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(71) Applicant *(for all designated States except US)*: **E. I. DU PONT DE NEMOURS AND COMPANY** [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **HALLAHAN, David, L.** [US/US]; 5117 New Kent Road, Wilmington, DE 19808 (US).

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(54) Title: DIHYDRONEPETALACTONE AS INSECT REPELLENT

(57) Abstract: Dihydronepetalactone, a minor natural constituent of the essential oil of catmints (Nepeta spp.) such as Nepeta cataria, has been identified as an effective insect repellent compound. Synthesis of dihydronepetalactone may be achieved by hydrogenation of nepetalactone, the major constituent of catmint essential oils. This compound, which also has fragrance properties, may be used commercially for its insect repellent properties.

TITLE

DIHYDRONEPETALACTONE AS INSECT REPELLENT

FIELD OF THE INVENTION

This application claims the benefit of U.S. Provisional Application No. 60/366,147, filed March 20, 2002, which is incorporated in its entirety as a part hereof for all purposes.

Field of the Invention

The present invention relates to the field of insect repellency, and the use of dihydronepetalactone stereoisomers generally as repellent materials.

Background of the Invention

Repellent substances generally cause insects to be driven away from, or to reject, otherwise insect-acceptable food sources or habitats. Most known repellents are only mildly toxic. A few of the known repellents, in fact, are not active poisons at all but rather prevent damage to plants/animals or articles of manufacture by making insect food sources or living conditions unattractive or offensive. Most current commercial insect repellents contain the synthetic chemical *N,N*-diethyl-m-toluamide (DEET) as their primary active ingredient. For instance, repellents sold under the major commercial brand names such as Off!®, Deep Woods Off!®, and Cutter® are all DEET based products and comprise 85% of insect repellent sales (Consumer Reports Buying Guide, 1994 Special Year- End Issue). Further, Consumer Reports tests indicated that products with the highest concentration of DEET lasted the longest against mosquitoes. Despite being an effective repellent, however, this compound has certain drawbacks. Specifically, it possesses an unpleasant odor and imparts a greasy feel to the skin. Although it has recently been re-registered for use in the US by the EPA, concerns have been raised as to its safety, particularly when applied to children (Briassoulis, G.; Narlioglou, M.; Hatzis, T. (2001) *Human & Experimental Toxicology* 20(1), 8-14). Studies have demonstrated that high concentrations of DEET may give rise to allergic or toxic reactions in some individuals. Other disadvantages associated with DEET include: 1) it is a synthetic chemical having a limited spectrum of activity; 2) DEET is a powerful plasticizer and will dissolve or mar many plastics and painted surfaces; and 3) DEET plasticizes the inert ingredients typically used in topical formulations in order to lengthen the time of effectiveness. This leads to DEET formulations with low user acceptability.

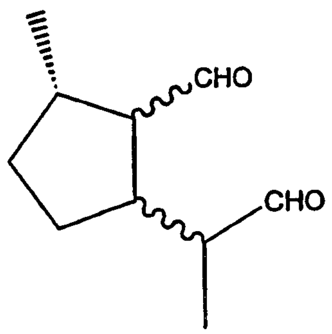
As a result of the above limitations, DEET-free products with repellent activity are finding favor with consumers, and demand for compositions containing natural products (versus synthetic chemicals such as DEET) is increasing. These DEET-free repellent compounds require a combination of excellent repellency, high residual activity and relatively little or no toxicity to humans (or pets) and the environment. In response to these consumer demands, there is an on-going need to develop new repellent compounds which can be obtained from, or synthesized from, natural plant materials and which are pleasant to use.

Many plant species produce essential oils (aromatic oils) which are used as natural sources of insect repellent and fragrant chemicals [Hay, R.K.M., Svoboda, K.P., *Botany*, in 'Volatile Oil Crops: their biology, chemistry and production'. Hay, R.K.M., Waterman, P.G. (eds.). Longman Group UK Limited (1993)]. Citronella oil, known for its general repellence towards insects, is obtained from the graminaceous plants *Cymbopogon winterianus* and *C. nardus*. Examples of plants used as sources of fragrant chemicals include *Melissa officinalis* (Melissa), *Perilla frutescens* (Perilla), *Posostemon cablin* (Patchouli) and various *Lavandula* spp. (Lavender). All of these examples of plants yielding oil of value to the fragrance industry are members of the Labiatae (*Lamiaceae*) family. Plants of the genus *Nepeta* (catmints) are also members of this family, and produce an essential oil which is a minor item of commerce. This oil is very rich in a class of monoterpenoid compounds known as iridoids [Inouye, H. *Iridoids. Methods in Plant Biochemistry* 7:99-143 (1991)], more specifically the methylcyclopentanoid nepetalactones [Clark, L.J. *et al. The Plant Journal*, 11:1387-1393 (1997)] and derivatives.

Iridoid monoterpenoids have long been known to be effective repellents to a variety of insect species (Eisner, T. *Science* 146:1318-1320 (1964); Eisner, T. *Science* 148:966-968 (1965); Peterson, C. and J. Coats, *Pesticide Outlook* 12:154-158 (2001); and Peterson, C. *et al. Abstracts of Papers American Chemical Society*, (2001) 222 (1-2): AGRO73). U.S. Patent 4,663,346 discloses insect repellants with compositions containing bicyclic iridoid lactones (e.g., iridomyrmecin). Further, U.S. Patent 4,869,896 discloses use of these bicyclic iridoid lactone compositions in potentiated insect repellent mixtures with DEET.

Formal studies concerning the repellency of dihydronepetalactones, a class of iridoid monoterpenoids derived from nepetalactones (shown in Figure 1), have been much less conclusive and have failed to teach or imply that these compounds exert a repellent effect on the common insect pests of human society. For example, a study of the composition of the secretion from anal glands of the ant *Iridomyrmex nitidus* showed that isodihydronepetalactone was present in appreciable amounts, together with isoiridomyrmecin (Cavill, G.W.K., and D.V. Clark. *J. Insect Physiol.* 13:131-135 (1967)). Although isoiridomyrmecin was known at the time to possess good 'knockdown' insecticidal activity, no evidence was provided in support of a similar activity for isodihydronepetalactone, and no investigation of this compound's repellent effect (as distinct from insecticidal activity) was made.

In a later publication by Cavill, G.W.K., et al. (*Tetrahedron* 38:1931-1938 (1982)), the presence of dihydronepetalactones in the defensive secretion of an ant was again reported, but the authors concluded that the compound iridodial (and not a dihydronepetalactone) was the basic repellent constituent.



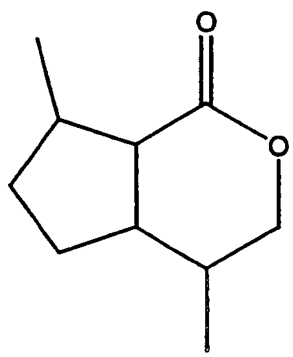
Iridodial

Most recently, Jefson, M., et al. (*J. Chemical Ecology* 9:159-180 (1983)) described the repellent effect of dihydronepetalactone. Initial repellency caused by the undiluted compound was measured with respect to the ant species *Monomorium destructor* during feeding. After 25 seconds of exposure to the pure dihydronepetalactone, approximately 50-60% of the ants ceased to feed. However, further analyses of the repellency over a longer time course were not presented, nor were analyses with anything other than the pure undiluted compound. Repellency observed over such short periods of time (seconds) with concentrated chemicals is insufficient to allow prediction of efficacy in practical applications such as in topical insect repellents.

There is consequently a continuing need for a biologically-based compound having improved insect repellent properties (with respect to DEET) and which is substantially non-toxic or only mildly toxic to humans. Preferred repellents will have activity against a wide variety of insects, including biting insects, wood-boring insects, noxious insects, household pests, and the like. Applicants have found that dihydronepetalactones perform well as a new class of effective insect repellent compounds without the disadvantageous properties characteristic of prior-art compositions.

Summary of the Invention

One embodiment of this invention is an insect repellent composition or article that contains a dihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, represented by the general formula:



Another embodiment of this invention is a process for fabricating an insect repellent composition or an insect repellent article of manufacture by providing as the composition or article, or incorporating into the composition or article, a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, as described above. A further embodiment of this invention is a method of imparting, augmenting or enhancing the insect repellent effect of an article by incorporating into the article a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, as described above.

Yet another embodiment of this invention is the use of a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, as described above as an insect repellent, and thus in a method of repelling insects, the insects are exposed to a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, as described above.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the chemical structures of the naturally-occurring iridoid (methylcyclopentanoid) nepetalactones.

Figure 2 shows the total ion chromatograms from combined gas chromatography/mass spectrometry (GC-MS) analysis of a distilled nepetalactone-enriched fraction from commercially-available catmint oil (A), together with that of the material produced from this fraction by hydrogenation (B).

Figure 3 shows the mass spectra of the major constituents of the nepetalactone-enriched fraction (A) and the hydrogenated material (B) identified by GC-MS analysis.

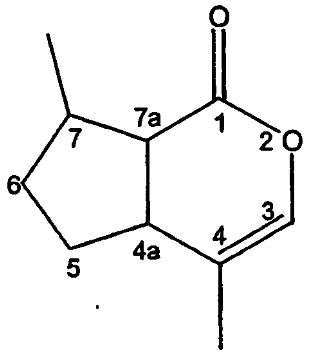
Figure 4 shows the ^{13}C NMR analysis performed on a distilled nepetalactone-enriched fraction of commercially-available catmint oil.

Figure 5 shows the ^{13}C NMR spectrum obtained from analysis of the dihydronepetalactones produced by hydrogenation of a distilled nepetalactone-enriched fraction of commercially-available catmint oil.

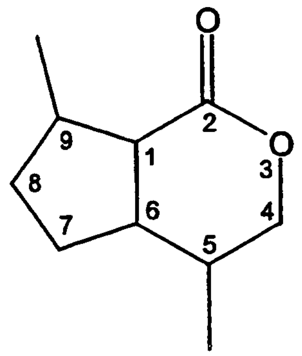
Figure 6 shows the distribution of landing density of female *Aedes aegyptii* mosquitoes on membranes treated with dihydronepetalactones over time in an *in vitro* repellency test.

Detailed Description of the Invention

As used herein, the term "nepetalactone" refers to the compound having the general structure:

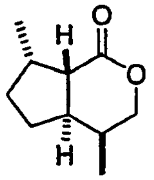


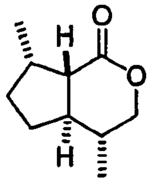
Four chiral centers are present within the methylcyclopentanoid backbone of nepetalactone at carbons 4, 4a, 7 and 7a as shown above; (7S)-nepetalactones are produced by several plants and insects. Dihydronepetalactones are defined by Formula 1:

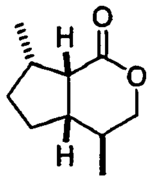


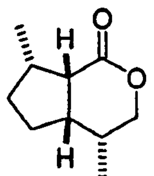
Formula 1

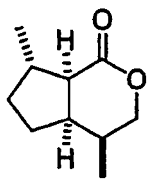
wherein 1, 5, 6 and 9 indicate the four chiral centers of the molecule and the structure encompasses all possible stereoisomers of dihydronepetalactone. The structures of dihydronepetalactone stereoisomers that may be derived from (7*S*)-nepetalactones are shown below.

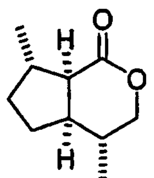

(1S,5S,9S,6R)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one

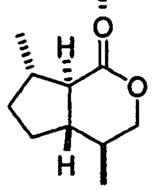

(1S,9S,5R,6R)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one

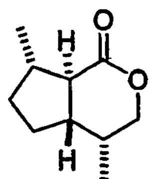

(1S,5S,9S,6S)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one


(1S,9S,6S,5R)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one


(9S,5S,1R,6R)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one


(9S,1R,5R,6R)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one


(9S,6S,1R,5S)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one


(9S,6S,1R,5R)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one

As used herein, the term "dihydronepetalactones" or "dihydronepetalactone mixtures" refers to any mixture of dihydronepetalactone stereoisomers. The molar or mass composition of each of these isomers relative to the whole dihydronepetalactone composition can be variable. Dihydronepetalactones are abbreviated as "DHN".

As used herein, the term "insect" refers to any member of a large group of invertebrate animals characterized, in the adult state (non-adult insect states include larva and pupa) by division of the body into head, thorax, and abdomen, three pairs of legs, and, often (but not always) two pairs of membranous wings. This definition therefore includes a variety of biting insects (e.g., ants, bees, black flies, chiggers, fleas, green head flies, mosquitoes, stable flies, ticks, wasps), wood-boring insects (e.g., termites), noxious insects (e.g., houseflies, cockroaches, lice, roaches, wood lice), and household pests (e.g., flour and bean beetles, dust mites, moths, silverfish, weevils).

As used herein, the term "host" hereinafter refers to any plant or animal affected by insects. Typically, hosts are considered to be insect-acceptable food sources or insect-acceptable habitats.

As used herein, the term "insect susceptible article" will refer to any item of commerce created by man, which is affected by insects. This may include buildings, furniture, and the like. Typically, these articles of manufacture are considered to be insect-acceptable food sources or insect-acceptable habitats.

As used herein, the term "insect repellent" or "insect repellent composition" or "repellent composition" will refer to a compound or composition which deters insects from their preferred hosts or insect-suitable articles of manufacture. Most known repellents are not active poisons at all, but rather prevent damage to plants/animals or articles of manufacture by making insect food sources or living conditions unattractive or offensive. Typically, insect repellents are a compound or composition that can be either topically applied to the host; or, the compound or composition is incorporated into an insect susceptible article to produce an insect repellent article that deters insects from the nearby 3-

dimensional space in which the host or article exists. In either case, the affect of the insect repellent is to drive the insects away from or to reject: 1.) the host, thereby minimizing the frequency of insect "bites" to the host; or 2.) the insect susceptible article, thereby protecting the article from insect damage. Repellents may be in the form of gases (olfactory), liquids, or solids (gustatory).

Some examples of well-known insect repellents include: benzil; benzyl benzoate; 2,3,4,5-bis(butyl-2-ene) tetrahydrofurfural (MGK Repellent 11); butoxypolypropylene glycol; N-butylacetanilide; normal-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate (Indalone); dibutyl adipate; dibutyl phthalate; di-normal-butyl succinate (Tabatrex); N,N-diethyl-meta-toluamide (DEET); dimethyl carbate (*endo,endo*)-dimethyl bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate); dimethyl phthalate; 2-ethyl-2-butyl-1,3-propanediol; 2- ethyl-1,3-hexanediol (Rutgers 612); di-normal-propyl isocinchomeronate (MGK Repellent 326); 2-phenylcyclohexanol; p-methane-3,8-diol, and normal-propyl N, N- diethylsuccinamate. Standard repellents for mosquitoes, ticks, and the like are citronella oil (discussed below), dimethyl phthalate, normal-butylmesityl oxide oxalate and 2-ethyl hexanediol-1,3 (See, Kirk-Othmer Encyclopedia of Chemical Technology, 2nd Ed., Vol. 11: 724-728; and The Condensed Chemical Dictionary, 8th Ed., p 756).

In addition to the chemical compositions above, a variety of effective insect repellents consist of essential oils and/or active ingredients of essential oils. "Essential oils" are defined as any class of volatile oils obtained from plants possessing the odor and other characteristic properties of the plant. Examples of repellent compounds that are essential oils include: almond bitter oil, anise oil, basil oil, bay oil, caraway oil, cardamom oil, cedar oil, celery oil, chamomile oil, cinnamon oil, citronella oil, clove oil, coriander oil, cumin oil, dill oil, eucalyptus oil, fennel oil, ginger oil, grapefruit oil, lemon oil, lime oil, mint oil, parsley oil, peppermint oil, pepper oil, rose oil, spearmint oil (menthol), sweet orange oil, thyme oil, turmeric oil, and oil of wintergreen. Examples of active ingredients in essential oils are: citronellal, methyl salicylate, ethyl salicylate, propyl salicylate, citronellol, safrole, and limonene.

In contrast to an "insect repellent", an "insecticide" is a compound or mixture which is capable of poisoning an insect via its oral ingestion, by contact with the insect cuticle, or by fumigant action through the air. Thus, an insecticide is a type of pesticide designed to control insect life which is harmful to man (i.e., directly harmful as disease vectors, or indirectly harmful by destruction of crops, food products, or textile fabrics). Several well-known insecticides include: inorganic compounds (such as arsenic, lead and copper); naturally occurring organic compounds (such as rotenone, pyrethrins, nicotine, copper naphthenate and petroleum derivatives); and synthetic organic compounds (such as DDT, dieldrin, endrin, chlordane, lindane, para-dichlorobenzene and parathion).

As used herein, the term "potentiated insect repellent composition" refers to a repellent composition which produces a result substantially in excess of that which reasonably could be expected or predicted from the known effect of the components either alone or additively. In the present invention, a potentiated insect repellent composition will include dihydronepetalactones or a mixture thereof, and at least one other insect repellent compound that is not itself dihydronepetalactone (sometimes referred to as a non-dihydronepetalactone insect repellent compound).

An "insect repellent composition" can be used as a component of an "insect repellent article", wherein the term "insect repellent article" refers to an article of manufacture possessing insect repellency that is enhanced, altered, or augmented by the insect repellent composition. As used herein with respect to insect repellency, the terms "alter" and "modify" in their various forms refer to a means of supplying or imparting insect repellency to a composition, or augmenting the existing insect repellency characteristics where natural repellency is deficient in some regard, or supplementing the existing insect repellency to modify its quality, or character. The term "enhance" is intended to mean the intensification (without effecting a change in kind or quality of repellency) of one or more repellency properties in an insect repellent composition or insect repellent article.

In a preferred embodiment, the insect repellent composition of this invention also functions as a fragrance composition since it is capable of imparting a pleasing fragrance or aroma to the insect repellent composition or to an insect repellent article. Dihydronepetalactones are useful in an insect repellent composition or article to enhance, alter, or augment the overall aroma or fragrance of the composition or article. With respect to fragrance, the terms "alter" and "modify" in their various forms refer to a means of supplying or imparting a fragrance or aroma character or note to otherwise bland substances or augmenting the existing aroma characteristics where natural aroma is deficient in some regard or supplementing the existing aroma impression to modify its quality, character, or aroma. The term "enhance" is intended to mean the intensification (without effecting a change in kind or quality of aroma) of one or more aroma nuances and their organoleptic impression of a fragrance, perfume composition, or one or more perfumed articles.

The term "fragrance composition" is used herein to mean a mixture of organic compounds including, for example, alcohols, aldehydes, ketones, nitriles, esters, lactones, natural essential oils, synthetic essential oils, and mercaptans, which are admixed so that the combined odors of the individual components produce a pleasant or desired fragrance. Such compositions usually contain: (1) the main note or the "bouquet" or foundation stone of the composition; (2) modifiers which round off and accompany the main note; (3) fixatives which include odorous substances which lend a particular note to the perfume throughout all stages of evaporation and substances which retard evaporation; and (4) top notes which are usually low-boiling, fresh-smelling materials.

In fragrance or aroma compositions, the individual component will contribute its particular olfactory characteristics, but the overall effect of the composition will be the sum of each of the effects of each of the ingredients. Thus, the dihydronepetalactones of this invention or mixtures thereof can be used to alter the aroma characteristics of such compositions, for example, by highlighting or moderating the olfactory reaction contributed by another ingredient in the composition.

Dihydronepetalactones are known in the literature, for example as minor constituents of the essential oils of several labiate plants of the genus *Nepeta* (Regnier, F.E., et al. *Phytochemistry* 6:1281-1289 (1967); DePooter, H.L., et al. *Flavour and Fragrance Journal* 3:155-159 (1988); Handjieva, N.V. and S.S. Popov *J. Essential Oil Res.* 8:639-643 (1996)).

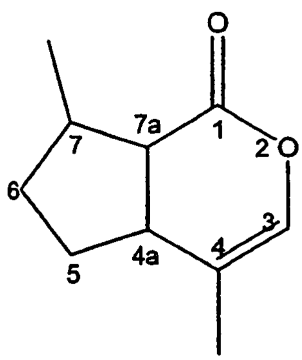
Additionally, dihydronepetalactones have been identified as constituents of the defensive secretions of certain insects, including rove beetles (Jefson, M., et al. *J. Chem. Ecol.* 9:159-180 (1983)) and ants, specifically *Iridomyrmex* species (Cavill, G.W.K. and D.V. Clark. *J. Insect Physiol.* 13:131-135 (1967)). In those species that possess dihydronepetalactones, it has been proposed that they are biosynthetically derived from the iridoid monoterpene iridodial.

The chemical synthesis of dihydronepetalactones and their related iridoid monoterpenoid compounds has been described and found to be conducted in a variety of ways. The following are useful references relating to synthesis:

1) Abelman, M.M. et al. *J. Am. Chem. Soc.* 104(14):4030-2 (1982)
2) Fleming, I. and N.K. Terrett. *Tetrahedron Lett.* 25(44): 5103-5104 (1984); *J. Chem. Soc., Perkin Trans.* 1:2645-2650 (1998).
3) Lee, E. and C.H. Yoon. *J. Chem. Soc., Chem. Commun.* 4: 479-81 (1994).
4) Nagata, H. and K. Ogasawara. *Tetrahedron Lett.* 40(36): 6617-6620 (1999).
5) Nangia, A. et al. *Tetrahedron Lett.* 35(22): 3755-8 (1994).
6) Tanimori, S. and M. Nakayama. *Agric. Biol. Chem.* 55(4): 1181-1184 (1991).
7) Uyehara, T. et al. *J. Chem. Soc., Chem. Commun.* 2:113-14 (1989); *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* 32: 441-6 (1990); *J. Org. Chem.* 57(11): 3139-3145 (1992).
8) Wolinsky, J. and E.J. Eustace. *J. Org. Chem.* 37(21): 3376-8 (1972).
9) Wolinsky, J. and D.L. Nelson. *Tetrahedron* 25(17): 3767-74 (1969).

