aldrich Chemical Company, Milan, Italy.

TEST ORGANISMS.—Psoroptic mites were isolated from naturally infested New Zealand white rabbits; the scabs and cerumen, collected from the infected ears, were observed by a stereoscopic microscope to identify the adult male and female mites of *Psoroptes cuniculi* used for testing in this work. A voucher specimen (No. 95) has been deposited at the Insect Collection of the Dipartimento di Patologia Animale Profilassi ed Igiene degli Alimenti in Pisa.

BIOLOGICAL TESTING.—Two different procedures were used to test the monoterpenes, direct contact and inhalation. In the former, dilutions of 1.0, 0.25, and 0.125% in physiological saline containing 20% vaseline oil were used. For each dilution, a dose of 0.5 ml was placed in a 6-cm petri dish containing 10 mites. Mites were placed in empty plates, in plates containing 0.5 ml of physiological saline, and in plates containing physiological saline plus 20% vaseline oil. Plates containing Acacerulen® (Teknofarma) were used as controls. Six replications for each dilution were made. All plates were placed at room temperature (22°) and 70% relative humidity. All the motionless mites, observed by a stereoscopic microscope after a period of 48 h, were stimulated with a needle. Lack of any reaction and persistent immobility was presumed to indicate death (15).

In the experiments carried out to test the acaricidal activity by inhalation of monoterpenes, mites (10/sample) were placed in 6-cm petri dishes covered with a filter paper disk which allowed gas exchange: each plate so prepared was inserted in a 9-cm petri dish containing the test substance and closed with the normal cover. Mites were exposed to dosages of 6, 3, and 1 µl of each compound, measured with a Gilson P2 microtiter pipette (standard error ±0.027 µl). At each dosage, 60 mites were tested. All plates were tested at room temperature (22°) and 70% relative humidity. In these experiments all compounds used were pure, except for thymol and menthol which, as solids, were dissolved (1:6, w/w) in vaseline oil and, therefore, were tested at dosages six times higher (36, 18, and 6 µl). The control mites were exposed to the action of Acacerulen® (Teknofarma), physiological saline, and vaseline oil, all at 0.5 ml, and to the action of no substance (untested controls). All the motionless mites, observed by stereoscope microscope after a period of 24 h, were transferred into fresh 6-cm petri dishes and observed again

after 24 h. Their mortality was evaluated as indicated before. The results are expressed as mean lethality percentage±standard deviation of six replications.

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