One preferred and convenient method for synthesis of the dihydronepetalactone mixtures of the present invention is by hydrogenation of nepetalactone. Catalysts such as platinum oxide and palladium supported on strontium carbonate give dihydronepalactone in 24-90% yields (Regnier, F.E., et al. *Phytochemistry* 6:1281-1289 (1967)). Nepetalactone is a known material that can be conveniently obtained in relatively pure form from the essential oils isolated by various means from plants of the genus *Nepeta* (catmints). Isolation of such oils is well known in the art, and examples of methodology for oil extraction include (but are not limited to) steam distillation, organic solvent extraction, microwave-assisted organic solvent extraction, supercritical fluid extraction, mechanical extraction and enfleurage (initial cold extraction into fats followed by organic solvent extraction).

The essential oils isolated from different *Nepeta* species are well known to possess different proportions of each naturally-occurring stereoisomer of nepetalactone (Regnier, F.E., et al. *Phytochemistry* 6:1281-1289 (1967); DePooter, H.L., et al. *Flavour and Fragrance Journal* 3:155-159 (1988); Handjieva, N.V. and S.S. Popov. *J. Essential Oil Res.* 8:639-643 (1996)). Thus, from oil derived from any *Nepeta* species containing a mixture of nepetalactones, a mixture of dihydronepetalactone stereoisomers will be generated upon hydrogenation. Four chiral centers are present within the methylcyclopentanoid backbone of the nepetalactone at carbons 4, 4a, 7 and 7a as shown:



Thus it is clear that a total of eight pairs of dihydronepetalactone enantiomers are possible after hydrogenation. Of these, the naturally occurring stereoisomers described thus far are (9*S*)-dihydronepetalactones. Preferred repellent materials in accordance with the present invention include a mixture of any or all of the possible stereoisomers of dihydronepetalactone. More preferred repellent materials include a mixture of (9*S*)-dihydronepetalactones. Most preferred are (9*S*)-dihydronepetalactone stereoisomers derived from (7*S*)-nepetalactones. This includes the compounds commonly known as *cis,trans*-nepetalactone, *cis,cis*-nepetalactone, *trans,cis*- nepetalactone, and *trans,trans*-nepetalactone, as illustrated in Figure 1.

Upon completion of the hydrogenation reaction, the resulting mixture of isomer products may be separated by a conventional method (e.g., preparative liquid chromatography) to yield each highly purified pair of dihydronepetalactone diastereomers.

In addition to variation in nepetalactone stereoisomer content between different *Nepeta* species, intra-species variation is also known to exist. Plants of a given species may produce oils with different compositions depending on the conditions of their growth or growth stage at harvest. Additionally, within a single species, *Nepeta racemosa*, variation in oil composition independent of growth conditions or growth stage at harvest has been demonstrated (Clark, L.J., et al. *The Plant Journal*, 11:1387-1393 (1997)). Plants of a single species exhibiting different oil compositions are termed chemotypes, and it has been shown that in *Nepeta racemosa*, chemotypes exhibiting marked differences in the proportion of different nepetalactone stereoisomers exist (Clark, L.J.,et al., *supra*). Thus, the preferred process for producing specific dihydronepetalactone enantiomers would be hydrogenation of an oil from a *Nepeta* chemotype known to contain specific nepetalactone stereoisomers.

The preferred process for producing the dihydronepetalactones represented by Formula I in the present invention, therefore, is by hydrogenation of nepetalactones from plants with oils of defined nepetalactone stereoisomer content, an industrially advantageous approach in terms of production cost and its biological basis. Other processes are as disclosed in U.S. Provisional Application No. 60/369,470, filed April 3, 2002.

The dihydronepetalactones of the present invention possess unique properties of insect repellency and are particularly effective against a wide spectra of common insect pests, including biting insects, wood-boring insects, noxious insects, and household-pests.

The insect repellent compositions of this invention containing dihydronepetalactones or mixtures thereof are effective against a variety of insects which interfere with human society. These insects include a variety of biting insects (e.g., ants, bees, black flies, chiggers, fleas, green head flies, mosquitoes, stable flies, ticks, wasps), wood-boring insects (e.g., termites), noxious insects (e.g., houseflies, cockroaches, lice, roaches, wood lice), and household pests (e.g., flour and bean beetles, dust mites, moths, silverfish, weevils). In the case of mosquitoes, which convey pathogenic microbes, these repellent properties are additionally effective for preventing infection with such diseases.

A wide variety of compounds possess insect repellent and/or mosquito repellent activity, as evidenced by: 1.) the diversity of chemical structures reported by the USDA to contain repellent activity (Chemicals Evaluated as Insecticides and Repellents at Orlando, Fla., compiled by W. V. King, U.S. Department of Agriculture, Agricultural Research Service, Agriculture Handbook No. 69); and 2.) by the variety of insect repellant active materials present in insect repellent formations (See, e.g., European patent applications 97,812 and 97,813, and U.S. 4,127,672, U.S. 4,756,905, U.S. 5,465,685, U.S. 5,489,433, U.S. 5,565,208, U.S. 5,672,337 and U.S. 5,716,602). In general, activity is found in alcohols, amides, esters, ketones, acids, lactones, and lactams; and to some degree, repellency activity does appear to depend on the physical properties of these compounds.

One property that is important to overall insect repellency is surface activity, as most (if not all) repellents contain both polar and non-polar regions in their structure. A second property is volatility. Insect repellents form an unusual class of compounds where evaporation of the active ingredient from the host's skin surface or from the insect repellent article is necessary for effectiveness, as measured by the host's protection from insect bites or the article's protection from insect damage. In the case of a topical insect repellent, a certain minimum concentration of repellent is needed in the air space directly above the skin surface of the host in order to repel insects, and this concentration is a measure of the potency of the repellent. However, evaporation rate is also affected by the rate of skin absorption--in most cases, penetration into and through the skin is an undesirable mode of loss of compound from the skin surface. Similar considerations must be made for insect repellent articles, concerning the minimum concentration of repellent required in the three-dimensional air space surrounding the article itself.

A variety of strategies are available to researchers attempting to balance these properties of evaporation (and optionally, penetration). First, it is possible to find a single active ingredient having the right balance of physical properties. Alternatively, the active ingredient could be formulated with polymers and inert ingredients added to the active ingredient for the purpose of modifying the persistence of the active ingredient on the host's skin surface or within the insect repellent article. However, adding inert ingredients to the active ingredient limits the number of molecules of active ingredient on the surface of the repellent film or article. Since a molecule must be on the surface in order to evaporate, the evaporation rate is lowered. This carries with it the negative consequence of diluting the concentration of active ingredient that can be applied to the host's skin surface or that is present on the surface of an insect repellent article. This, in turn, reduces the overall potency of a formulation containing inert ingredients. In a third alternative, the active ingredient can be contained in microcapsules to control rates of loss from the host's skin surface or insect repellent article. Finally, another technique of limiting the evaporation rate of active ingredient is to synthesize a precursor molecule, which slowly disintegrates on the skin surface or insect repellent article to release the active ingredient.

For example, release of the active ingredient may be, for example, by sub-micron encapsulation, in which the active ingredient is encapsulated (surrounded) within a skin nourishing protein just the way air is captured within a balloon. The protein may be used at, for example, a 20% concentration. An application of repellent contains many of these protein capsules that are suspended in either a water-based lotion, or water for spray application. After contact with skin the protein capsules begin to breakdown releasing the encapsulated dihydronepetalactone. The process continues as each microscopic capsule is depleted then replaced in succession by a new capsule that contacts the skin and releases its active ingredient. The process may take up to 24 hours for one application. Because protein's adherence to the skin is so effective, these formulas are very resistant to perspiration (sweat-off), and water. When applied they are dry and comfortable with no greasiness. This system results in very effective protection, but it is only effective when used on skin because clothing does not have the capability to release the proteins. An alternative system uses a polymer to encase the repellent, which slows down early evaporation leaving more dihydronepetalactone available for later evaporation. This system can often increase a repellent's length of effectiveness by 25% to 50% over comparable non-entrapped products, but often feels greasy because of the presence of the polymer. In another alternative, a synergist is used to keep stimulating the evaporation of the dihydronepetalactone in the composition.

Regardless of the particular strategy applied to control volatility of an insect repellent, each repellent must have a minimum effective evaporation rate (MEER) from the skin surface or insect repellent article to maintain the necessary minimum concentration of repellent in the air space directly above the skin surface/article for effective insect repellency. An evaporation rate greater than the minimum effective evaporation rate (MEER) results in a significant and undesirable mode of loss. The issue is further complicated, however, since the MEER will change as a function of conditions in the field. Both the avidity or biting tendency of an insect and the concentration of insects in the host's environment must be considered. For example, as the avidity of mosquitoes increases, a higher MEER will be required. In an environment having a low concentration of mosquitoes where those mosquitoes are not hungry, the MEER could be as low as 2, or more commonly 5, or 6. In contrast, in an environment having a high concentration of hungry mosquitoes, the MEER might be as high as 12-15. Preferred in the present invention are insect compositions wherein the skin surface evaporation rate is at least equal to a minimum effective evaporation rate over a period of time where a preferred period of time is about 5 hours.

In the present invention, a variety of carriers or diluents for the above-disclosed dihydronepetalactones can be used. The carrier allows the formulation to be adjusted to an effective concentration of repellant molecules. When formulating a topical insect repellent, preferably, the repellant molecules are mixed in a dermatologically acceptable carrier. The carrier may further provide water repellency, prevent skin irritation, and/or soothe and condition skin. Factors to consider when selecting a carrier(s) for any formulation of insect repellent include commercial availability, cost, repellency, evaporation rate, odor, and stability. Some carriers can themselves have repellent properties.

For the present invention, the specific choice of a carrier, if any, to be utilized in achieving the desired intimate admixture with the final product can be any carrier conventionally used in insect repellent formulations. The carrier, moreover, should preferably also be one that will not be harmful to the environment. Accordingly, the carrier can be any one of a variety of commercially available organic and inorganic liquid, solid, or semi- solid carriers or carrier formulations usable in formulating insect repellent products. For example the carrier may include silicone, petrolatum, lanolin or many of several other well known carrier components.

Examples of organic liquid carriers include liquid aliphatic hydrocarbons (e.g., pentane, hexane, heptane, nonane, decane and their analogs) and liquid aromatic hydrocarbons. Examples of other liquid hydrocarbons include oils produced by the distillation of coal and the distillation of various types and grades of petrochemical stocks, including kerosene oils which are obtained by fractional distillation of petroleum. Other petroleum oils include those generally referred to as agricultural spray oils (e.g., the so-called light and medium spray oils, consisting of middle fractions in the distillation of petroleum and which are only slightly volatile). Such oils are usually highly refined and may contain only minute amounts of unsaturated compounds. Such oils, moreover, are generally paraffin oils and accordingly can be emulsified with water and an emulsifier, diluted to lower concentrations, and used as sprays. Tall oils, obtained from sulfate digestion of wood pulp, like the paraffin oils, can similarly be used. Other organic liquid carriers can include liquid terpene hydrocarbons and terpene alcohols such as alpha-pinene, dipentene, terpineol, and the like.

Other carriers include silicone, petrolatum, lanolin, liquid hydrocarbons, agricultural spray oils, paraffin oil, tall oils, liquid terpene hydrocarbons and terpene alcohols, aliphatic and aromatic alcohols, esters, aldehydes, ketones, mineral oil, higher alcohols, finely divided organic and inorganic solid materials.

In addition to the above-mentioned liquid hydrocarbons, the carrier can contain conventional emulsifying agents which can be used for causing the dihydronepetalactone compounds to be dispersed in, and diluted with, water for end-use application.

Still other liquid carriers can include organic solvents such as aliphatic and aromatic alcohols, esters, aldehydes, and ketones. Aliphatic monohydric alcohols include methyl, ethyl, normal-propyl, isopropyl, normal-butyl, sec-butyl, and tert-butyl alcohols. Suitable alcohols include glycols (such as ethylene and propylene glycol) and pinacols. Suitable polyhydroxy alcohols include glycerol, arabitol, erythritol, sorbitol, and the like. Finally, suitable cyclic alcohols include cyclopentyl and cyclohexyl alcohols.

Conventional aromatic and aliphatic esters, aldehydes and ketones can be used as carriers, and occasionally are used in combination with the above-mentioned alcohols. Still other liquid carriers include relatively high-boiling petroleum products such as mineral oil and higher alcohols (such as cetyl alcohol). Additionally, conventional or so-called "stabilizers" (e.g., tert-butyl sulfinyl dimethyl dithiocarbonate) can be used in conjunction with, or as a component of, the carrier or carriers comprising the compositions of the present invention.

Solid carriers which can be used in the compositions of the present invention include finely divided organic and inorganic solid materials. Suitable finely divided solid inorganic carriers include siliceous minerals such as synthetic and natural clay, bentonite, attapulgite, fuller's earth, diatomaceous earth, kaolin, mica, talc, finely divided quartz, and the like, as well as synthetically prepared siliceous materials, such as silica aerogels and precipitated and fume silicas. Examples of finely divided solid organic materials include cellulose, sawdust, synthetic organic polymers, and the like. Examples of semi-solid or colloidal carriers include waxy solids, gels (such as petroleum jelly), lanolin, and the like, and mixtures of well-known liquid and solid substances which can provide semi-solid carrier products, for providing effective repellency within the scope of the instant invention.

Insect repellent compositions of the present invention containing the dihydronepetalactones may also contain adjuvants known in the art of personal care product formulations, such as thickeners, buffering agents, chelating agents, preservatives, fragrances, antioxidants, gelling agents, stabilizers, surfactants, emolients, coloring agents, aloe vera, waxes, other penetration enhancers and mixtures thereof, and therapeutically or cosmetically active agents.

Additionally, the compositions of the present invention may also contain other adjuvants such as one or more therapeutically or cosmetically active ingredients. Exemplary therapeutic or cosmetically active ingredients useful in the compositions of the invention include fungicides, sunscreening agents, sunblocking agents, vitamins, tanning agents, plant extracts, anti-inflammatory agents, anti-oxidants, radical scavenging agents, retinoids, alpha- hydroxy acids, emollients, antiseptics, antibiotics, antibacterial agents or antihistamines, and may be present in an amount effective for achieving the therapeutic or cosmetic result desired.

Dihydronepetalactones may be utilized in the present invention individually or combined in any proportion. As is conventional in the art, the desired amount of an insect repellent composition to be added to a given insect susceptible article with properties of insect repellency is determined by the nature of the product and other factors. These factors include both considerations of cost and the nature of the other ingredients in the insect repellent composition or repellent article, their amounts, and the desired repellency effect. In general, the composition of the repellent should contain sufficient amounts of active insect repellant material to be efficacious in repelling the insect from the host over a prolonged period of time (preferably, for a period of at least several hours).

The amount of each dihydronepetalactone of Formula I or mixtures thereof in an insect repellent composition or repellent article in accordance with the present invention will generally not exceed about 80% by weight based on the weight of the final product, however, greater amounts may be utilized in certain applications and this amount is not limiting. More preferably, a suitable amount of dihydronepetalactone will be at least about 0.001% by weight and preferably about 0.01% up to about 50% by weight; and more preferably, from about 0.01% to about 20% weight percent, based on the weight of the composition or article. Specific compositions will depend on the intended use.

The dihydronepetalactone repellent compositions of the present invention can be formulated without a carrier and be effective. More often, however, the insect repellent composition will include a carrier and contain about 0.001-50% weight of the disclosed compounds, and such compound is usually in intimate mixture with the carrier to bring the active material into position for repelling common insect pests, such as biting insects, wood-boring insects, noxious insects, household pests, and the like.

The compositions of the invention may be formulated and packaged so as to deliver the product in a variety of forms including as a solution, suspension, cream, ointment, gel, film or spray, depending on the preferred method of use. The carrier may be an aerosol composition adapted to disperse the dihydronepetalactone into the atmosphere by means of a compressed gas.

In some cases, the dihydronepetalactone repellent compositions of the present invention can be formulated with at least one other insect repellent that is not itself dihydronepetalactone to create a potentiated insect repellent composition (see, for example, U.S. 4,869,896). In this case, the effect of the dihydronepetalactone and the at least one other insect repellent produce a repellency result substantially in excess of that which reasonably could be expected or predicted from the known effect of the components either alone or additively. Exemplary other insect repellent compounds which may be used in a potentiated insect repellent composition with dihydronepetalactones include, but are not limited to: benzil; benzyl benzoate; 2,3,4,5-bis(butyl-2-ene) tetrahydrofurfural (MGK Repellent 11); butoxypolypropylene glycol; N-butylacetanilide; normal-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate (Indalone); dibutyl adipate; dibutyl phthalate; di-normal-butyl succinate (Tabatrex); N,N-diethyl-meta-toluamide (DEET); dimethyl carbate (*endo,endo*)-dimethyl bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate); dimethyl phthalate; 2-ethyl-2-butyl-1,3-propanediol; 2- ethyl-1,3-hexanediol (Rutgers 612); di-normal-propyl isocinchomeronate (MGK Repellent 326); 2-phenylcyclohexanol;normal-propyl N, N- diethylsuccinamate, 1-piperidinecarboxylic acid 2-(2-hydroxymethyl) 1-methylpropylester (Bayrepel) and p-menthane-3,8-diol. Preferred is a potentiated insect repellent composition comprising dihydronepetalactones (or a mixture thereof) and DEET, Bayrepel or p-menthane-3,8-diol. Most preferred is a potentiated insect repellent composition comprising dihydronepetalactones (or a mixture thereof) and other natural repellent molecules such as p-menthane-3,8-diol.

Desirable properties of a topical insect repellent article include low toxicity, resistance to loss by water immersion or sweating, low or no odor or at least a pleasant odor, ease of application, and rapid formation of a dry tack-free surface film on the host's skin. In order to obtain these properties, the formulation for a topical insect repellent article should permit insect-infested animals (e.g., dogs with fleas, poultry with lice, cows with ticks, and humans) to be treated with an insect repellent composition of the present invention by contacting the skin, fur or feathers of such an animal with an effective amount of the repellent article for repelling the insect from the animal host. Thus, dispersing the article into the air or dispersing the composition as a liquid mist or fine dust will permit the repellent composition to fall on the desired host surfaces. Likewise, directly spreading of the liquid/semi-solid/solid repellent article on the host is an effective method of contacting the surface of the host with an effective amount of the repellent composition.

Embodiments of the present invention which may be used as a topical insect repellent articles, include (but are not limited to): colognes, lotions, sprays, creams, gels, ointments, bath and shower gels, foam products (e.g., shaving foams), makeup, deodorants, shampoo, hair lacquers/hair rinses, and personal soap compositions (e.g., hand soaps and bath/shower soaps).

This invention also relates to the use of dihydronepetalactone mixtures as insect repellents in a variety of articles which are susceptible to attack by insects. These outdoor consumable products will generally, but not necessarily, comprise an insect repellent composition of the invention, but will contain an effective amount of dihydronepetalactone. Typical articles that can be improved by the use of dihydronepetalactones and mixtures thereof include, but are not limited to: air fresheners, candles, other scented articles, fibers, sheets, cloth [e.g., for clothing, nettings (mosquito netting), and other fabrics], paper, paint, ink, clay, woods, furniture (e.g.; for patios and decks), carpets, sanitary goods, plastics, polymers, and the like.

The dihydronepetalactone compositions of this invention may be blended with polymers, which may also involve a controlled release systems. Compatible polymers may or may not be biodegradable. Exemplary polymers are high density polyethylene or low density polyethylene, biodegradable thermoplastic polyurethanes, biodegradable ethylene polymers, and poly(epsilon caprolactone) homopolymers and compositions containing the same as disclosed in U.S. 4,496,467, U.S. 4,469,613 and U.S. 4,548,764.

Dihydronepetalactones are particularly advantageous for use as repellent materials in preparations of the present invention for a variety of reasons.

First, the compounds are cost effective to produce, an important consumer consideration when choosing an effective repellent. Many commerically available repellent products are only effective in relatively concentrated form, including as much as 5-30% (or more) repellent in a carrier, based on total weight. U.S. 4,416, 881 to McGovern et al., for example, discloses repellent concentrations of 6.25-25% repellent in a carrier.

Secondly, the compounds are known natural compounds, thus overcoming concerns raised against synthetic chemicals such as DEET as the primary active ingredient in repellent compositions.

Finally, in addition to the natural repellent properties of the dihydronepetalactone mixtures thus prepared, the compositions also possess a unique and pleasant fragrance. The fragrance notes of the subject compounds make them useful in imparting, altering, augmenting or enhancing the overall olfactory component of an insect repellent composition or article, for example, by utilizing or moderating the olfactory reaction contributed by one or more other ingredients in the composition. Specifically, the dihydronepetalactones of the invention or mixtures thereof may be utilized to either mask or modify the odor contributed by other ingredients in the formulation of the final repellent composition or article, and/ or to enhance consumer appeal of a product by imparting a characteristic perfume or aroma. It is expected that the pleasant fragrance of dihydronepetalactones will possess much greater appeal to consumers than other insect repellent compounds, particularly DEET.

Dihydronepetalactones and their uses are also described in U.S. SN 10/349,865, filed January 23, 2003, which is incorporated in its entirety as a part hereof for all purposes.

Examples

The present invention is further defined in the following examples. It should be understood that these examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. The notation below of w/v refers to the weight of the active ingredient in grams in 100 mL of solution.

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "mL" means milliliters, "L" means liters, "m/z" means mass (m) to charge (z) ratio, "ppm" means parts per million, "mol %" means percentage expressed on a molar basis, "Hz" means Hertz (1/sec), and "psig" means pounds per square inch guage.

EXAMPLE 1

Preparation of nepetalactones by fractional steam distillation of oil of *Nepeta cataria*

A sample of commercially-available catnip oil, prepared by steam distillation of herbaceous material from the catmint *Nepeta cataria*, was obtained (Berjé, Bloomfield, NJ, USA). Combined gas chromatography –

mass spectrometry (GC-MS) of this oil indicated that the principal constituents were nepetalactone stereoisomers (data not shown).

The nepetalactone fraction was further purified by fractional distillation of this oil. Figure 2A presents the GC-MS total ion chromatogram of the nepetalactone-enriched fraction prepared by fractional distillation of the commercial sample of *Nepeta cataria* essential oil. The conditions employed were: column HP5-MS, 25m x 0.2mm; oven 120°C, 2 min, 15°C/min, 210°C, 5 min.; He @ 1 ml/min. Peaks with m/z 166 are nepetalactones). The unlabelled peaks correspond to minor sesquiterpenoid contaminants. In Figure 3A, the mass spectrum of the major peak (6.03 min, nepetalactone) in Figure 2A is shown. ^{1}H and ^{13}C NMR analysis of the oil and the purified material was also carried out, and the ^{13}C data is presented (Figure 4). The ^{13}C chemical shifts for the four possible stereoisomers reported in the literature were compared to the spectra taken for the sample. Three stereoisomers were detected and the amounts were quantified based on the carbonyl region at around 170 ppm. The chemical shifts, for both the original oil and the enriched material, are provided in Table 1. Each carbon atom of nepetalatone is identified, as shown in Figure 4.

Table 1
^{13}C Chemical Shifts and Mol% Values of
Nepetalactone Stereoisomers Present in Commercial Sample of
Essential Oil of Catmint (*Nepeta cataria*) and in
Fraction Purified by Steam Distillation

	ESSENTIAL OIL			PURIFIED FRACTION		
	cis, trans-	*trans, cis-*	*cis, cis-*	*cis, trans-*	*trans, cis-*	*cis, cis-*
ATOM	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)
a	170.9	170.1		170.8	170.1	
b	133.7	135.9	134.2	133.7	135.9	134.2
c	115.3	120.4		115.3	120.4	
d	40.8	37.3	39.6	40.8	37.4	39.5
e	49.4	49.1	46.4	49.5	49.1	46.3
f	39.7	32.1	38.4	39.8	32.1	38.4
g	33.0	30.0	32.7	33.1	30.0	32.7
h	30.9	26.1	30.4	31.0	26.1	30.5
j	20.2	17.5	17.1	20.3	17.6	17.2
i	15.4	14.2	14.7	15.5	14.2	14.8
Mol%	80.20%	17.70%	2.10%	84.50%	14.30%	1.20%

This analysis indicated that in the oil, nepetalactones were present in the following proportions: 80.2 mol% *cis,trans*-nepetalactone, 17.7 mol% *trans,cis*-nepetalactone and 2.1 mol% *cis,cis*-nepetalactone. The data indicated the proportions of nepetalactones in the purified material were 84.5 mol% *cis,trans*-nepetalactone, 14.3 mol% *trans,cis*-nepetalactone and 1.2 mol% *cis,cis*-nepetalactone. GC-MS analysis of this purified fraction indicated that it consisted predominantly of these nepetalactones (m/z 166), accompanied by trace amounts of the sesquiterpenoids caryophyllene and humulene (data not shown).

EXAMPLE 2

Preparation of dihydronepetalactones

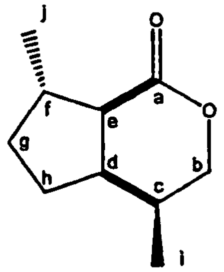
107 g of the distilled nepetalactone fraction of the catmint oil prepared as described in Example 1 was dissolved in ethanol (200 ml) and placed in a Fisher-Porter bottle with 12.7g 2% Pd/$SrCO_3$ (Aldrich 41,461-1). The tube was evacuated and backfilled with H_2 two times, then

charged with H_2 at 30 psig. After 48 hr stirring at room temperature, the tube was vented and the contents filtered over Celite to remove catalyst. The solvent was removed under vacuum, yielding a clear oil.

GC-MS analysis (column HP5-MS, 25m x 0.2mm; Oven 120°C, 2 min, 15°C/min, 210°C, 5 min.; He @ 1 ml/min) was conducted on this material. The total ion chromatogram is presented in Figure 2B. This analysis indicated that the principal component (65.43% area; Rt 7.08 min) represented a dihydronepetalactone isomer, with m/z 168; the mass spectrum of this component is presented in Figure 3B. This spectrum contains an ion with m/z 113, diagnostic for dihydronepetalactones (Jefson, M., *et al. J. Chemical Ecology* 9:159-180 (1983)). Five additional peaks, representing the remaining dihydronepetalactone diastereomers which might be derived from the three nepetalactones present in the starting material were also represented in the chromatogram. These occurred at Rt 5.41 min, 6.8% area, m/z 168; Rt 5.93 min, area 1.2%, m/z 168; Rt 6.52min, 4.88% area, mass 168; Rt 6.76min, 13.8% area, m/z 168 and Rt 7.13 min, 1.25% area, m/z 168. No residual nepetalactones were detected by GC-MS.

^{1}H, ^{13}C and a series of 2D NMR analyses were also performed. The carbonyl region of the ^{13}C NMR spectrum (Figure 5) showed at least five spin systems, one of them in larger amounts than the other four (ca. 75%). Very little residual nepetalactone was detected.

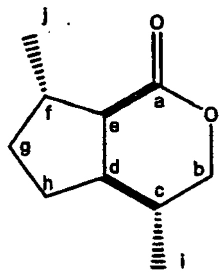
Based on the analysis of coupling constants and the intensities of the different NOE cross peaks observed, the stereochemistry of the principal component of the material was determined to be the dihydronepetalactone of Formula 2 (9*S*,5*S*,1*R*,6*R*)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one).



Formula 2

The distance between the methyl group (i) and proton (d) is longer than the distance between the methyl group (j) and the proton (e), an observation consistent with the *cis-trans* stereochemical configuration.

The stereoisomer isodihydronepetalactone (9*S*,5*R*,1*R*,6*R*)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one; (Formula 3) was similarly identified by ^{13}C chemical shifts and is present in 3.6%.



Formula 3

Thus the GC-MS and NMR data indicate that hydrogenation of the mixture of nepetalactone stereoisomers yielded the corresponding dihydronepetalactone diastereomers, as expected. The pair of diastereomers (Formula 2 and Formula 3) derived from *cis,trans*-nepetalactone (84.5 Mol% of the starting material) were the predominant dihydronepetalactones, at 78.6% of the mixture following hydrogenation.

EXAMPLE 3

Repellency testing of a dihydronepetalactone mixture

The dihydronepetalactone mixture prepared in accordance with Example 2 was evaluated for its repellent effects against female *Aedes* *aegypti* mosquitoes. These tests were carried out under contract by Insect Control & Research, Inc. (Baltimore, MD).

Approximately 250 female *Aedes aegypti* mosquitoes were introduced into a chamber containing 5 wells, each covered by a Baudruche membrane. Wells were filled with bovine blood, containing sodium citrate (to prevent clotting) and ATP (72 mg ATP disodium salt per 26 ml of blood), and heated to 37°C. A volume of 25 µl of isopropyl alcohol, containing putative repellent chemicals (Table 2), was applied to each membrane.

Table 2

Experimental Design Applied for Repellency Testing

Purpose	Compound	Concentration
Untreated Control	Isopropyl alcohol	100%
Positive Control	Isopropyl alcohol with DEET	1.0% (w/v)
Experimental Samples	Isopropyl alcohol with	1.0% (w/v)
	Dihydronepetalactone	2.5% (w/v)
		5.0% (w/v)

After 5 min, 4 day-old female mosquitoes were added to the chamber. The number of mosquitoes probing the membranes for each treatment was recorded at 2 min intervals over 20 min. All data presented is from three replicate experiments.

Table 3 presents the effect of dihydronepetalactone concentration with respect to the amount of time taken before the female *A. aegypti* mosquitoes first probed each membrane.

Table 3
Effect of Dihydronepetalactone Concentraton on
Mean Time to "First Probing"

Repellent Concentration	Mean Time (min)
Isopropyl alcohol (untreated control)	4.6
1% DEET (positive control)	12
1% DHN	8
2.5% DHN	9.3
5% DHN	18

Mosquitoes began landing on the untreated control well within 4.6 min. Dihydronepetalactone at 5% concentration was found to discourage mosquito "first probing" for approximately 18 min, compared to 12 min for DEET (at 1% w/v). Lower concentrations of dihydronepetalactone (1% and 2.5% w/v) were found to inhibit first probing for an average of 8 and 9.3 min, respectively.

The distribution of landing density by female *A. aegypti* on membranes treated with dihydronepetalactones was analyzed over time, as shown graphically in Figure 6. Dihydronepetalactone at 5% concentration was found to almost eliminate mosquito landings for 20 minutes, while DEET (1% w/v) permitted 2 mosquitoes to land in this experiment. Again, lower concentrations of dihydronepetalactone (1% and 2.5% w/v) were found to exhibit repellency (as compared to the untreated control), but at lower levels than the positive control (DEET at 1% w/v).

The total number of landings permitted on each membrane during the course of the experiments were determined, and the results are summarized in Table 4.

Table 4
Number of Landings Permitted
According to Repellency Concentration

Repellent Concentration	Mean Number of Landings
Isopropyl alcohol (untreated control)	58.99
1% DEET (positive control)	4.66
1% DHN	14
2.5% DHN	6.33
5% DHN	0.33

Again, the data shows that at all concentrations tested dihydronepetalactone was repellent, although significantly increased repellency with respect to1% DEET was observed only at 5% (w/v).

Cumulatively, this data show that at all three concentrations tested, treatment with dihydronepetalactone significantly reduced probing of the membranes over the course of the experiment (20 min) as compared to the isopropyl alcohol treatment. At concentrations of 2.5% and 5.0%, dihydronepetalactones had effectiveness as a repellent of mosquitoes for the entire observation period. There was also a direct relationship between dihydronepetalactone concentration and the time elapsed for the first mosquito to land and begin probing the membrane. Overall, the data indicates that the mixture of dihydronepetalactones employed in these experiments was an effective repellent, although repellency equivalent to DEET was observed only with higher concentrations.

EXAMPLE 4

Preparation of
dihydronepetalactones from
trans,cis-nepetalactone

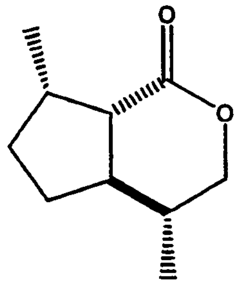
It has been shown that *trans,cis*-nepetalactone (4a*S*, 7*S*, 7a*S*- or *E,Z*-nepetalactone), is more effective than either the *cis,trans*- (*Z,E*-)

nepetalactone or unfractionated catmint essential oil in repelling the german cockroach (Peterson, C.J. *et al.* (2002) *Household and Structural Insects*, 95 (2), 377-380). Accordingly, we determined to purify *trans,cis*-nepetalactone for hydrogenation to the corresponding dihydronepetalactones and repellency testing of these derivatives. A number of plants were grown from seed of the catmint *Nepeta racemosa* (Chiltern Seeds, Cumbria, UK). Leaf pairs plucked from individual plants were immersed in ethyl acetate and after 2h the solvent was removed and the extracts analyzed by gas chromatography. Plants producing preponderantly *trans,cis*-nepetalactone in their oils were thus identified (Clark, L.J., *et al.* (1997) *The Plant Journal*, 11:1387-1393), and grown to maturity. Leaf material from these plants was harvested, freeze-dried, extracted into ethyl acetate, and the extracts concentrated. Nepetalactone was purified from the concentrated extract by silica gel chromatography in hexane/ethyl acetate (9:1) followed by preparative thin-layer chromatography on silica using the same solvent mixture. After removal of the solvent and re-dissolving in hexane, the *trans,cis*-nepetalactone was crystallized on dry ice. GC-MS and NMR (^{1}H and ^{13}C) analysis confirmed the identity of the crystalline material as *trans,cis*-nepetalactone. The ^{13}C chemical shifts, compared to the chemical shifts of Table 1, are shown in Table 5.

Table 5 ^{13}C chemical shifts of the nepetalactone sample prepared in Example 4, compared to the chemical shifts of *trans,cis*-nepetalactone (from Table 1)

Atom	*trans, cis*-nepetalactone	Sample
	δ (ppm)	δ (ppm)
a	170.1	170.3
b	135.9	136.0
c	120.4	120.5
d	37.3	37.5
e	49.1	49.3
f	32.1	32.2
g	30.0	30.1
h	26.1	26.3
j	17.5	17.7
i	14.2	14.4

Hydrogenation of the *trans,cis*-nepetalactone thus prepared was carried out in ethanol using ESCAT#142 catalyst (Englehart) at 50°C for 4h. GC-MS and NMR (^1H and ^{13}C) confirmed that the *trans,cis*-nepetalactone had been quantitatively converted to the corresponding dihydronepetalactone stereoisomers, with one in significant excess. NMR analysis of the major diastereomer: ^1H NMR (500 MHz, $CDCl_3$): d 0.97 (d, 3H, J = 6.28 Hz), 0.98 (d, 3H, J = 6.94 Hz) d 1.24 (m, 2H), 1.74 (m, 1H), 1.77 (m, 2H), 1.99 (m, 2H), 2.12 (dd, 1H, J = 6.86 and 13.2 Hz), 2.51 (m, 1H), 3.78 (tr, 1H, J = 11.1 Hz), 4.33 (dd, 1H, J = 5.73 and 11.32 Hz); ^{13}C (500 MHz, $CDCl_3$): d 15.43, 18.09, 27.95, 30.81, 31.58, 35.70, 42.51, 51.40, 76.18, 172.03. The ^{13}C NMR spectrum indicated that this major diastereomer constituted ca. 93.7% of the preparation. Based on the observed couplings for the methylene to the lactone oxygen, the stereogenic methine carbon bearing methyl group, the methyl group itself and the bridgehead methine, it is concluded that the diastereomer is most likely the (1*S*,9*S*,5*R*,6*R*)-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one) of Formula 4.



Formula 4

The magnitude of the observed couplings are consistent with dihedral angles between the protons on vicinal carbon atoms in the above structure according to the Karplus equation (ref. Spectrophotometric Identification of Organic Compounds, 4th. edition, Robert M. Silverstein, G. Clayton Bassler and Terence C. Morill, 1981, page 208-210).

EXAMPLE 5

Repellency Testing of Dihydronepetalactones prepared by Hydrogenation of *trans,cis*-Nepetalactone

The dihydronepetalactone prepared according to Example 4, consisting predominantly of 1*S*,9*S*,5*R*,6*R*-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one; Formula 4, was tested for repellency against *Aedes aegypti* mosquitoes essentially as described in Example 3. The experimental design is summarized in Table 6, and all data presented is from five replicate experiments.

Table 6
Experimental Design Applied for Repellency Testing

Purpose	Compound	Concentration
Untreated Control	Isopropyl alcohol	100%
Positive Control	Isopropyl alcohol with DEET	1.0% (w/v)
Experimental Samples	Isopropyl alcohol with	1.0% (w/v)
	Dihydronepetalactone	0.5% (w/v)
		0.2% (w/v)

Table 7 presents the effect of dihydronepetalactone concentration with respect to the amount of time taken before the female *A. aegyptii* mosquitoes first probed each membrane.

Table 7
Effect of Dihydronepetalactone Concentraton on
Mean Time to "First Probing"

Repellent Concentration	Mean Time (min)
Isopropyl alcohol (untreated control)	8.0
1% DEET (positive control)	14.8
1% DHN	16.0
0.5% DHN	10.4
0.2% DHN	8.4

Dihydronepetalactone at 1% concentration was found to discourage mosquito "first probing" for approximately 16 min, marginally better than (but statistically indistinguishable from) DEET at the same concentration, where mean time to first probe was 14.8 min. Lower concentrations of dihydronepetalactone (0.5% and 0.2% w/v) were found to inhibit first probing for an average of 10.4 and 8.4 min, respectively.

The distribution of landing density by female *A. aegypti* on membranes treated with dihydronepetalactones was analyzed over time, as shown graphically in Figure 7. Dihydronepetalactone at 1.0% concentration was found to completely eliminate mosquito landings for 10 minutes, while DEET (1% w/v) permitted mosquitoes to land by 8 min. Again, lower concentrations of dihydronepetalactone (0.5% and 0.2% w/v) were found to exhibit repellency (as compared to the untreated control), but at lower levels than the positive control (DEET at 1% (w/v)).

The total number of landings permitted on each membrane during the course of the experiments were determined, and the results are summarized in Table 8.

Table 8
Number of Landings Permitted
According to Repellent and Concentration
During 2 minute Observation Periods

Repellent Concentration	Mean Number of Landings
Isopropyl alcohol (untreated control)	18.17
1% DEET (positive control)	4.8
1% DHN	4.0
0.5% DHN	16.2
0.2% DHN	23.2

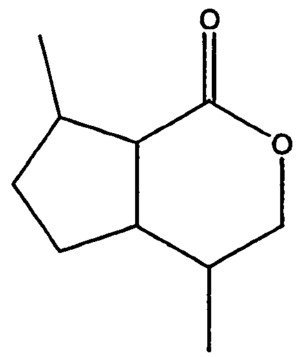
Again, this data indicates that 1% dihydronepetalactone exhibited equivalent repellent activity to1% DEET.

Cumulatively, this data show that at all three concentrations tested, treatment with dihydronepetalactone significantly reduced probing of the membranes over the course of the experiment (20 min) as compared to the isopropyl alcohol treatment. At all concentrations tested, dihydronepetalactones had effectiveness as a repellent of mosquitoes for the entire observation period. There was also a direct relationship between dihydronepetalactone concentration and the time elapsed for the first mosquito to land and begin probing the membrane. Overall, the data indicate that the dihydronepetalactones derived from hydrogenation of *trans,cis*-nepetalactone are an effective repellent, and equivalent to DEET in efficacy in these tests.

CLAIMS

What is claimed is:

1. An insect repellent composition comprising a dihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, represented by the general formula:



2. The composition of Claim 1 wherein the dihydronepetalactone stereoisomers are (9*S*)-dihydronepetalactone stereoisomers derived from (7*S*)-nepetalactones.

3. The composition of Claim 1 which comprises 1*S*,9*S*,5*R*,6*R*-5,9-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one.

4. The composition of Claim 1 which comprises dihydronepetalactone in an amount of at least about 0.001% by weight of the total weight of the composition.

5. The composition of Claim 1 which comprises dihydronepetalactone in an amount of from about 0.001% to about 80% by weight of the total weight of the composition.

6. The composition of Claim 1 which comprises dihydronepetalactone in an amount of from about 0.01% to about 50% by weight of the total weight of the composition.

7. The composition of Claim 1 which comprises one or more of the members of the group consisting of an adjuvant, a carrier and an insect repellent compound that is not a dihydronepetalactone.

8. The composition of Claim 7, wherein the adjuvant is selected from the group consisting of thickeners, buffering agents, chelating agents, preservatives, fragrances, antioxidants, gelling agents, stabilizers, surfactants, emolients, coloring agents, aloe vera, waxes, and therapeutically or cosmetically active ingredients.

9. The composition of Claim 7, wherein the carrier is selected from the group consisting of silicone, petrolatum, lanolin, liquid hydrocarbons, agricultural spray oils, paraffin oil, tall oils, liquid terpene hydrocarbons and terpene alcohols, aliphatic and aromatic alcohols, esters, aldehydes, ketones, mineral oil, higher alcohols, finely divided organic and inorganic solid materials.

10. The composition of Claim 7, wherein the carrier comprises an aerosol composition adapted to disperse the dihydronepetalactone into the atmosphere by means of a compressed gas.

11. The composition of Claim 7, wherein the non-hydronepetalactone insect repellent is selected from the group consisting of: benzil, benzyl benzoate, 2,3,4,5-bis(butyl-2-ene) tetrahydrofurfural, butoxypolypropylene glycol, N-butylacetanilide, normal-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate, dibutyl adipate, dibutyl phthalate, di-normal-butyl succinate, N,N-diethyl-meta-toluamide, dimethyl carbate, dimethyl phthalate, 2-ethyl-2-butyl-1,3-propanediol, 2- ethyl-1,3-hexanediol, di-normal-propyl isocinchomeronate, 2-phenylcyclohexanol, p-methane-3,8-diol, and normal-propyl N, N- diethylsuccinamate.

12. The composition of Claim 1 which is repellent to insects comprising biting insects, wood-boring insects, noxious insects, and household pest insects.

13. The composition of Claim 1 which is repellent to mosquitoes.

14. The composition of Claim 1 which has a skin surface evaporation rate of at least a minimum effective evaporation rate.

15. The composition of Claim 1 which has a skin surface evaporation rate of at least a minimum effective evaporation rate for at least five hours.

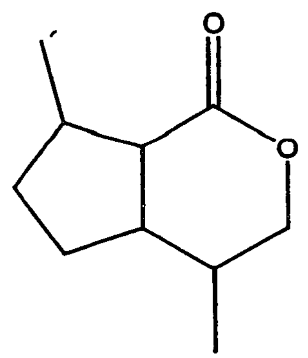
16. The composition of Claim 1 which is in the form of a cologne, a lotion, a spray, a cream, a gel, an ointment, a bath or shower gel, a foam product, makeup, a deodorant, shampoo, a hair lacquer or rinse or a personal soap.

17. The composition of Claim 1 which is in the form of an insect repellent article of manufacture.

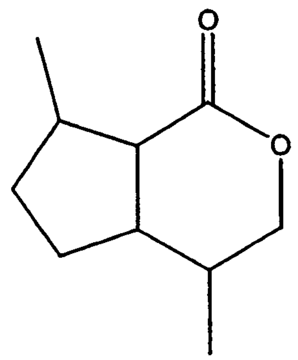
18. The article of Claim 17 which is applied to a human or animal host.

19. The article of Claim 17 which is selected from the group consisting of air freshener, a candle, a scented articles, a fiber, a sheets, cloth, paper, paint, ink, clay, wood, furniture, carpeting, sanitary goods, a plastic, and a polymer.

20. A process for fabricating an insect repellent composition or an insect repellent article of manufacture, comprising providing as the composition or article, or incorporating into the composition or article, a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, represented by the general formula:



21. A method of imparting, augmenting or enhancing the insect repellent effect of an article, comprising the step of incorporating into the article a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, represented by the general formula:

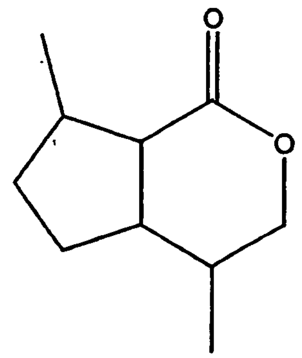


22. A method according to Claim 20 wherein the dihydronepetalactone stereoisomers are (9*S*)-dihydronepetalactone stereoisomers derived from (7*S*)-nepetalactones.

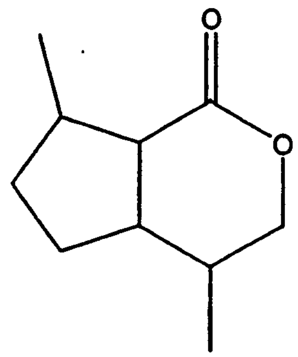
23. A method according to Claim 20 wherein the article is selected from the group consisting of a cologne, a lotion, a spray, a cream, a gel, an ointment, a bath or shower gel, a foam product, makeup, a deodorant, shampoo, a hair lacquer or rinse, a personal soap composition, air freshener, a candle, a scented article, a fiber, a sheet, cloth, paper, paint, ink, clay, wood, furniture, carpeting, sanitary goods, a plastic, and a polymer.

24. A method according to Claim 23, wherein the article is applied to a human or animal host.

25. A method of repelling insects comprising exposing the insects to a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, represented by the general formula:

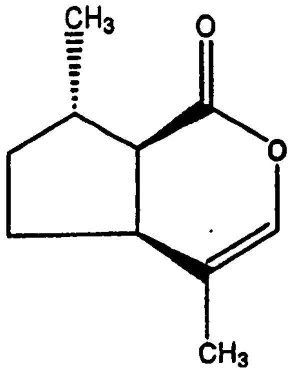


26. The use of a diihydronepetalactone, or a mixture of dihydronepetalactone stereoisomers, represented by the general formula:

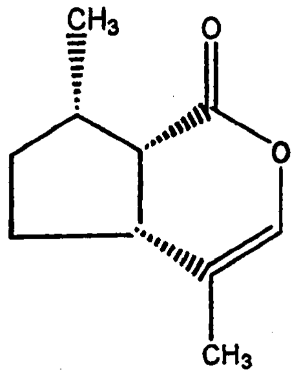


as an insect repellent.

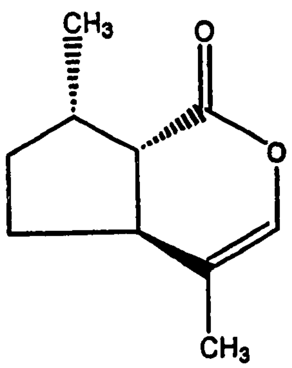
Figure 1



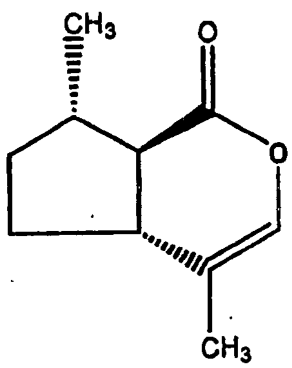
(4a*S*,7*S*,7a*R*) nepetalactone
(*cis,trans*-nepetalactone)



(4a*R*,7*S*,7a*S*) nepetalactone
(*cis,cis*-nepetalactone)

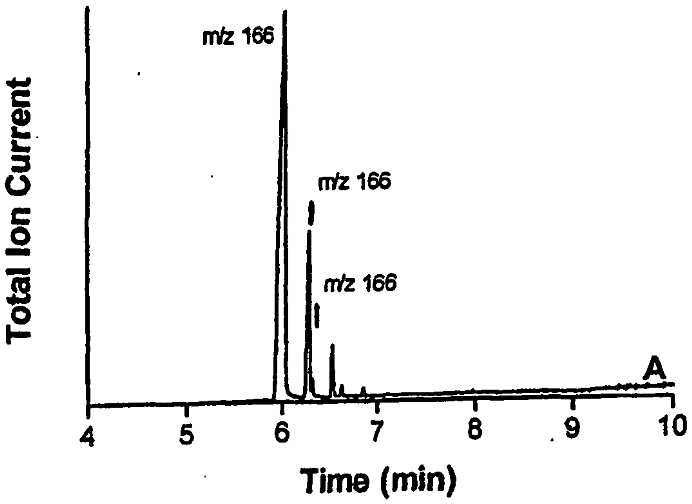


(4a*S*,7*S*,7a*S*) nepetalactone
(*trans,cis*-nepetalactone)



(4a*R*,7*S*,7a*R*) nepetalactone
(*trans,trans*-nepetalactone)

Figure 2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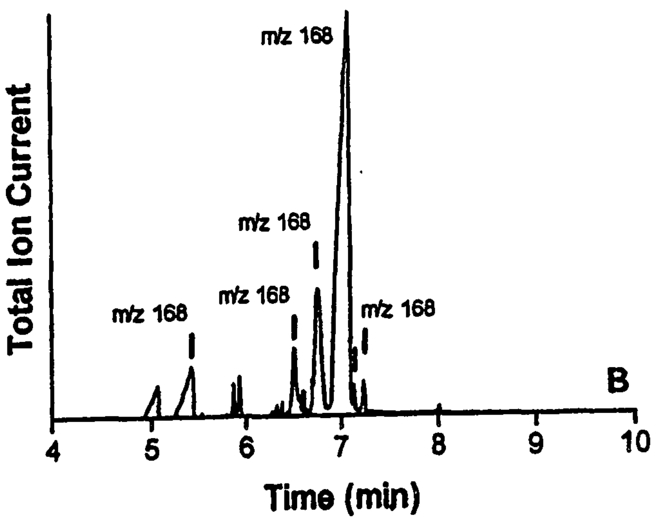
5
6
7
8
9
10
Time (min)


m/z 168
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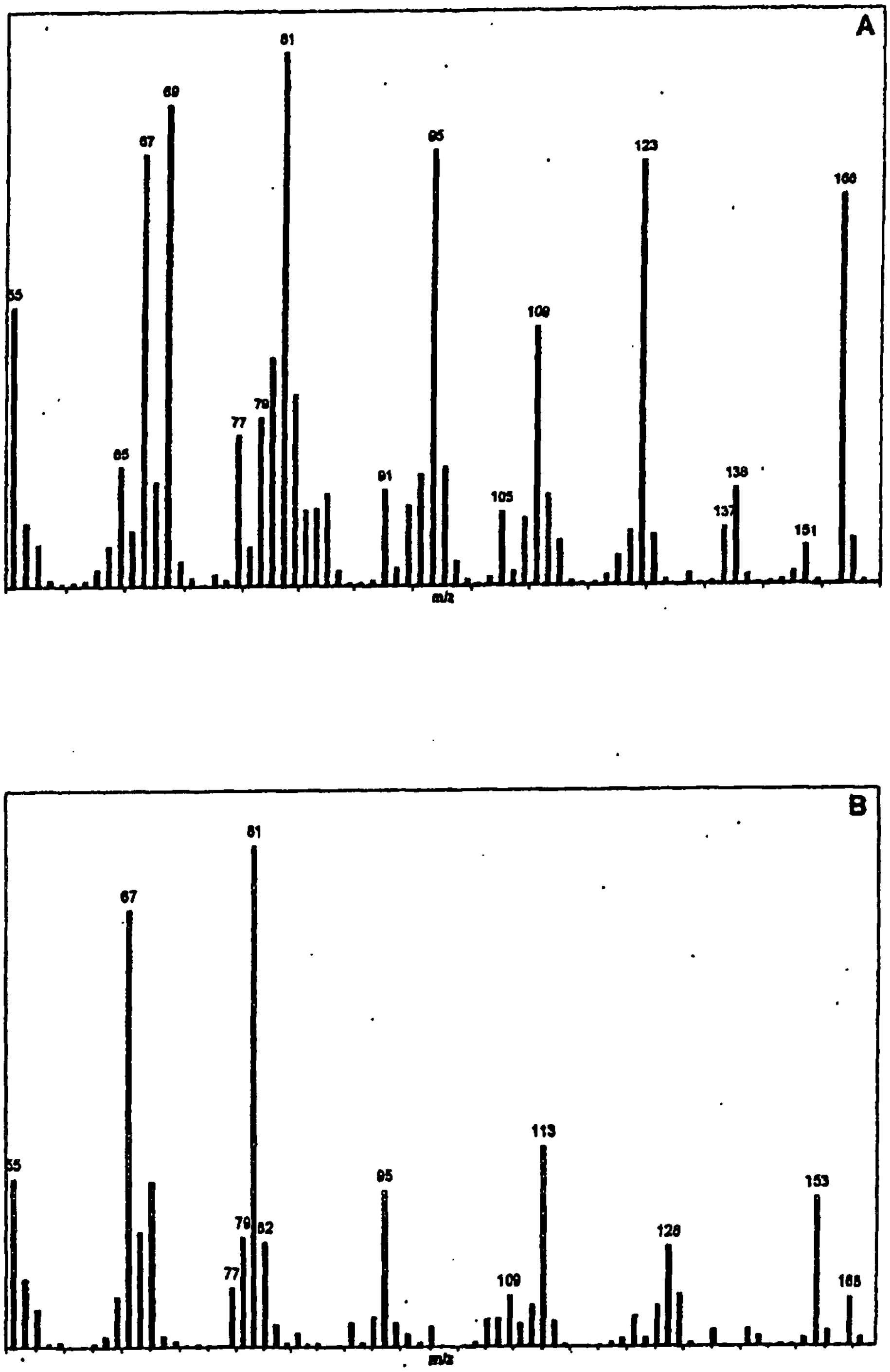
B
Total Ion Current
4
5
6
7
8
9
10
Time (min)

Figure 3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m/z

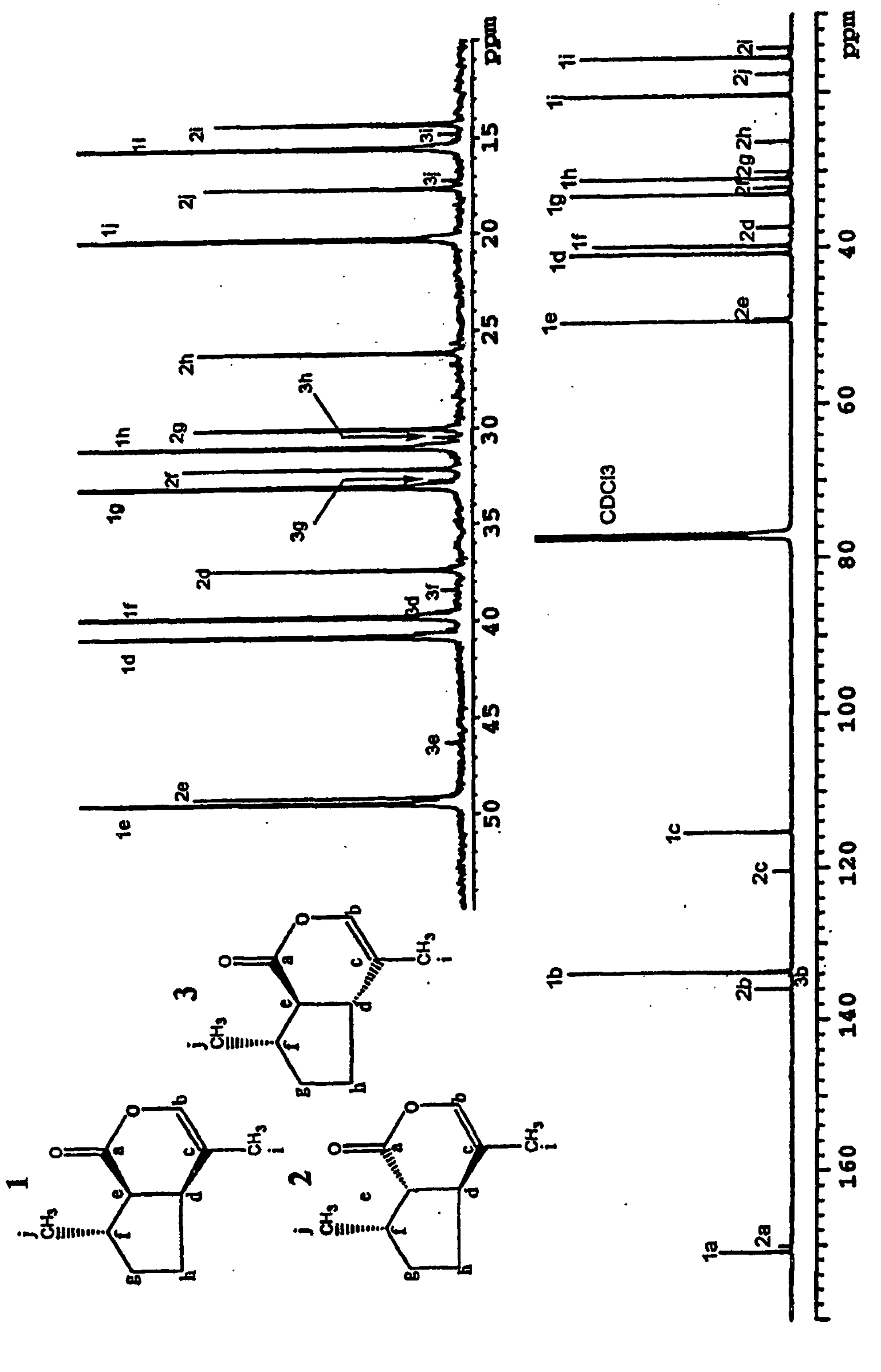
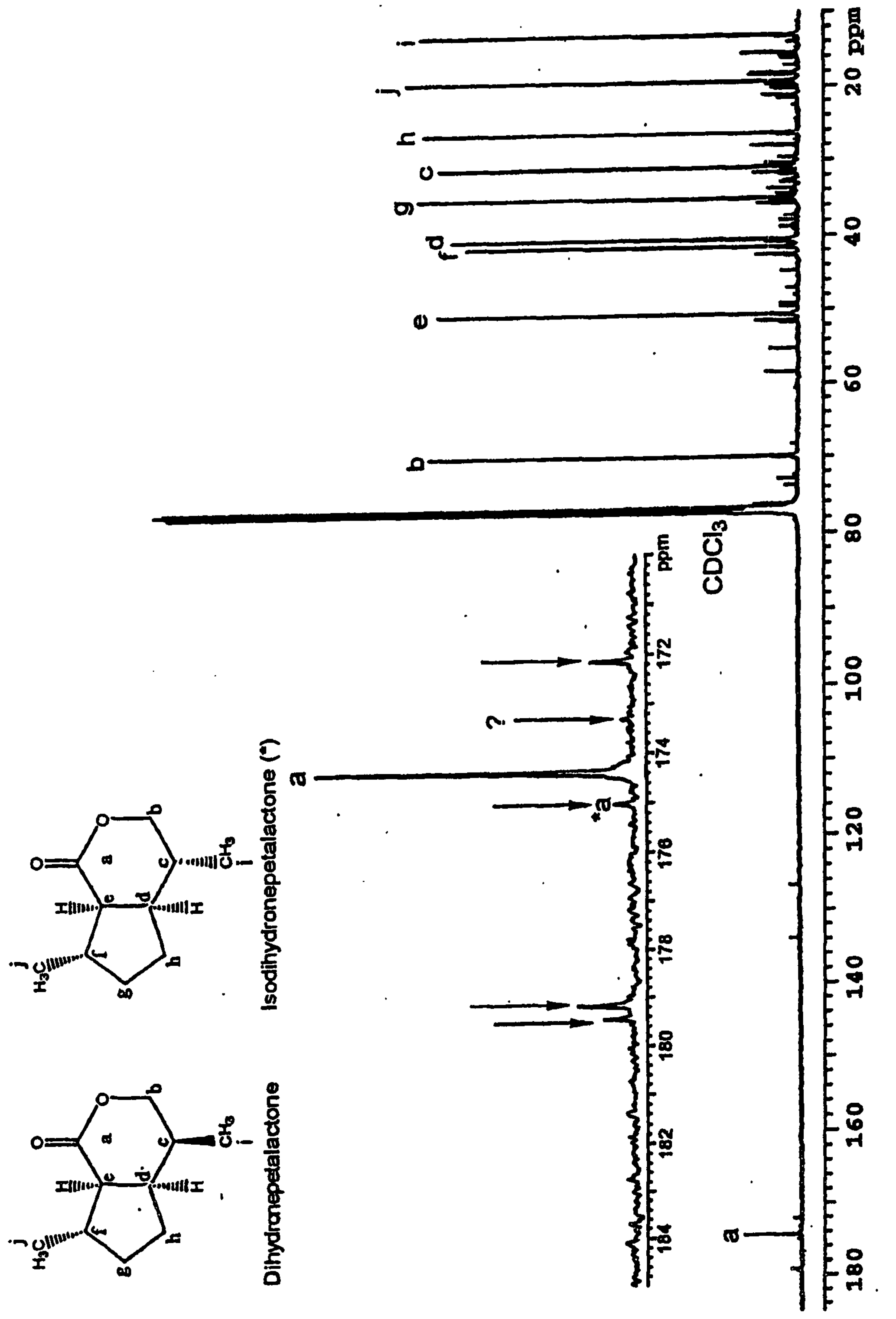


Figure 4


Dihydronepetalactone
Isodihydronepetalactone (*)
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80
100
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160
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176
178
180
182
184
?
*a

Figure 5

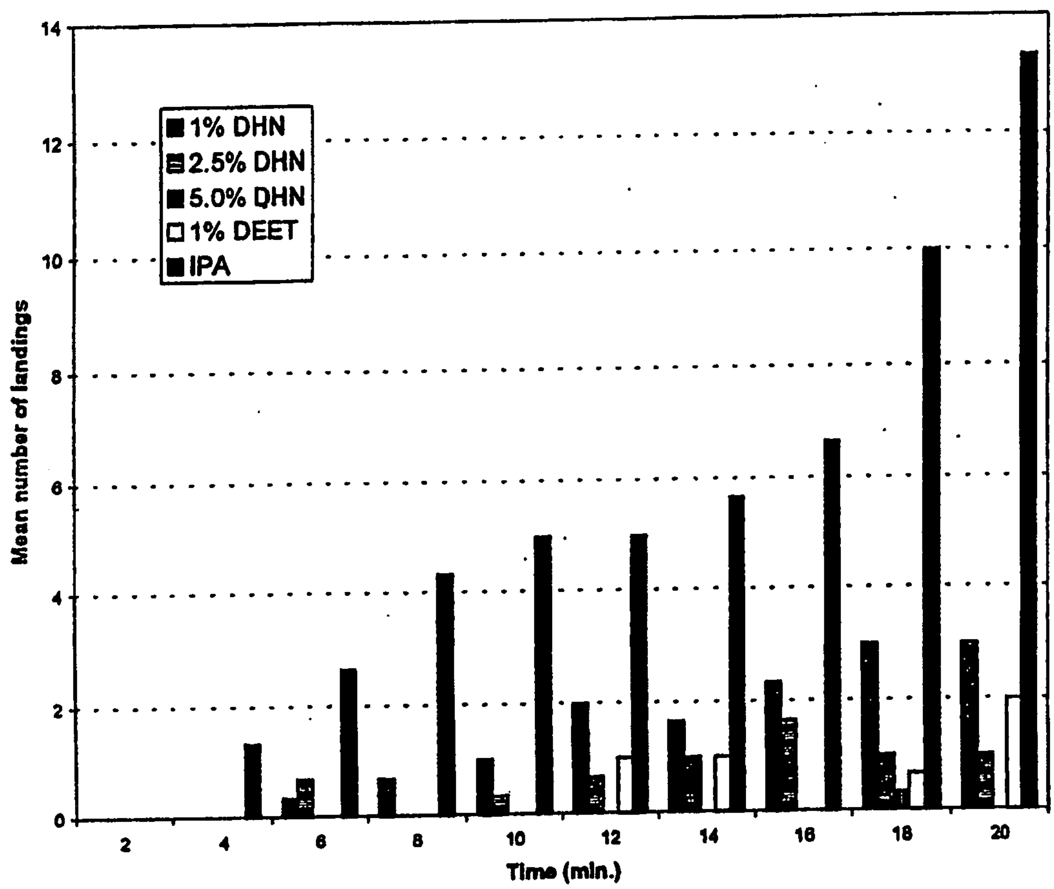
1% DHN
2.5% DHN
5.0% DHN
1% DEET
IPA
Mean number of landings
Time (min.)
0
2
4
6
8
10
12
14
16
18
20

Figure 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/08516

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N43/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JEFSON, M. ET AL.: J. CHEMICAL ECOLOGY, vol. 9, 1983, pages 159-180, XP009012673 cited in the application page 159; figure 8 page 175, paragraph 2 page 178, paragraph 4 - paragraph 5	1-26
X	CAVILL G.W.K. ET AL.: TETRAHEDRON, vol. 38, 1982, pages 1931-1938, XP001153144 cited in the application abstract	1,2,4-7, 12-15
X	CAVILL, G.W.K. ET AL.: J. INSECT PHYSIOL., vol. 13, 1967, pages 131-135, XP009012674 cited in the application abstract	1,2,4-7, 12-15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2003	11/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Molina de Alba, J

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 167 265 A (ANGUS CHEMICAL) 8 January 1986 (1986-01-08) cited in the application the whole document	1-26
Y	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 2001 PETERSON CHRIS ET AL: "Examination of two essential oils as mosquito repellents." Database accession no. PREV200100468328 XP002245714 abstract & ABSTRACTS OF PAPERS AMERICAN CHEMICAL SOCIETY, vol. 222, no. 1-2, 2001, page AGR073 222nd National Meeting of the American Chemical Society;Chicago, Illinois, USA; August 26-30, 2001 ISSN: 0065-7727	1-26

International Application No
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[71] 申请人 纳幕尔杜邦公司
地址 美国特拉华州威尔明顿
[72] 发明人 D·L·哈拉汗
[74] 专利代理机构 中国专利代理(香港)有限公司
代理人 邹雪梅 段晓玲

权利要求书4页 说明书28页 附图6页

[54] 发明名称 作为驱虫剂的二氢荆芥内酯

[57] 摘要

已经证实猫薄荷(荆芥属)例如土荆芥的香精油的微量天然成分－二氢荆芥内酯可作为有效的驱虫化合物。通过氢化猫薄荷香精油的主要成分－荆芥内酯可以合成得到二氢荆芥内酯。同时还具有芳香性的这种化合物由于其驱虫性能因而可在商业上使用。

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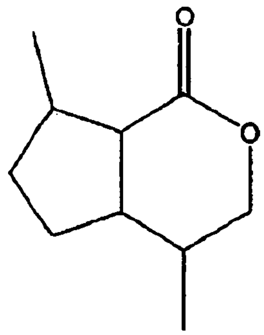
1. 驱虫组合物，含有下述通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物：



2. 权利要求 1 的组合物，其中二氢荆芥内酯立体异构体是由(7S)-荆芥内酯衍生而来的(9S)-二氢荆芥内酯立体异构体。

3. 权利要求 1 的组合物，它含有 1S,9S,5R,6R-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮。

4. 权利要求 1 的组合物， 它含有含量为组合物总重量的至少大约 0.001 重量%的二氢荆芥内酯。

5. 权利要求 1 的组合物，它含有含量为组合物总重量的大约 0.001 重量%至大约 80 重量%的二氢荆芥内酯。

6. 权利要求 1 的组合物，它含有含量为组合物总重量的大约 0.01 重量%至大约 50 重量%的二氢荆芥内酯。

7. 权利要求 1 的组合物，它含有一种或多种选自辅剂、载体和非二氢荆芥内酯驱虫化合物的物质。

8. 权利要求 7 的组合物，其中辅剂选自增稠剂、缓冲剂、螯合剂、防腐剂、芳香剂、抗氧化剂、胶凝剂、稳定剂、表面活性剂、软化剂、着色剂、芦荟液、蜡以及治疗活性成分或化妆用活性成分。

9. 权利要求 7 的组合物，其中载体选自硅酮，凡士林，羊毛脂，液态烃类，农业喷淋油，石蜡油，松浆油，液态萜烯烃类以及萜烯醇类，脂肪族和芳香族醇类、酯类、醛类、酮类，矿物油，高级醇，粉碎的有机和无机固态材料。

10. 权利要求 7 的组合物，其中载体含有适合于通过压缩气体

使二氢荆芥内酯分散于空气中的气雾剂组合物。

11. 权利要求 7 的组合物，其中非二氢荆芥内酯驱虫化合物选自：二苯乙二酮；苯甲酸苄酯；2,3,4,5-双(丁-2-烯基)四氢糠醛；丁氧基聚丙二醇；N-丁基乙酰苯胺；6,6-二甲基-5,6-二氢-1,4-吡喃酮-2-羧酸正丁酯；己二酸二丁酯；邻苯二甲酸二丁酯；琥珀酸二正丁酯；N,N-二乙基-间-甲苯酰胺；卡百酸二甲酯；邻苯二甲酸二甲酯；2-乙基-2-丁基-1,3-丙二醇；2-乙基-1,3-己二醇；异辛可部酸二正丙酯；2-苯基环己醇；对苤烷-3,8-二醇以及 N,N-二乙基琥珀酰胺酸正丙酯。

12. 权利要求 1 的组合物，它驱逐各种昆虫，包括咬啮型昆虫、钻木类昆虫、有毒昆虫以及家居害虫。

13. 权利要求 1 的组合物，它驱蚊。

14. 权利要求 1 的组合物，它具有至少等于最低有效挥发速率的皮肤表面挥发速率。

15. 权利要求 1 的组合物，它在至少 5 小时内具有至少等于最低有效挥发速率的皮肤表面挥发速率。

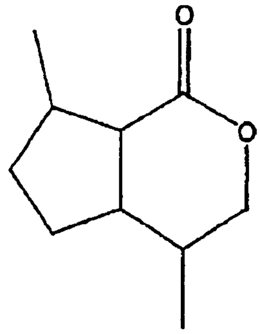
16. 权利要求 1 的组合物，它为古龙香水、洗液、喷剂、乳膏、凝胶、软膏、沐浴或淋浴凝胶、起泡制品、化妆品、除臭剂、香波、头发光洁剂或润发剂或者个人肥皂的形式。

17. 为驱虫制品形式的权利要求 1 的组合物。

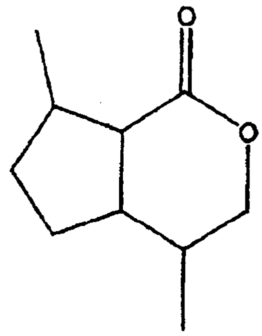
18. 权利要求 17 的制品，它被施用于人或动物寄主。

19. 权利要求 17 的制品，它选自空气清新剂、蜡烛、加香制品、纤维、被单、布料、纸张、油漆、墨水、粘土、木材、家具、地毯、卫生用品、塑料以及聚合物。

20. 制造驱虫组合物或驱虫制品的方法，所述方法包括提供下述通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物作为该组合物或制品，或者将下述通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物合并入该组合物或制品中：



21. 赋予、增加或增强制品驱虫效果的方法，包括将下述通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物合并入该制品中的步骤：

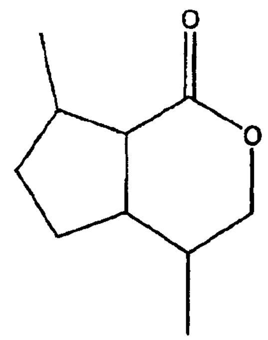


22. 根据权利要求 20 的方法，其中二氢荆芥内酯立体异构体是由(7S)-荆芥内酯衍生而来的(9S)-二氢荆芥内酯立体异构体。

23. 根据权利要求 20 的方法，其中制品选自古龙香水、洗液、喷剂、乳膏、凝胶、软膏、沐浴或淋浴凝胶、起泡制品、化妆品、除臭剂、香波、头发光洁剂或润发剂、个人肥皂组合物、空气清新剂、蜡烛、加香制品、纤维、被单、布料、纸张、油漆、墨水、粘土、木材、家具、地毯、卫生用品、塑料以及聚合物。

24. 根据权利要求 23 的方法，其中制品被施用于人或动物寄主。

25. 驱虫方法，它包括使昆虫曝露于下述通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物中：



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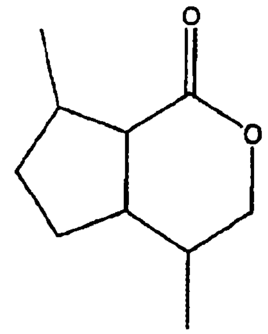
26. 下述通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物作为驱虫剂的应用：



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作为驱虫剂的二氢荆芥内酯

本申请要求享受 2002 年 3 月 20 日提交的美国临时申请 60/366,147 的权益，在此将其全部内容引入作为本发明的一部分以满足各种需要。

发明领域

本发明涉及驱虫领域，以及二氢荆芥内酯立体异构体作为驱虫材料的广泛应用。

发明背景

驱虫物质往往使得昆虫远离或者拒绝那些原本可以接受的食物源或者生活环境。大多数已知驱虫剂仅仅是略微有些毒性。实际上，少数已知驱虫剂根本就不是活性毒剂，而只是用于通过使昆虫食物源或生活环境失去吸引力或者令其望而生厌从而防止植物/动物或各种制品受损害。目前大多数的驱虫剂商品中含有合成化学品 N,N-二乙基-间甲苯酰胺(DEET)作为其主要的活性成分。比如说，销售商品名为诸如 Off!®、Deep Woods Off!®以及 Cutter®的驱虫剂都是含 DEET 的产品并且它们占驱虫产品销售额的 85%（消费者报告购买指南，1994 年岁末特刊）。另外，消费者报告测试还表明含有 DEET 浓度最高的产品驱蚊效果持续最久。尽管 DEET 是有效的驱虫剂，但是这种化合物仍然存在某些缺点。具体地说，它具有令人不快的气味并且给皮肤的感觉比较油腻。尽管该化合物最近再次被环保署(EPA)登记在美国使用，但是对其安全性特别是当应用于儿童时的关注程度越来越高(Briassoulis, G.; Narlioglou, M.; Hatzis, T. (2001) 人类和实验毒理学(Human & Experimental Toxicology) 20 (1), 8-14)。研究表明，高浓度的 DEET 在某些个体体内可能引起急性或毒性反应。其它与 DEET 相关的缺点包括：1) 这种合成化学品活性谱比较窄；2) DEET 是强有力的塑化剂，可以溶解或者破坏多种塑料制品和涂漆表层；以及 3) DEET 可以塑化通常应用于局部用制剂中以延长有效时间的惰性成分。这导致使用 DEET 制剂的接受程度降低。

由于上述的各种限制因素，具有驱虫活性但不含 DEET 的产品开

始受到消费者的青睐，同时对要求含有天然产物(相对于合成化学品例如 DEET 而言)的组合物的呼声越来越高。这样的不含 DEET 的驱虫化合物要求同时具有优秀的驱虫活性、高的残留活性以及对人体(或宠物)和环境的毒性相对较低或者没有。为了满足消费者的上述要求，目前正需要开发可由天然植物原料得到或者合成得到的并且使用起来使人愉快的新驱虫化合物。

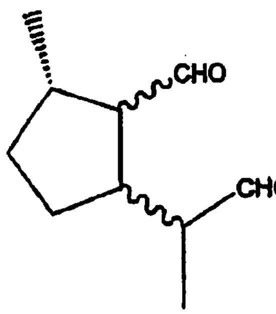
很多草本植物物种可以产生用作驱虫和芳香化学品天然来源的香精油(芳香油) [Hay, R. K. M., Svoboda, K. P., Botany 的'Volatile Oil Crops: their biology, chemistry and production'. Hay, R. K. M., Waterman, P. G. (著); Longman Group UK Limited (1993)]。因其广泛的驱虫活性而闻名的香茅油可以由冬香茅(Cymbopogon winterianus)和亚香茅(C. nardus)植物得到。用作芳香化学品来源的植物实例包括香蜂花(Melissa officinalis(Melissa))、白苏(Perilla frutescens(Perilla))、Pogostemon cablin (Patchouli)以及各种薰衣草属植物(Lavandula spp. (Lavender))。所有的这些实例均是唇形科(Lamiaceae)的成员。荆芥(Nepeta)属植物(猫薄荷(catmints))也是该科的成员，它们产生作为商业上少量产品的香精油。这种油富含于一类被称作环烯醚萜的类单萜化合物中[Inouye, H. Iridoids. Methods in Plant Biochemistry 7:99-143 (1991)]，更具体地说，就是甲基环戊烷样(methylcyclopentanoid)荆芥内酯化合物(Clark, L.J.等人. The Plant Journal, 11:1387-1393 (1997))及其衍生物中。

长期以来一直认为环烯醚萜类单萜类可以有效地驱逐各类昆虫物种(Eisner, T. Science 146:1318-1320 (1964); Eisner, T. Science 148:966-968 (1965); Peterson, C.和 J. Coats, Pesticide Outlook 12:154-158 (2001); 以及 Peterson, C.等人. Abstracts of Papers American Chemical Society, (2001) 222 (1-2): AGRO73)。美国专利 4,663,346 公开了混有含双环环烯醚萜内酯(例如蚁素)组合物的驱虫剂。另外，美国专利 4,869,896 公开了上述双环环烯醚萜内酯组合物在混合有 DEET 的增效驱虫混合物中的应用。

对于由荆芥内酯(如图 1 所示)衍生而来的一类环烯醚萜类单萜类——二氢荆芥内酯的驱虫活性的正式研究一直不是很明确，也没有教

导或者暗示这些化合物对于人类社会所常见的虫害具有驱逐效果。例如，对 Iridomyrmex nitidus 蚂蚁的肛腺分泌物的组成进行研究，发现其含有可观含量的二氢异荆芥内酯和异蚁素(Cavill, G. W. K.和 D. V. Clark. J. Insect Physiol. 13:131-135 (1967))。尽管当时认为异蚁素具有良好的“致命性”杀虫活性，但是并不能提供出证据以支持异二氢荆芥内酯也具有相似的活性，也没有对该化合物的驱虫效果(明显不同于杀虫活性)进行过研究。

随后在 Cavill, G. W. K. 等人的文献(Tetrahedron 38: 1931-1938 (1982))中，再次报道了蚂蚁的防御性分泌物中存在二氢荆芥内酯，但是作者推断认为化合物琉蚁二醛(而不是二氢荆芥内酯)是基本的驱虫成分。



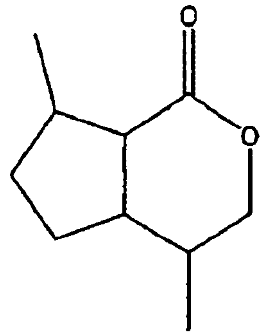
琉蚁二醛

最近，Jefson, M. 等人(J. Chemical Ecology 9:159-180 (1983))描述了二氢荆芥内酯的驱虫效果。针对正在进食的 Monomorium destructor 蚂蚁对由未稀释的化合物所具有的原始驱虫效果进行了测试。曝露于纯的二氢荆芥内酯下 25 秒钟之后，大约有 50-60%的蚂蚁停止进食。然而，作者对于经历更长时间过程的驱虫效果缺乏进一步的分析，并且也没有分析除上述未稀释的纯化合物之外的其它情况。对于浓缩化学品在如此短时间(几秒钟)内所观察到的驱虫效果并不足以使人预料到其在实际应用(例如局部用驱虫剂)中的效果。

因此，仍然需要一种驱虫性能提高了的(相对于 DEET 而言)同时对人体基本上无毒或者仅仅略微有些毒性的生物学化合物。优选的驱虫剂具有驱逐各种昆虫的活性，包括咬啮型昆虫、钻木类昆虫、有毒昆虫、家居害虫等。申请人发现，二氢荆芥内酯类在作为新的一类不具有现有组合物的不良特性的有效驱虫化合物方面表现尤其出色。

发明概述

本发明一实施方案为一种驱虫组合物或制品，它含有如下面通式所示的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物：



本发明另一实施方案为制造驱虫组合物或驱虫制品的方法，所述方法通过如下方式进行：提供上述的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物作为该组合物或制品，或者将上述的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物合并入该组合物或者制品中。本发明另一实施方案为赋予、增加或增强制品驱虫效果的方法，它通过将上述的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物合并入该制品中进行。

本发明再一实施方案为上述的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物作为驱虫剂的应用，因此在一种驱虫方法中，昆虫被曝露于上述的二氢荆芥内酯或者二氢荆芥内酯立体异构体混合物中。

附图简述

图 1 示出了天然存在的环烯醚萜(甲基环戊烷样)荆芥内酯的化学结构。

图 2 示出了对可购得的猫薄荷油蒸馏后的富含荆芥内酯的馏分(A)以及由该馏分通过氢化反应得到的物质(B)进行气相色谱/质谱(GC-MS)联用分析得到的总离子色谱图。

图 3 示出了通过 GC-MS 分析鉴定的富含荆芥内酯的馏分(A)以及氢化物质(B)的主要成分的质谱。

图 4 示出了对可购得的猫薄荷油蒸馏后的富含荆芥内酯的馏分进行的 ^{13}C NMR 分析。

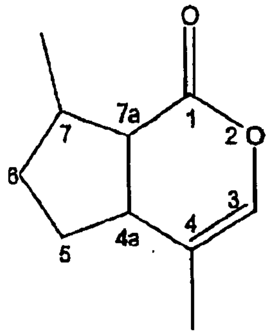
图 5 示出了对二氢荆芥内酯进行分析所获得的 ^{13}C NMR 谱，其中所述二氢荆芥内酯通过氢化可购得的猫薄荷油蒸馏后的富含荆芥内酯

的馏分得到。

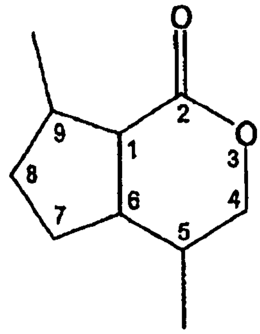
图 6 示出了体外驱虫活性试验中雌性 Aedes aegyptii 蚊子在经二氢荆芥内酯处理过的薄膜上的降落密度对时间的分布图。

发明详述

本文所用的术语"荆芥内酯"是指具有下述通式结构的化合物:

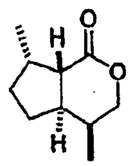


如上所示，在荆芥内酯的甲基环戊烷样骨架上的碳 4、4a、7 以及 7a 位有四个手性中心；(7S)-荆芥内酯可以由很多植物和昆虫得到。二氢荆芥内酯如通式 1 定义:

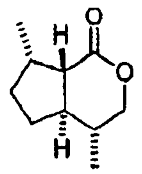


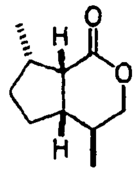
通式 1

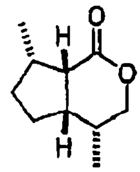
其中，1、5、6 以及 9 表明该分子的四个手性中心，同时该结构包括了二氢荆芥内酯所有可能的立体异构体。可由(7S)-荆芥内酯衍生而来的二氢荆芥内酯立体异构体的结构如下所示。

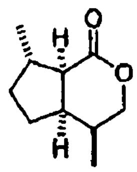


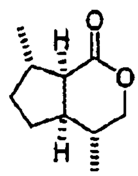
(1S,5S,9S,6R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮

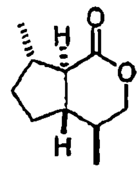

(1S,9S,5R,6R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮

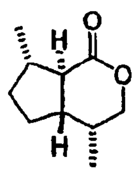

(1S,5S,9S,6S)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮


(1S,9S,6S,5R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮


(9S,5S,1R,6R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮


(9S,1R,5R,6R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮


(9S,6S,1R,5S)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮


(9S,6S,1R,5R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮

本文所用的术语“二氢荆芥内酯”或者“二氢荆芥内酯混合物”是指二氢荆芥内酯立体异构体的任意混合物。相对于整个二氢荆芥内酯组成而言，每种异构体的摩尔数或质量组成是可以改变的。二氢荆芥内酯缩写为“DHN”。

本文所用的术语“昆虫”是指任意的一种无脊椎动物，其特征在于在成年期(非成虫期包括幼虫和蛹)躯体被划分为头部、胸腔和腹

部、三对脚以及通常是(但也不尽是)两对膜翼。因此该定义包括各种咬啮型昆虫(例如蚂蚁、蜜蜂、黑蝇、恙螨、跳蚤、绿头蝇、蚊子、厩蝇(stable flies)、扁虱、黄蜂)、钻木类昆虫(例如白蚁)、有毒昆虫(例如家蝇、蟑螂、虱子、木虱)以及家居害虫(例如面粉和大豆甲虫、尘螨、蛀虫、蠹虫、象鼻虫)。

本文所用的术语"寄主"是指任意的易受昆虫影响的植物或者动物。一般认为寄主就是可为昆虫接受的食物源或者生活环境。

本文所用的术语"昆虫敏感制品"是指任意的易受昆虫影响的人造商品。它可能包括建筑物、家具等。一般认为这种制品就是可为昆虫接受的食物源或者生活环境。

本文所用的术语"驱虫剂"或者"驱虫组合物"或者"驱逐组合物"是指可以阻止昆虫靠近其优选寄主或者所适合制品的化合物或者组合物。多数已知驱虫剂根本就不是活性毒剂，而只是用于通过使昆虫食物源或生活环境失去吸引力或者令其望而生厌从而防止植物/动物或者各种制品受损害。一般情况下，驱虫剂或者局部应用于寄主；或者被合并入昆虫敏感制品中从而得到驱虫制品的化合物或者组合物，该驱虫制品可以阻止昆虫靠近该寄主或制品所处的三维空间。在另一种情形下，驱虫剂的效果就是驱使昆虫离开或者拒绝：1.）寄主，从而使昆虫"叮咬"该寄主的频率降至最低；或者 2.）昆虫敏感制品，从而保护该制品防止被昆虫损坏。驱虫剂可以是气体(作用于嗅觉)、液体、或者固体(作用于味觉)。

一些众所周知的驱虫剂实例包括：二苯乙二酮；苯甲酸苄酯；2,3,4,5-双(丁-2-烯基)四氢糠醛(MGK 驱虫剂 11)；丁氧基聚丙二醇；N-丁基乙酰苯胺；6,6-二甲基-5,6-二氢-1,4-吡喃酮-2-羧酸正丁酯(Indalone)；己二酸二丁酯；邻苯二甲酸二丁酯；琥珀酸二正丁酯(Tabatrex)；N,N-二乙基-间-甲苯酰胺(DEET)；卡百酸二甲酯(dimethyl carbate)((内,内)-双环[2.2.1]庚-5-烯-2,3-二羧酸二甲酯)；邻苯二甲酸二甲酯；2-乙基-2-丁基-1,3-丙二醇；2-乙基-1,3-己二醇(Rutgers 612)；异辛可部酸二正丙酯(MGK 驱虫剂 326)；2-苯基环己醇；对蓋烷-3,8-二醇以及 N,N-二乙基琥珀酰胺酸正丙酯。用于蚊子、扁虱等的常用驱虫剂有香茅油(将在下文讨论)、邻苯二甲酸二甲酯、正丁基异亚丙基丙酮草酸酯(n-butyl mesityl oxide oxalate)

以及 2-乙基-1,3-己二醇(参见 Kirk-Othmer Encyclopedia of Chemical Technology, 第二版, Vol. 11:724-728; 以及 The Condensed Chemical Dictionary, 第八版, 第756页)。

除了上述化学组合物外，还有很多有效驱虫剂由香精油和/或香精油活性成分组成。“香精油”是指任何一类由具有气味或者其它植物特性的植物得到的挥发油。香精油驱虫剂化合物的实例包括：苦杏仁油、茴香油、罗勒油、月桂油、香菜油、豆寇油、雪松油、芹菜油、春黄菊油、肉桂油、香茅油、丁香油、胡荽油、 枯茗油、莳萝油、桉树油、茴香油、生姜油、柚子油、柠檬油、酸橙油、薄荷油、欧芹油、胡椒薄荷油、胡椒油、玫瑰油、荷兰薄荷油(薄荷醇)、甜橙油、百里香油、姜黄油以及鹿蹄草油。香精油活性成分的实例有：香茅醛、水杨酸甲酯、水杨酸乙酯、水杨酸丙酯、香茅醇、黄樟油素以及柠檬油精。

相对于“驱虫剂”，“杀虫剂”是指通过口食、接触昆虫表皮或者通过空气烟薰作用能够将昆虫毒死的化合物或者混合物。因此，杀虫剂是一类用来控制昆虫生命并且对人也有害的农药(即，作为疾病媒介物产生直接的害处，或者经由破坏农作物、食品或者纺织品产生间接的害处)。一些众所周知的杀虫剂包括：无机化合物(例如砷、铅和铜)；天然存在的有机化合物(例如鱼藤酮、除虫菊酯、烟碱、环烷酸铜和石油衍生物)；以及合成有机化合物(例如 DDT、狄氏剂、异狄氏剂、氯丹、林丹、对-二氯苯以及对硫磷)。

本文所用的术语“增效驱虫组合物”是指其产生的效果实质上超过由单独或联合这些成分的已知效果所能期望或者预料得到的效果的驱虫组合物。在本发明中，增效驱虫组合物包括二氢荆芥内酯及其混合物，以及至少一种不是二氢荆芥内酯的其它驱虫化合物(有时也称作非二氢荆芥内酯驱虫化合物)。

“驱虫组合物”可用作“驱虫制品”的成分，其中术语“驱虫制品”是指具有被该驱虫组合物增强、改变或者增加的驱虫性能的制品。关于本文所描述的驱虫性能，各种形式的术语“改变”和“调节”意思是指给组合物补充或者赋予驱虫性能，或者是指在某方面天然驱虫性能欠缺的情况下增加已有的驱虫特性，或者是指补充已有的驱虫性能从而调节其性质或者特性。术语“增强”意思是指加强(不影响驱

虫性能的类型和性质)驱虫组合物或驱虫制品的一种或多种驱虫特性。

在一优选实施方案中，本发明的驱虫组合物还具有芳香组合物的功能，因为它能够赋予该驱虫组合物或驱虫制品令人愉快的芳香或香气。二氢荆芥内酯可用于驱虫组合物或制品中以增强、改变或者增加该组合物的整体香气或芳香. 关于芳香，各种形式的术语“改变”和“调节”意思是给没有气味的物质补充或者赋予芳香、香气特性或香韵，或者是指在某方面自然香气不足的情况下增加已有的香气特性，或者是指补充已有的香气效果从而调节其性质、特性或者香气. 术语“增强”意思是指加深(不影响香气的类型和性质)芳香、香料组合物、或者一种或多种加香制品的一种或多种香气的细微差别以及它们的器官感觉效果.

本文所用的术语“芳香组合物”是指一种有机化合物的混合物，所述有机化合物包括例如醇、醛、酮、腈、酯、内酯、天然香精油、合成香精油以及硫醇在内，这些有机化合物被混合在一起，使得单独成分的气味混合后产生让人愉悦的或者合意的芳香. 这类组合物通常包含: (1) 组合物的主香或“香簇(bouquet)”或者根基; (2) 调节剂，它对主香进行修整并与主香相伴; (3) 定香剂，它包括有气味的物质和延缓蒸发的物质，其中所述有气味物质在整个蒸发的全部步骤中可以给香料带来一种独特的香韵; 以及(4) 顶香，它们通常是低沸点、闻起来清新的物质.

在芳香或者香气组合物中，单个成分都会发挥其特定的嗅觉特性，但是组合物的整体效果是每种成分的每种效果的总和. 因此，本发明的二氢荆芥内酯或其混合物可用于改变这类组合物的芳香特性，例如通过突出或缓和由组合物中另一成分提供的嗅觉反应.

从文献资料已知二氢荆芥内酯是荆芥属中的多种唇形科植物的香精油中的微量成分(Regnier, F.B.,等人. Phytochemistry 6:1281-1289 (1967); DePooter, H.L.,等人. Flavour and Fragrance Journal 3:155-159 (1988); Handjieva, N.V.和 S.S. Popov J. Essential Oil Res. 8:639-643 (1996)). 另外，它们也被鉴定出是某些昆虫的防御性分泌物中的成分，这类昆虫包括隐翅虫(Jefson, M.等人. J. Chem. Ecol. 9:159-180 (1983))和蚂蚁，特别是虹蚁属(Iridomyrmex)蚂蚁

(Cavill, G.W.K.和 D.V.Clark. J. Insect Physiol. 13:131-135 (1967))。在这些含有二氢荆芥内酯的物种中，有人曾经提出它们可以由环烯醚萜类单萜类琉蚁二醛通过生物合成途径衍生得到。

已经描述和介绍过以各种方式进行的二氢荆芥内酯及其相关环烯醚萜类单萜化合物的化学合成。下面是涉及合成的有用参考文献:

1) Abelman, M.M.等人. J. Am. Chem. Soc. 104 (14): 4030-2 (1982)。

2) Fleming, I.和 N.K. Terrett. Tetrahedron Lett. 25 (44): 5103-5104 (1984); J. Chem. Soc., Perkin Trans. 1: 2645-2650 (1998)。

3) Lee, E.和 C.H. Yoon. J. Chem. Soc., Chem. Commun. 4: 479-81 (1994)。

4) Nagata, H.和 K. Ogasawara. Tetrahedron Lett. 40 (36): 6617-6620 (1999)。

5) Nangia, A.等人. Tetrahedron Lett. 35 (22): 3755-8 (1994)。

6) Tanimori, S.和 M. Nakayama. Agric. Biol. Chem. 55 (4): 1181-1184 (1991)。

7) Uyehara, T.等人. J. Chem. Soc., Chem. Commun. 2: 113-14 (1989); Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 32: 441-6 (1990); J. Org. Chem. 57 (11): 3139-3145 (1992)。

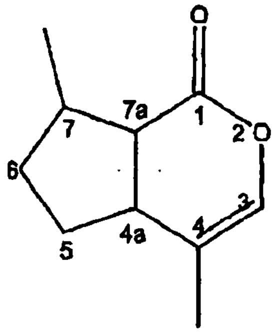
8) Wolinsky, J.和 E.J. Eustace. J. Org. Chem. 37 (21): 3376-8 (1972)。

9) Wolinsky, J.和 D.L. Nelson. Tetrahedron 25 (17): 3767-74 (1969)。

一种用于合成本发明的二氢荆芥内酯混合物的优选和简便方法是通过氢化荆芥内酯。诸如氧化铂和钯(负载于碳酸锶上)的催化剂可以提供产率为 24-90%的二氢荆芥内酯(Regnier, F.E. 等人. Phytochemistry 6:1281-1289 (1967))。荆芥内酯是一种已知物质，其相对纯的形式可以由通过各种手段从荆芥属植物(猫薄荷)中分离得到的香精油中方便地得到。分离这类香精油的方法是本领域所熟知的，用于提取所述油的方法实例包括(但不仅仅限制于此)蒸汽蒸馏

法、有机溶剂提取法、微波辅助的有机溶剂提取法、超临界流体提取法、机械提取法以及花香吸取法(先冷提到油脂中，接着再用有机溶剂提取)。

众所周知，从不同荆芥属物种分离出来的香精油具有不同组成比例的天然存在的各个荆芥内酯立体异构体(Regnier, F.E. 等人. Phytochemistry 6:1281-1289 (1967); DePooter, H.L. 等人. Flavour and Fragrance Journal 3:155-159 (1988); Handjieva, N.V. 和 S.S. Popov. J. Essential Oil Res. 8:639-643 (1996))。因此，从由含有荆芥内酯混合物的任意荆芥属物种得到的香精油经氢化可以得到二氢荆芥内酯立体异构体混合物。如下所示，在荆芥内酯的甲基环戊烷样骨架上的碳 4、4a、7 以及 7a 位有四个手性中心:



因此，很明显氢化后可能有总共 8 对二氢荆芥内酯对映体。在这些对映体中，迄今为止记载过的天然存在的立体异构体是(9S)-二氢荆芥内酯。根据本发明优选的芳香物质包括任一或者所有可能的二氢荆芥内酯立体异构体混合物。更优选的芳香物质包括(9S)-二氢荆芥内酯的混合物。最优选的是由(7S)-荆芥内酯衍生而来的(9S)-二氢荆芥内酯立体异构体。如图 1 所示，它包括通常称作顺式,反式-荆芥内酯、顺式,顺式-荆芥内酯、反式,顺式-荆芥内酯、以及反式,反式-荆芥内酯。

氢化反应结束后，所得到的异构体产物的混合物可以通过常规方法，例如制备性液相色谱法进行分离，从而得到每对经高度纯化的二氢荆芥内酯非对映体。

除了在不同的荆芥属物种之间存在着荆芥内酯立体异构体的含量差异之外，还已知在同一物种内也存在差异。指定植物物种可以产生具有不同组成的香精油，这取决于它们生长的条件或者在收获时所处

生长阶段。另外，已经证实，在单一物种 Nepeta racemosa 内，存在着与生长条件或者收获时所处生长阶段无关的油组成差异(Clark, L.J. 等人. The Plant Journal, 11:1387-1393 (1997))。表现出不同油组成的单一植物物种被称作化学型(chemotypes)，已经表明，在 Nepeta racemosa 中，化学型在所含各种荆芥内酯立体异构体的比例方面存在明显差异(Clark, L.J.等人，同上)。因此，制备特定二氢荆芥内酯对映体的优选方法应该是氢化由已知含有特定荆芥内酯立体异构体的荆芥属化学型提取出来的油。

因此，制备本发明如通式 1 所示的二氢荆芥内酯的优选方法是通过氢化由具有规定荆芥内酯立体异构体含量的油的植物得到的荆芥内酯，这种方法就生产成本和其生物基础而言是一种具有工业优点的制备方法。其它的方法公开在 2002 年 4 月 3 日提交的美国临时申请 60/369,470 中。

本发明的二氢荆芥内酯具有独特的驱虫特性，尤其是可有效对抗较宽范围的常见虫害，包括咬啮型昆虫、钻木类昆虫、有毒昆虫、以及家居害虫。

含有二氢荆芥内酯或其混合物的本发明驱虫组合物可有效对抗各种干扰人类社会的昆虫。这类昆虫包括各种咬啮型昆虫(例如蚂蚁、蜜蜂、黑蝇、恙螨、跳蚤、绿头蝇、蚊子、厩蝇、扁虱、黄蜂)、钻木类昆虫(例如白蚁)、有毒昆虫(例如家蝇、蟑螂、虱子、蜚蠊、木虱)以及家居害虫(例如面粉和大豆甲虫、尘螨、蛀虫、蠹虫、象鼻虫)。对于传播致病菌的蚊子，这种驱虫特性还可以有效地预防传染上这类疾病。

很多化合物具有驱虫和/或驱蚊活性，其证据如下：1.) 美国农业部(USDA)报道的各种具有驱虫活性的化学结构(Chemicals Evaluated as Insecticides and Repellent at Orlando, Fla., W. V. King 编辑，美国农业部，农业研究服务机构，农业手册第 69 期)；以及 2.) 驱虫制剂中的各种具有不同驱虫活性的原料(参见例如欧洲专利申请 97,812 和 97,813、和美国专利 4,127,672、美国专利 4,756,905、美国专利 5,465,685、美国专利 5,489,433、美国专利 5,565,208、美国专利 5,672,337 以及美国专利 5,716,602)。总的来说，已在醇类、酰胺类、酯类、酮类、酸类、内酯类以及内酰胺类中

发现该活性；并且驱虫活性在某种程度上似乎依赖于这些化合物的物理性质。

对于总体驱虫活性而言，比较重要的一个性质是表面活性，因为大多数(并非全部)驱虫化合物在其结构中既含有极性又含有非极性区域。第二个性质是挥发性。驱虫剂是这样一类与众不同的化合物，为了保证其有效性，活性成分必须从寄主的皮肤表面或者驱虫制品挥发开去，有效性就是通过看其是否保护寄主免于被昆虫叮咬或者看其是否保护制品免于被昆虫损坏来评价的。至于局部用驱虫剂，为了驱逐昆虫，在直接位于寄主皮肤表面上的空间需要适宜的最低浓度的驱虫剂，该浓度就是驱虫剂效力的一个评价标准。当然，挥发速率还受皮肤的吸收能力影响——在多数情况下，渗入皮肤和透过皮肤并不是适用于从皮肤表面释出化合物的理想模式。对于驱虫制品也必须考虑这些因素，即考虑在制品周围的三维空间里所需驱虫剂的最低浓度。

有各种手段可供试图在这些挥发特性（和任选渗透特性）之间寻求平衡的研究人员采用。第一，可以寻找一种具有平衡的物理特性的单一活性成分。作为其替代方式，为了改善活性成分在寄主皮肤表面上或驱虫制品中的存留能力，该活性成分可以与加入其中的聚合物和惰性成分制成制剂。然而，向活性成分中加入惰性成分使得活性成分在驱虫薄膜或制品表面上的分子数目受到限制。由于为了挥发，分子必须附在表面上，因此使得挥发速率降低。这导致一个消极的结果，那就是使得施用到寄主皮肤表面上或者存在于驱虫制品表面上的活性化合物的浓度被稀释。这进而降低了含惰性成分的制剂的整体效力。在第三种替代方式中，可以制得活性成分的微囊从而控制从寄主皮肤表面或者驱虫制品释出的速率。最后，限制活性成分挥发速率的另一种方法是合成其前体分子，它可以在皮肤表面上或者驱虫制品中慢慢分解从而释出活性成分。

例如，活性成分可以通过例如亚微米封装技术释出，其中使活性成分被包封(环绕)于皮肤营养蛋白质内，这就好比空气填充在气球里。这种蛋白质可以以例如 20%的浓度使用。含有很多上述蛋白质胶囊的驱虫剂其应用之一就是将它悬浮在含水洗液或者水中供喷雾用。与皮肤接触后，该蛋白质胶囊开始崩解释放出被包封的二氢荆芥内酯。上述步骤连续进行，各微囊耗尽之后接着替代以新胶囊，该新胶

囊与皮肤接触从而释出其活性成分。该方法使用一次可以持续 24 小时。由于蛋白质对皮肤的粘附性很强，所以这种制剂很耐排汗(出汗)以及水分。使用的时候，它们很干爽并且舒适无油腻感。这种方法可以很有效地保护，但其仅仅是在用于皮肤时有效，因为衣服并不具备释放蛋白质的能力。替代方法使用聚合物包裹该驱虫剂，这样减慢了早期的挥发而余下更多的二氢荆芥内酯供后期挥发。与没有封装的产品相比，这种方法通常可以使驱虫剂的效力时间延长 25%-50%，但是由于存在聚合物所以通常会感觉油腻。在另一替代方式中，使用增效剂以不断刺激组合物中的二氢荆芥内酯挥发。

不管施用何种特殊手段来控制驱虫剂的挥发性，每种驱虫剂必须具有一个从皮肤表面或者驱虫制品挥发开去的最低有效挥发速率(MEER)以维持在直接位于该皮肤表面/制品上的空间内必需的最低浓度的驱虫剂，以便有效地驱逐昆虫。如果挥发速率高于最低有效挥发速率(MEER)，会导致不希望的显著损失。然而，由于 MEER 可以随该领域的条件而改变，因此这个问题被进一步复杂化。既要考虑到昆虫的饥渴性或叮咬能力，还要考虑到寄主环境下的昆虫密集程度。例如，随着蚊子的饥渴性增加，也要求 MEER 增大。在蚊子密集程度较低的环境下并且那些蚊子并不饥饿，MEER 可以低至为 2，或者更通常为 5 或 6。相反，在蚊子又饥饿其密集程度又高的环境下，MEER 可高达 12-15。在本发明中优选这样的驱虫组合物，其中在一段时间内皮肤表面挥发速率至少等于最低有效挥发速率，所述时间段优选为 5 小时左右。

在本发明中，可以使用各种各样的适合上述二氢荆芥内酯的载体或稀释剂。该载体可以将制剂调节至含有有效浓度的驱虫剂分子。如果制备成局部用驱虫剂的话，优选将驱虫剂分子混合在皮肤学上可接受的载体中。载体还可以提供拒水性、防止刺激皮肤和/或缓和和调理皮肤。选择一种或多种载体用于任何驱虫制剂中时需要考虑的因素包括商业实用性、成本、驱虫能力、挥发速率、气味以及稳定性。有些载体本身就具有驱虫特性。

对于本发明来说，如果使用载体，则用于实现与终产品的理想紧密混合效果的载体可具体选择通常用于驱虫制剂中的任意载体。此外，载体应该优选那种不会对环境产生危害的载体。因此，载体可以

是任意一种可用于制备驱虫产品的可商购得到的各种有机和无机液态、固态或半固态载体或者载体制剂。例如，载体可以包括硅酮、凡士林、羊毛脂或者其它已知载体成分中的多种成分。

有机液态载体的实例包括液态脂肪族烃类(例如戊烷、己烷、庚烷、壬烷、癸烷及其类似物)以及液态芳香族烃类。其它液态烃类的实例包括由煤和由各种类别的石化原料蒸馏蒸馏得到的油，包括由石油分馏得到的煤油。其它的石油类包括那些通常称作农业喷淋油的油类(例如所谓的既有轻油性质又显中油性质的喷淋油（light and medium spray oil)，它由石油蒸馏物的中间馏分组成，仅具有轻微的挥发性)。这类油通常是高度精制的，并且可能仅仅含有微量不饱和化合物。此外，这类油通常是石蜡油，因此可以用水和乳化剂乳化，稀释至低浓度，从而用作喷淋油。使用硫酸浸提木浆所得到的松浆油和石蜡油一样，也可以按照类似方法使用。其它有机液态载体可以包括液态萜烯烃类和萜烯醇类例如α-蒎烯、二戊烯、萜品醇等。

其它的载体包括硅酮，凡士林，羊毛脂，液态烃类，农业喷淋油，石蜡油，松浆油，液态萜烯烃类以及萜烯醇类，脂肪族和芳香族醇类、酯类、醛类、酮类，矿物油，高级醇，粉碎的有机和无机固态材料。

除了上述液态烃类外，载体还可以含有传统的乳化剂，乳化剂可用来使二氢荆芥内酯在水中分散从而被水稀释以供终端应用。

其余的液态载体还可以包括有机溶剂例如脂肪族和芳香族醇类、酯类、醛类、酮类。脂肪族单羟基醇包括甲醇、乙醇、正丙醇、异丙醇、正丁醇、仲丁醇以及叔丁醇。适宜的醇类包括二元醇(例如乙二醇和丙二醇)以及频哪醇。适宜的多羟基醇包括丙三醇、阿拉伯糖醇、赤藻糖醇、山梨醇等。最后，适宜的环状醇包括环戊醇和环己醇。

普通的芳香族和脂肪族酯类、醛类以及酮类也可用作载体，有时它们也可以与上述醇类联合使用。其余的液态载体包括相对较高沸点的石油产品，例如矿物油和高级醇(例如十六醇)。另外，普通的或者所谓的“稳定剂”(例如叔丁基亚硫酰基二甲基二硫代碳酸酯)可以与含有本发明组合物的一种或多种载体联合使用或作为其成分之一。

可用于本发明组合物中的固态载体包括粉碎的有机和无机固态材料。适宜的粉碎的固态无机载体包括硅质矿石例如合成和天然的粘土、蒙脱石、硅镁土、漂白土、硅藻土、高岭土、云母、滑石、粉碎

的石英等，以及合成得到的硅质材料例如白炭黑以及沉淀硅和煅制二氧化硅。粉碎的固态有机材料的实例包括纤维素、锯屑、合成有机聚合物等。半固态或者胶质载体的实例包括蜡状固体(例如凡士林油)、羊毛脂等，以及可以提供半固态载体产品的已知液态和固态物质的混合物，它可以提供本发明所述的有效的驱虫性能。

含有二氢荆芥内酯的本发明驱虫组合物还可以含有个人护理产品制剂领域所公知的各种辅剂，例如增稠剂、缓冲剂、螯合剂、防腐剂、芳香剂、抗氧化剂、胶凝剂、稳定剂、表面活性剂、软化剂、着色剂、芦荟液、蜡、其它的助渗透剂及其混合物、以及治疗活性剂或化妆用活性剂。

此外，本发明组合物还可以含有其它的辅剂例如一种或多种治疗活性成分或化妆用活性成分。可用于本发明组合物中的治疗活性成分或化妆用活性成分包括杀真菌剂、遮光剂、防晒剂、维他命、鞣剂、植物提取物、抗炎剂、抗氧化剂、自由基清除剂、类维生素 A、α-羟基酸、软化剂、消毒剂、抗生素、抗菌剂或者抗组胺剂，它们可以以达到所需治疗或化妆效果的有效含量存在。

二氢荆芥内酯在本发明中可以单独或者以任何比例联合使用。按照本领域常规，加入至特定昆虫敏感制品中使其具有驱虫性能所需的驱虫组合物用量取决于产品性质以及其它的因素。这些因素既包括对成本的考虑，又要考虑到驱虫组合物或者驱虫制品中其它成分的性质、它们的用量以及所希望达到的驱虫效果。总的来说，该驱虫组合物的组成应该含有足够量的活性驱虫物质以有效地使昆虫在较长的时间(优选至少几个小时)内远离寄主。

每种通式 I 的二氢荆芥内酯或其混合物在本发明驱虫组合物或驱虫制品中的用量通常不超过最终产品重量的大约 80 重量%，然而在某些应用中可能会用到更大的用量，因此对该用量并无限制。更优选的是，二氢荆芥内酯的适宜用量应该是组合物或制品重量的至少大约 0.001 重量%，优选为大约 0.01 重量%至大约 50 重量%；更优选为大约 0.01 重量%至大约 20 重量%。具体的组成将取决于目标用途。

本发明的二氢荆芥内酯组合物可以不用载体而制成制剂并且有效。然而，更普遍的情形是，驱虫组合物包括载体同时含有大约 0.001-50 重量%的所述化合物，这类化合物通常是以与载体紧密混合

的形式使得活性物质可以驱逐常见的昆虫害虫，例如咬啮型昆虫、钻木类昆虫、有毒昆虫、家居害虫等。

本发明组合物可以制成制剂并被包装起来从而使得可以将产品以各种形式递送，包括溶液剂、混悬剂、乳膏、软膏、凝胶、膜剂或者喷剂，这取决于所使用的优选方法。载体可以是适合于通过压缩气体使二氢荆芥内酯分散于空气中的气雾剂组合物。

在某些情形下，本发明的二氢荆芥内酯驱虫组合物可以与至少一种另外的非二氢荆芥内酯的驱虫剂制成制剂从而得到增效驱虫组合物(参见例如美国专利 4,869,896)。在这种情况下，由二氢荆芥内酯和至少一种另外的驱虫剂产生的效果实质上超过由单独或联合这些成分的已知效果所能期望或者预料得到的效果。可与二氢荆芥内酯用于增效驱虫组合物中的其它驱虫剂的实例包括但不限制于：二苯乙二酮；苯甲酸苄酯；2,3,4,5-双(丁-2-烯基)四氢糠醛(MGK 驱虫剂 11)；丁氧基聚丙二醇；N-丁基乙酰苯胺；6,6-二甲基-5,6-二氢-1,4-吡喃酮-2-羧酸正丁酯(Indalone)；己二酸二丁酯；邻苯二甲酸二丁酯；琥珀酸二正丁酯(Tabatrex)；N,N-二乙基-间-甲苯酰胺(DEET)；卡百酸二甲酯((内,内)-双环[2.2.1]庚-5-烯-2,3-二羧酸二甲酯)；邻苯二甲酸二甲酯；2-乙基-2-丁基-1,3-丙二醇；2-乙基-1,3-己二醇(Rutgers 612)；异辛可部酸二正丙酯(MGK 驱虫剂 326)；2-苯基环己醇；N,N-二乙基琥珀酰胺酸正丙酯；1-哌啶羧酸 2-(2-羟甲基)-1-甲基丙基酯(Bayrepel)以及对蓋烷-3,8-二醇。优选含有二氢荆芥内酯(或其混合物)和 DEET、Bayrepel 或者对蓋烷-3,8-二醇的增效驱虫组合物。最优选含有二氢荆芥内酯(或其混合物)以及另外的天然驱虫分子例如对蓋烷-3,8-二醇的增效驱虫组合物。

局部用驱虫制品具有的令人满意的特性包括低毒性、抵抗因水浸或出汗而损失、味淡或者无味或者至少是具有令人愉悦的气味、易于施用以及可以在寄主皮肤上迅速形成干燥不粘的表面薄膜。为了获得这些特性，用于局部驱虫制品的配方应当满足下面的要求：通过用有效量的驱虫制品接触动物的皮肤、皮毛或者羽毛的方式将滋生有大量昆虫的动物(例如长有跳蚤的狗、长有白虱的家禽、长有扁虱的母牛、以及人)用本发明的驱虫组合物处理之后，可以将这些昆虫从动物寄主赶走。因此，将制品分散于空气中或者将组合物以液态薄雾或者细

粉尘的形式分散于空气可以使得驱虫组合物落在所需寄主表面上。同样地，直接向寄主喷洒液态/半固态/固态驱虫制品也是一种用有效量的驱虫组合物接触寄主表面的有效方法。

可用作局部用驱虫制品的本发明实施方案包括(但不限制于)：古龙香水、洗液、喷剂、乳膏、凝胶、软膏、沐浴和淋浴凝胶、起泡制品(例如刮胡泡沫体)、化妆品、除臭剂、香波、头发光洁剂/润发剂以及个人肥皂组合物(例如洗手皂和沐浴/淋浴肥皂)。

本发明还涉及二氢荆芥内酯混合物在各种容易遭受昆虫攻击的制品中用作驱虫剂的应用。室外消费品通常可以含有但不必一定含有本发明的驱虫组合物，但是将含有有效量的二氢荆芥内酯。通过使用二氢荆芥内酯或其混合物而得到改良的典型制品包括但不限制于：空气清新剂、蜡烛、其它的加香制品、纤维、被单、布料[例如衣服、网织品(蚊帐)以及其它织物]、纸张、油漆、墨水、粘土、木材、家具(例如家用和装饰用)、地毯、卫生用品、塑料、聚合物等。

本发明的二氢荆芥内酯组合物可以与具有控释系统的聚合物混合。相容的聚合物可以是或者不是生物可降解的。聚合物实例有高密度聚乙烯或者低密度聚乙烯、可生物降解的热塑性聚氨酯、可生物降解的乙烯聚合物、聚(ε-己内酯)均聚物以及含有所述聚合物的组合物，如美国专利4,496,467、美国专利4,469,613和美国专利4,548,764中所公开。

二氢荆芥内酯因为下述各种理由而特别适合用作本发明制备方法中的驱虫原料。

首先，该化合物制造成本低，这是消费者在选择有效驱虫剂时所要考虑的重要方面。很多可商购得到的驱虫产品仅仅是相对较浓时才有效，这样的浓度包括以总重量计在载体中含有多达5-30%(或者更多)的驱虫剂。例如，McGovern等人的美国专利4,416,881公开了载体中驱虫剂浓度为6.25-25%的驱虫剂。

其次，该化合物作为已知的天然化合物，因此不用担心有关反对合成化学品(例如DEET)作为驱虫组合物主要活性成分的顾虑。

最后，除了这样制备得到的二氢荆芥内酯混合物具有天然的驱虫特性之外，该组合物还具有独特的令人愉悦的芳香。主题化合物具有的这种芳香香韵由此使得其可用于赋予、调节、增加或者增强驱虫组

合物的总体嗅觉要素，例如通过利用或者缓和由组合物中一种或多种其它成分提供的嗅觉反应. 具体地说，本发明的二氢荆芥内酯或其混合物可用于掩盖或调节由最终驱虫组合物或制品配方中的其它成分所发出来的气味，和/或用于通过赋予其香味(perfume)或香气而提升产品对消费者的吸引力. 可以预料得到的是，二氢荆芥内酯的这种令人愉悦的芳香将比其它驱虫化合物特别是DEET更能吸引消费者.

美国专利 SN 10/349,865 (2003 年 1 月 23 日申请)中也描述了二氢荆芥内酯及其应用，在此将其全部内容引入作为本发明的一部分以满足各种需要.

实施例

在下面的实施例中进一步描述本发明. 应该理解，这些实施例在描述本发明优选实施方案的同时，仅仅是以示例说明的方式给出的. 根据上面的讨论以及这些实施例，本领域技术人员可以理解本发明的实质特征，并且可以对本发明作出各种各样的变化和修饰以使其适合于各种各样的用法和条件，而不偏离本发明的精神和范围。下面的符号 w/v 是指活性成分(单位是克)在 100 mL 溶液中的重量.

各缩略语的意思如下: "h"表示小时，"min"表示分钟，"sec"表示秒，"d"表示天，"mL"表示毫升，"L"表示升，"m/z"表示质量（m）与电荷（z）之比，"ppm"表示每百万分之一，"mol %"表示以摩尔计的百分比，"Hz"表示赫兹(1/秒)，以及"psig"表示磅/平方英寸表压.

实施例 1 通过蒸汽分馏土荆介(Nepeta cataria)油制备荆芥内酯

获得通过将来源于猫薄荷土荆芥的草本材料蒸汽蒸馏制备的商购猫薄荷油试样(Berjé, Bloomfield, New Jersey, USA)。所述油经联用气相色谱-质谱分析仪(GC-MS)分析，表明其主要成分是荆芥内酯立体异构体(数据没有给出)。

通过分馏这种油将荆芥内酯馏分进一步纯化. 图 2A 表示通过分馏土荆芥香精油商品试样得到的富含荆芥内酯的馏分的 GC-MS 总离子色谱图. 操作条件是: HP5-MS 柱, 25 m x 0.2 mm; 120℃烤箱, 2 min, 15℃/min, 210℃, 5 min.; He @ 1 ml/min. m/z 为 166 的峰是荆芥内酯。未标记峰对应于微量的倍半萜杂质. 在图 3A 中示出了图 2A 中主峰(6.03 min, 荆芥内酯)的质谱. 对油和纯化物质还进行了 ^{1}H 和 ^{13}C

NMR 分析，列出了 ^{13}C 数据(图 4)。将文献报道的四种可能立体异构体的 ^{13}C 化学位移与试样得到的光谱进行对比。测出三种立体异构体，以位于 170 ppm 附近的羰基区域为基础进行定量。原始油和经富集物质的化学位移均记录在表 1 中。对荆芥内酯的每个碳原子进行了鉴别，如图 4 所示。

表 1

猫薄荷(土荆芥)香精油商品试样和经蒸汽蒸馏纯化后的馏分中存在的荆芥内酯立体异构体的 ^{13}C 化学位移和摩尔%值

原子	香精油			纯化馏分		
	顺式,反式-	反式,顺式-	顺式,顺式-	顺式,反式-	反式,顺式-	顺式,顺式-
	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)
a	170.9	170.1		170.8	170.1	
b	133.7	135.9	134.2	133.7	135.9	134.2
c	115.3	120.4		115.3	120.4	
d	40.8	37.3	39.6	40.8	37.4	39.5
e	49.4	49.1	46.4	49.5	49.1	46.3
f	39.7	32.1	38.4	39.8	32.1	38.4
g	33.0	30.0	32.7	33.1	30.0	32.7
h	30.9	26.1	30.4	31.0	26.1	30.5
j	20.2	17.5	17.1	20.3	17.6	17.2
i	15.4	14.2	14.7	15.5	14.2	14.8
摩尔%	80.20%	17.70%	2.10%	84.50%	14.30%	1.20%

该分析表明，荆芥内酯以下述的比例存在于所述油中：80.2 摩尔% 顺式,反式-荆芥内酯，17.7 摩尔% 反式,顺式-荆芥内酯和 2.1 摩尔% 顺式,顺式-荆芥内酯。这些数据表明，在纯化物质中的荆芥内酯比例为：84.5mol% 顺式,反式-荆芥内酯，14.3 摩尔% 反式,顺式-荆芥内酯和 1.2 摩尔% 顺式,顺式-荆芥内酯。对该纯化馏分的 GC-MS 分析表明其主要由这些荆芥内酯(m/z 为 166)组成，伴有微量倍半萜类石竹烯和葎草烯(数据没有给出)。

实施例 2

二氢荆芥内酯的制备

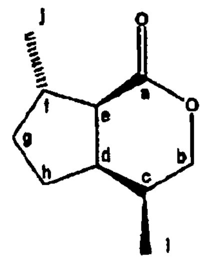
将如实施例 1 所述由猫薄荷油经蒸馏得到的 107 g 荆芥内酯馏分溶解于乙醇(200 ml)，并置于装有 12.7 g 2% $Pd/SrCO_3$(Aldrich 41,461-1)的 Fisher-Porter 瓶中。试管排空和用 H_2 充气两次，然后

充入 30 psig 的 H_2。在室温下搅拌 48 h 之后，将试管放气，混合物用硅藻土过滤除去催化剂。真空除去溶剂，得到透明油状物。

对该物质进行 GC-MS 分析(HP5-MS 柱，25 m x 0.2 mm；120℃烤箱，2 min,15℃/min，210℃，5 min.；He @ 1 ml/min)。总离子色谱图如图 2B 所示。该分析显示，m/z 为 168 的主要成分(65.43%面积；Rt 7.08 min)代表二氢荆芥内酯异构体；该成分的质谱如图 3B 所示。该质谱含有一个 m/z 为 113 的离子，被确认为二氢荆芥内酯(Jefson, M. 等人. J. Chemical Ecology 9:159-180 (1983))。代表剩余二氢荆芥内酯非对映体的五个附加峰也出现在色谱中，它们可能是由存在于原料中的三种荆芥内酯衍生而来的。它们出现在 Rt 5.41 min，6.8%面积，m/z 168；Rt 5.93 min，1.2%面积，m/z 168；Rt 6.52 min，4.88%面积，m/z 168；Rt 6.76 min，13.8%面积，m/z 168 和 Rt 7.13 min，1.25%面积，m/z 168。该分析中没有检测到残留的荆芥内酯。

对该物质进行 ^1H，^{13}C 和一系列的 2D NMR 方法分析。^{13}C NMR 光谱(图 5)中的羰基区域表明该试样中至少存在 5 个自旋体系，其中一个自旋体系的数量比其余四个大(大约 75%)。检测到极少的残留荆芥内酯。

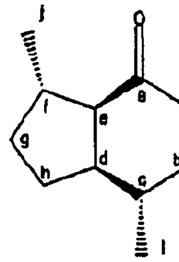
根据对偶合常数的分析以及所观察到的不同 NOE 交叉峰的强度，认为该物质主要成分的立体化学结构为通式 2 的二氢荆芥内酯：(9S,5S,1R,6R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮)。



通式 2

甲基基团(i)和质子(d)之间的距离大于甲基基团(j)和质子(e)之间的距离，该观察结果与其顺-反立体化学构型是一致的。

类似地，利用 ^{13}C 化学位移鉴别出立体异构体异二氢荆芥内酯(9S,5R,1R,6R)-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮；(通式 3)，它是以 3.6%存在。



通式 3

因此，这些 GC-MS 和 NMR 数据表明，正如所预料到的那样，将荆芥内酯立体异构体混合物氢化后得到了相应的二氢荆芥内酯非对映体。由顺式,反式-荆芥内酯(起始原料的 84.5 Mol%)衍生而来的非对映体对是主要的二氢荆芥内酯，占氢化后混合物的 78.6%。

实施例 3

二氢荆芥内酯混合物的驱虫活性试验

评价由实施例 2 制备得到的二氢荆芥内酯混合物对于雌性 Aedes aegypti 蚊子的驱虫效果。根据 Insect Control & Research, Inc. (Baltimore, MD)协议完成这些试验。

将大约 250 只雌性 Aedes aegypti 蚊子引入至含有 5 个井孔的容器中，每个井孔用 Baudruche 薄膜罩盖住。井孔中均充满含有柠檬酸钠(用于防止凝结)和 ATP (每 26 ml 血使用 72 mg ATP 二钠盐)的牛血，加热至 37℃。给各薄膜涂覆 25 μl 含有既定量驱虫化学品的异丙醇(表 2)。

表 2

用于测量驱虫活性的试验设计

目的	化合物	浓度
空白对照	异丙醇	100%
阳性对照	异丙醇和 DEET	1.0% (w/v)
试验样本	异丙醇和二氢荆芥内酯	1.0% (w/v)
		2.5% (w/v)
		5.0% (w/v)

5 分钟后，将出生了 4 天的蚊子放入容器中。在 20 分钟内，每隔 2 分钟记录一次那些试探经处理后薄膜的蚊子数目。重复三次，得到全部数据。

表 3 显示了二氢荆芥内酯浓度对于雌性 Aedes aegypti 蚊子首

次试探各薄膜之前所经历时间的影响。

表 3

二氢荆芥内酯浓度对于“首次试探”之前平均时间的影响

驱虫剂浓度	平均时间(分钟)
异丙醇(空白对照)	4.6
1% DEET (阳性对照)	12
1% DHN	8
2.5% DHN	9.3
5% DHN	18

蚊子在 4.6 分钟后开始降落在空白对照井孔上。发现 5%浓度的二氢荆芥内酯阻止蚊子“首次试探”的时间为大约 18 分钟，而 DEET (1% w/v) 为 12 分钟。发现浓度更低的二氢荆芥内酯(1%和 2.5% w/v)阻止首次试探的时间分别平均为 8 和 9.3 分钟。

分析了雌性 Aedes aegypti 蚊子在经二氢荆芥内酯处理过的薄膜上降落密度对时间的分布情况，如图 6 的图表所示。发现 5%浓度的二氢荆芥内酯几乎可在 20 分钟之内避免蚊子降落，而在该试验中 DEET (1% w/v)允许 2 只蚊子降落。另外，发现低浓度的二氢荆芥内酯(1%和 2.5% w/v)也具有驱虫活性(与空白对照相对比)，但是驱虫水平低于阳性对照(1% w/v 的 DEET)。

测量了试验过程中每张薄膜允许降落蚊子的总数目，其结果汇总在表 4 中。

表 4

根据驱虫剂浓度允许降落的数目

驱虫剂浓度	平均降落数
异丙醇(空白对照)	58.99
1% DEET (阳性对照)	4.66
1% DHN	14
2.5% DHN	6.33
5% DHN	0.33

上述数据再次表明所有浓度的试验二氢荆芥内酯均具有驱虫活性，尽管观察到只有在 5% (w/v)时才具有比 1% DEET 显著高的驱虫活性。

最后，这些数据表明以所试验的三种浓度用二氢荆芥内酯处理后，相对于用异丙醇处理后，在试验过程中(20 分钟)显著抑制了对薄膜的试探。在 2.5%和 5.0%浓度下，二氢荆芥内酯在整个观察期间均

具有驱虫活性。在二氢荆芥内酯浓度和第一只蚊子降落并开始试探薄膜所经历的时间之间还存在直接关系。总的来说，上述数据表明这些试验中所使用的二氢荆芥内酯混合物是有效的驱虫剂，尽管只有在高浓度时才观察到与 DEET 相当的驱虫活性。

实施例 4

由反式,顺式-荆芥内酯制备二氢荆芥内酯

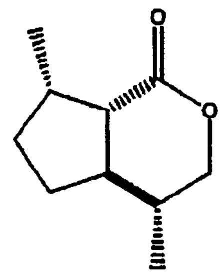
已经表明在驱逐 german 蟑螂中，反式，顺式-荆芥内酯(4aS,7S,7aS 或者 E,Z-荆芥内酯)比顺式,反式-(Z,E-)荆芥内酯或者未经分馏的猫薄荷香精油均更有效(Peterson, C. J.等人. (2002) Household and Structural Insects, 95(2), 377-380)。因此，我们决定将反式,顺式-荆芥内酯进行纯化以氢化成相应的二氢荆芥内酯并对这些衍生物进行驱虫活性试验。许多植物是由猫薄荷 Nepeta racemosa 的种子(奇尔特恩种，坎布里亚郡，UK)长成而来的。将由不同植物采摘而来的树叶浸渍于乙酸乙酯中，2 小时后除去溶剂，提取物用气相色谱分析。其油中主要生成反式,顺式-荆芥内酯的植物由此鉴别出来(Clark, L. J.等人. (1997) The Plant Journal, 11:1387-1393)然后培育成熟。采摘这些植物的叶原料，冷冻干燥，在乙酸乙酯中提取，浓缩提取物。由浓缩提取物中先后经硅胶色谱(己烷/乙酸乙酯(9:1))和使用相同溶剂混合物的制备性薄层色谱将荆芥内酯进行纯化。除去溶剂后，重新溶解于己烷，在干冰上使反式,顺式-荆芥内酯结晶。GC-MS 和 NMR(^{1}H 和 ^{13}C)分析证实该结晶物质为反式,顺式-荆芥内酯。对比于表 1 中的化学位移，该 ^{13}C 化学位移如表 5 所示。

表 5

与反式,顺式-荆芥内酯(表 1)的化学位移相对比，实施例 4 所制备的荆芥内酯样本的 ^{13}C 化学位移

原子	反式,顺式-荆芥内酯	样本
	δ (ppm)	δ (ppm)
a	170.1	170.3
b	135.9	136.0
c	120.4	120.5
d	37.3	37.5
e	49.1	49.3
f	32.1	32.2
g	30.0	30.1
h	26.1	26.3
j	17.5	17.7
i	14.2	14.4

在乙醇中使用 ESCAT # 142 催化剂(Englehart)于 50℃下将上述制备得到的反式,顺式-荆芥内酯氢化 4 小时。GC-MS 和 NMR(^1H 和 ^{13}C)证实反式,顺式-荆芥内酯已经定量转化成相应的二氢荆芥内酯立体异构体,其中一个明显过量.对该主要非对映体进行 NMR 分析: ^1H NMR (500 MHz, $CDCl_3$): d 0.97 (d, 3H, J = 6.28 Hz), 0.98 (d, 3H, J = 6.94 Hz) d 1.24 (m, 2H), 1.74 (m, 1 H), 1.77 (m, 2H), 1.99 (m, 2H), 2.12 (dd, 1H, J = 6.86 和 13.2 Hz), 2.51 (m, 1 H), 3.78 (tr, 1H, J = 11.1 Hz), 4.33 (dd, 1 H, J = 5.73 和 11.32 Hz); ^{13}C (500 MHz, $CDCl_3$): d 15.43, 18.09, 27.95, 30.81, 31.58, 35.70, 42.51, 51.40, 76.18, 172.03. ^{13}C NMR 谱表明该主要非对映体占产物的大约 93.7%. 根据所观察到的亚甲基与内酯氧、携带有甲基的立体异构次甲基、甲基本身以及桥头甲基的偶合，推定该非对映体最可能是通式 4 的(1S, 9S, 5R, 6R)-5, 9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮)。



通式 4

所观察到偶合值与根据 Karplus 方程(参见 Spectrophotometric Identification of Organic Compounds, 第四版, Robert M. Silverstein, G. Clayton Bassler 和 Terence C. Morill, 1981, 第208-210 页)计算得到的上述结构中相邻碳原子上质子之间的二面角是相一致的。

实施例 5

通过氢化反式,顺式-荆芥内酯制得的二氢荆芥内酯的驱虫活性试验

基本上按照实施例 3 所述的那样，测试根据实施例 4 制备得到二氢荆芥内酯(主要由 1S,9S,5R,6R-5,9-二甲基-3-氧杂二环[4.3.0]壬烷-2-酮；通式 4)对于 Aedes aegypti 蚊子的驱虫活性。试验设计汇总在表 6 中，所列出的数据来源于五次重复试验。

表 6

用于测量驱虫活性的试验设计

目的	化合物	浓度
空白对照	异丙醇	100%
阳性对照	异丙醇和 DEET	1.0% (w/v)
试验样本	异丙醇和二氢荆芥内酯	1.0% (w/v)
		0.5% (w/v)
		0.2% (w/v)

表 7 显示了二氢荆芥内酯浓度对于雌性 Aedes aegyptii 蚊子首次试探各薄膜之前所经历时间的影响。

表 7

二氢荆芥内酯浓度对于“首次试探”之前平均时间的影响

驱虫剂浓度	平均时间(分钟)
异丙醇(空白对照)	8.0
1% DEET (阳性对照)	14.8
1% DHN	16.0
0.5% DHN	10.4
0.2% DHN	8.4

发现 1%浓度的二氢荆芥内酯阻止蚊子“首次试探”的时间为大约 16 分钟，比相同浓度的 DEET 略好(但在统计学上不存在差别)，后者首次试探的平均时间是 14.8 分钟。发现浓度更低的二氢荆芥内酯(0.5%和 0.2% w/v)阻止首次试探的时间分别平均为 10.4 和 8.4 分钟。

分析了雌性 Aedes aegypti 蚊子在经二氢荆芥内酯处理过的薄膜上降落密度对时间的分布情况，如图 7 的图表所示。发现 1.0%浓度的二氢荆芥内酯完全可以在 10 分钟之内避免蚊子降落，而 DEET (1% w/v)允许蚊子降落的时间为 8 分钟。另外，发现低浓度的二氢荆芥内酯(0.5%和 0.2% w/v)也具有驱虫活性(与空白对照相对比)，但是驱虫水平低于阳性对照(1% w/v 的 DEET)。

测量了试验过程中每张薄膜允许降落蚊子的总数目，其结果汇总在表 8 中。

表 8

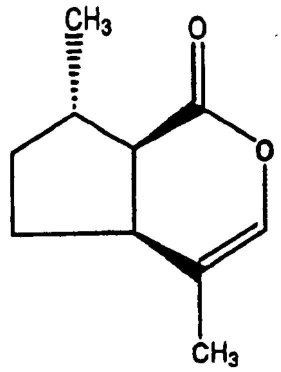
根据驱虫剂和浓度在 2 秒的观察期间内允许降落的数目

驱虫剂浓度	平均降落数
异丙醇(空白对照)	18.17
1% DEET (阳性对照)	4.8
1% DHN	4.0
0.5% DHN	16.2
0.2% DHN	23.2

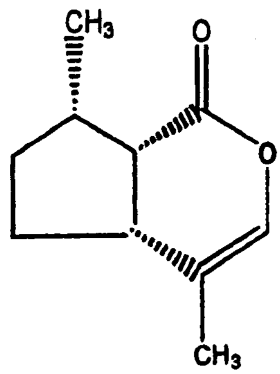
这些数据再次表明 1%浓度的二氢荆芥内酯具有与 1% DEET 相当的驱虫活性。

最后，这些数据表明以所试验的三种浓度用二氢荆芥内酯处理后，相对于用异丙醇处理后，在试验过程中(20 分钟)显著抑制了对薄膜的试探。在所有测试浓度下，二氢荆芥内酯在整个观察期间均具有驱虫活性。在二氢荆芥内酯浓度和第一只蚊子降落并开始试探薄膜之

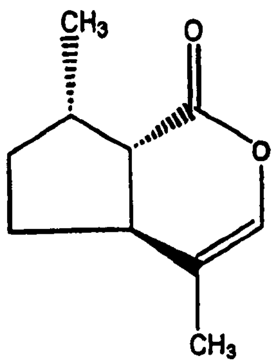
前所经历的时间之间还存在直接关系。总的来说，上述数据表明由氢化反式,顺式-荆芥内酯衍生而来的二氢荆芥内酯是有效的驱虫剂，并且在这些试验中与 DEET 效力相当。



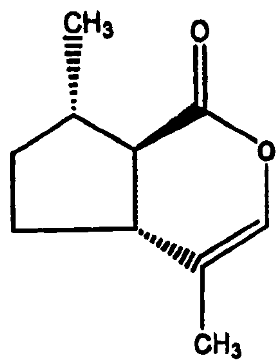
(4a*S*,7*S*,7a*R*) 荆芥内酯
(顺式，反式-荆芥内酯)



(4a*R*,7*S*,7a*S*) 荆芥内酯
(顺式，顺式-荆芥内酯)



(4a*S*,7*S*,7a*S*) 荆芥内酯
(反式，顺式-荆芥内酯)



(4a*R*,7*S*,7a*R*) 荆芥内酯
(反式，反式-荆芥内酯)

图 1

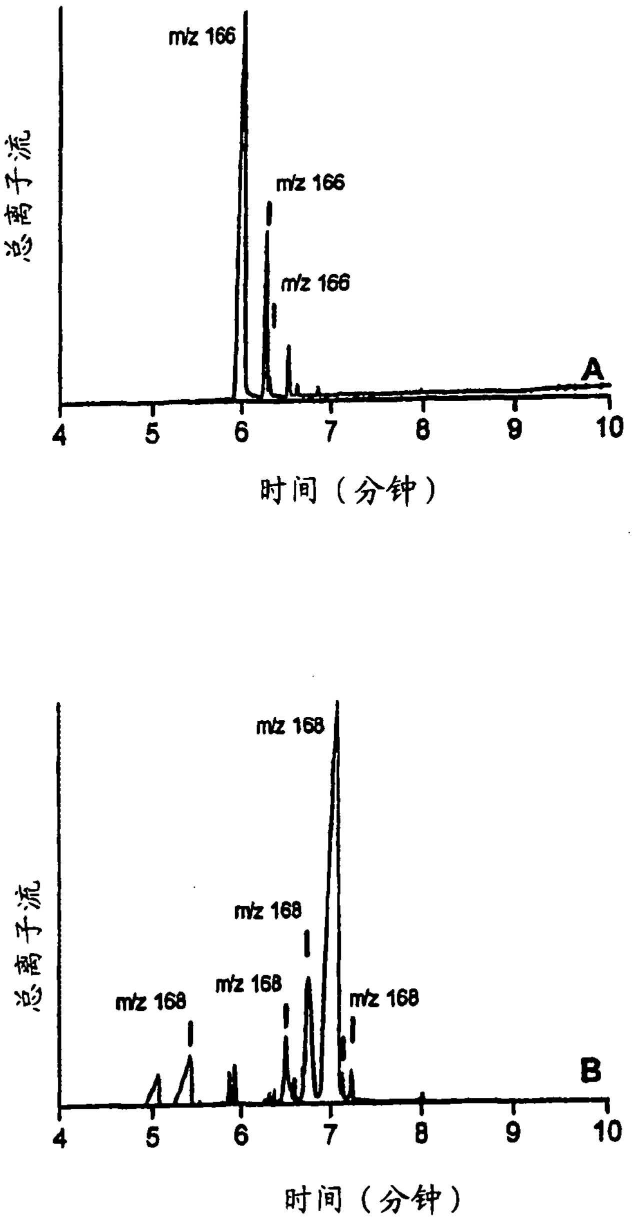

m/z 166
m/z 166
m/z 166
A
总离子流
4 5 6 7 8 9 10
时间（分钟）
m/z 168
m/z 168
m/z 168
m/z 168
m/z 168
B
总离子流
4 5 6 7 8 9 10
时间（分钟）

图 2

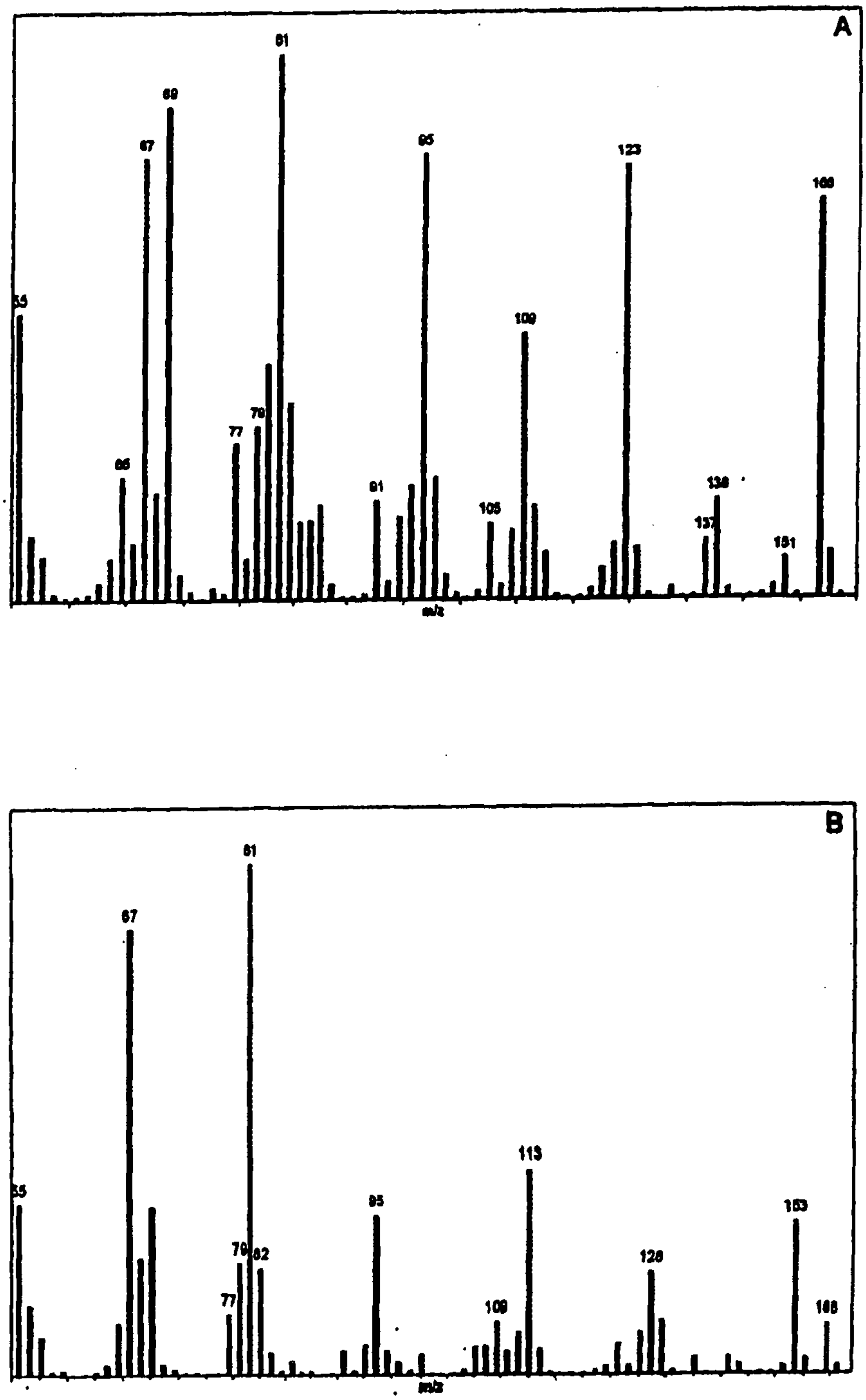

A
55
65
67
69
77
79
81
91
95
105
109
123
m/z
B
55
67
77
79
81
82
95
109
113
128
153
m/z

图 3

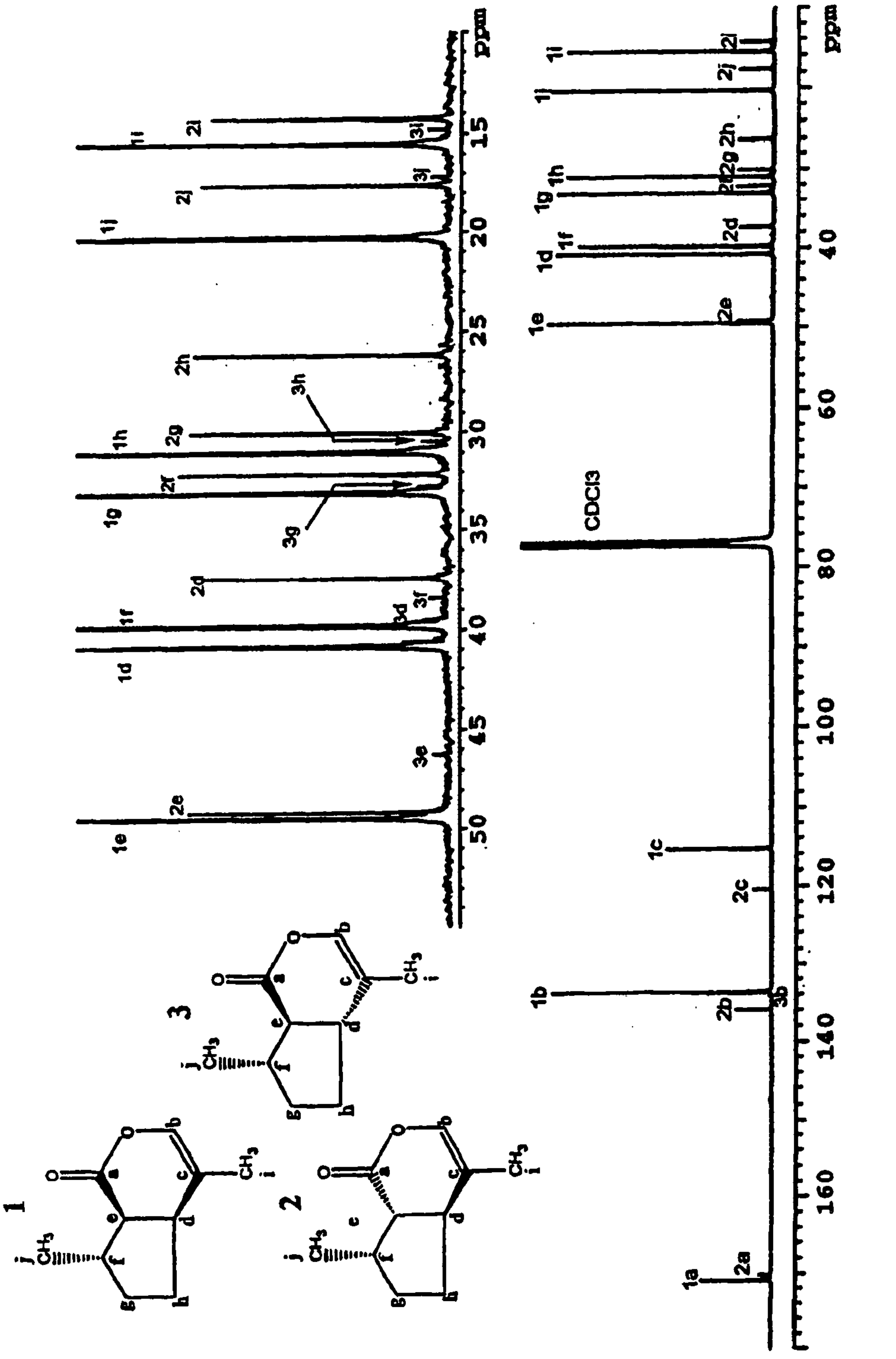


图 4

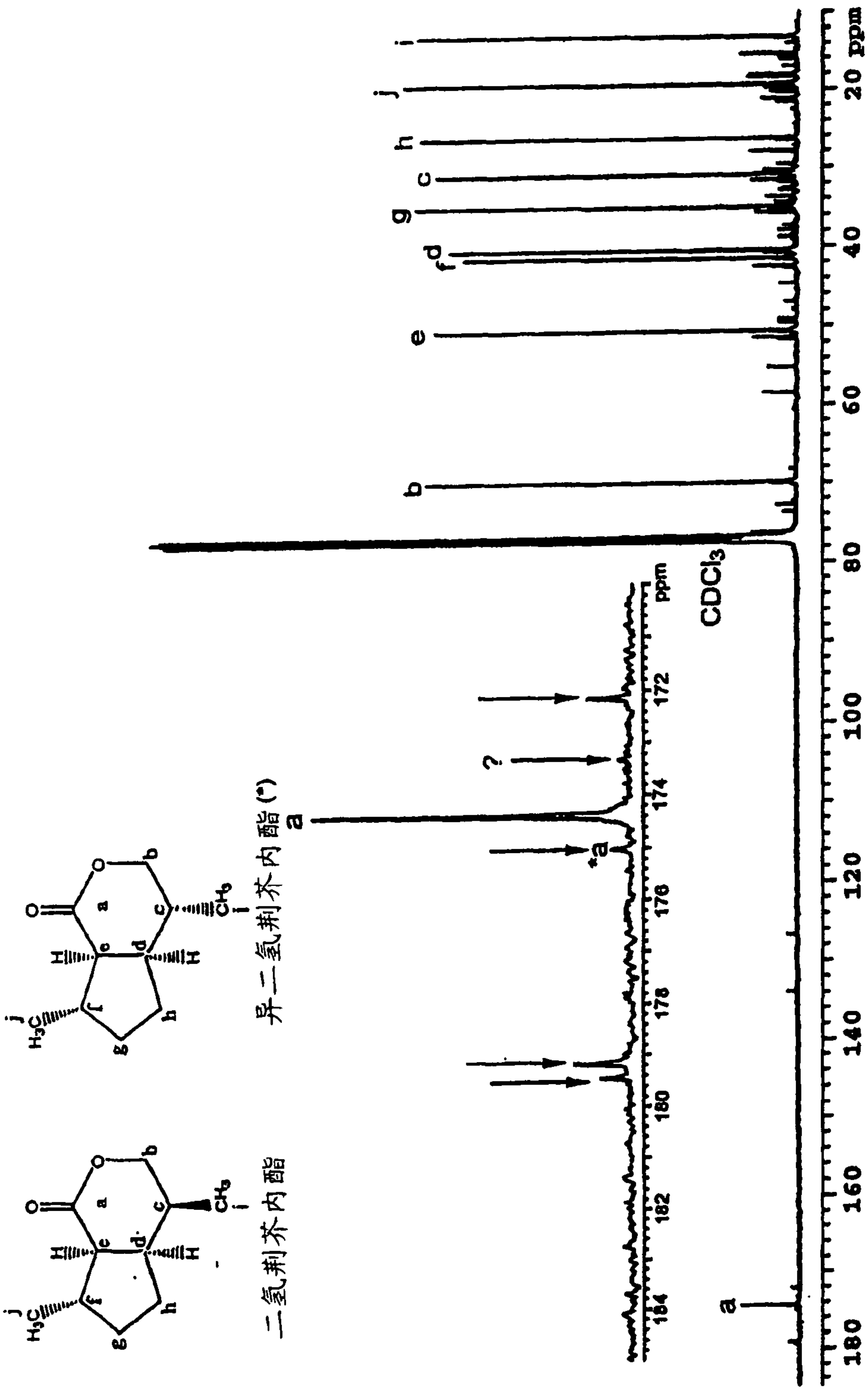


图 5

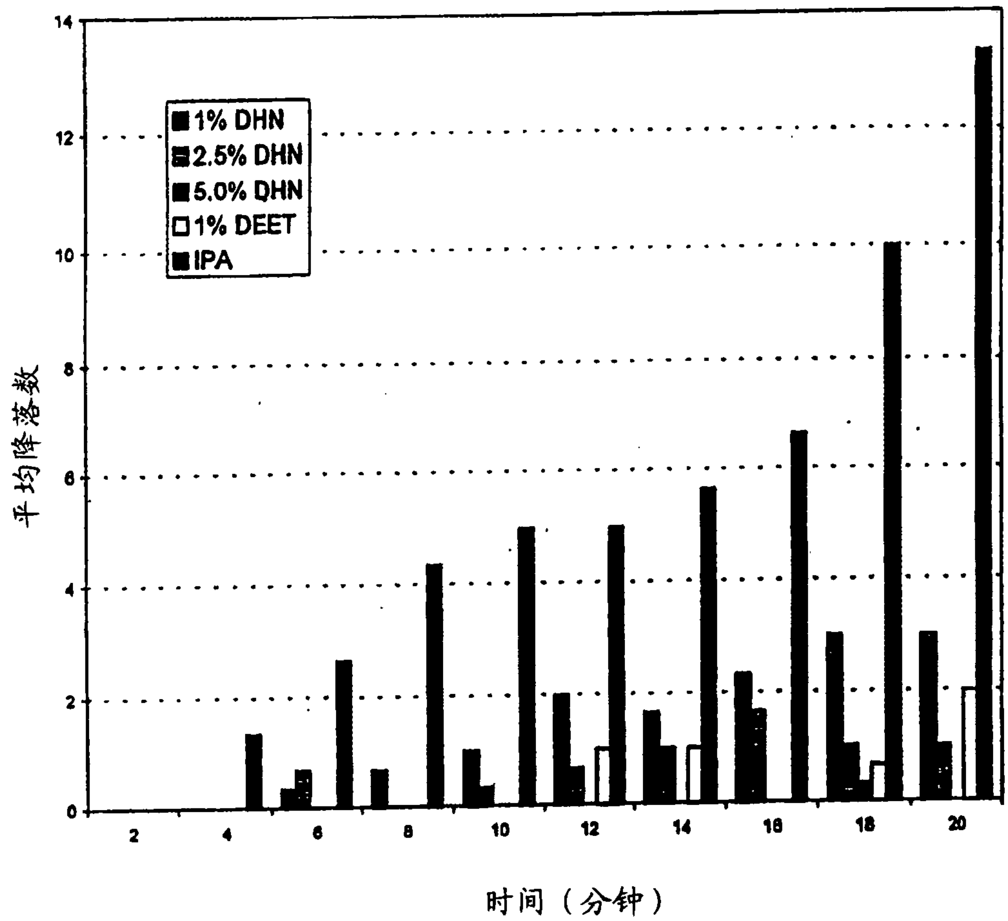


图 6