METHODS OF USING EMU OIL AND ACTIVE FRACTIONS THEREOF AS AN INSECT REPELLENT

TECHNICAL FIELD

This invention is in the field of topical insect repellents. More particularly, effective, natural and safe mosquito repellents comprising emu oil, active fractions of emu oil and compositions comprising emu oil and citronella are provided.

BACKGROUND ART

This invention relates to a method of repelling insects, and more particularly to a method for repelling mosquitoes using a natural ingredient, emu oil and active fractions thereof.

Known natural oils that repel insects include rotundial (from the leaves of *Vitex rotundifolia*, Watanabe K *et al.* (1995) *Biotech Biochem* **59(10)**:1979-1980); citronella oil (*e.g.* U.S. Patent No. 5,346,922); eucalyptus oil (Watanabe *et al.* (1993) *J. Agric. Food Chem.* **41**:2164-2166); neem oil (Sharma VP *et al.* (1993) *J. American Mosquito Control Association* **9(3)**:359-360); and oil of *Hedeoma pulgioides*, oil of anisum and oil of chrysanthemum (U.S. Patent No. 5,208,209).

However, the compound most widely used as a topically-applied insect repellent is N,N-diethyl-m-toluamide (DEET). When applied to children's skin, DEET has been implicated in causing convulsions. DEET is also known to react with certain plastics and synthetic rubber and cause skin irritation (Watanabe *et al.* (1993), *supra*). As a result of these problems and other side effects, New York State had banned products comprised of 100% DEET.

The active fractions of the naturally occurring insect repellents are also largely unknown. Methods of resolving heterogeneous compounds into chemical species are well-known in the art. For example, silica gel flash chromatography provides for high speed resolution of organic compounds (see, e.g., U.S. Patent No. 4,293,422). After separation, the eluted fractions can be recovered and tested for the activity of interest.

Accordingly, there remains a need for a natural, safe substance which acts to repel biting insects when topically applied to the skin. The active fraction(s) of such a substance is also needed.

SUMMARY OF THE INVENTION

The present invention provides a method for repelling biting insects comprising the step of topically applying emu oil, active fractions of emu oil or compositions comprising emu oil and citronella to the skin of a subject.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the number of mosquitoes present on filter paper treated with water, sucrose or sucrose and diluted emu oil (sample 776) at 2.5 minute intervals. The solid triangles show water-treated filter paper controls. The open squares show sucrose-treated filter paper. The solid squares show sucrose-treated paper overlaid with undiluted sample 776. The open triangles show sucrose-treated paper overlaid with a 50% dilution of sample 776. The open circles show sucrose-treated paper overlaid with sample 776 diluted to 25%.

Figure 2 shows the number of mosquitoes present on filter paper treated with water, sucrose, or sucrose and fractions of sample 776 at 5 minute intervals. The solid squares show water-treated paper. The open squares show sucrose-treated paper overlaid. The solid triangles show sucrose-treated paper overlaid with a sample 776. The open triangles show sucrose-treated paper overlaid with fraction F1. The solid circles show sucrose-treated paper overlaid with fraction F2.

Figure 3 shows the ^{1}H NMR spectrum of the F1 fraction of emu oil.

Figure 4 shows the ^{1}H NMR spectrum of the F2 fraction of emu oil.

Figure 5 shows the average number of mosquitoes biting test limbs per ten minute intervals..

BEST MODE FOR CARRYING OUT THE INVENTION

Throughout this application, various publications, patents and published patent applications are referred to by an identifying citation. The disclosures of these publications, patents and published patent applications are hereby incorporated by

reference into this application to more fully describe the state of the art to which this invention pertains.

The present invention provides a method of repelling biting insects using emu oil, a natural and safe substance. In a preferred embodiment, pure emu oil is applied to the skin. In another preferred embodiment, diluted emu oil is topically applied. In yet another preferred embodiment, an active fraction of emu oil is applied to the skin. In a further preferred embodiment, a composition comprising emu oil and citronella is applied to the skin.

The following examples are presented as a further guide to the practitioner of ordinary skill in the art, and are not to be construed as limiting the invention in any way.

Examples

Example 1:

The effect of emu oil on frequency of mosquito lands and bites

To determine if emu oil was an effective mosquito repellent, pure emu oil (Zoogen, Inc., Davis, Ca) was applied to one hand of a volunteer. The other hand was left untreated. Each hand was placed in a nylon mesh cage containing mosquitoes (*Aedes aegypti*) and the number of mosquitoes which landed and/or bit in 30 seconds was recorded. The experiment was performed in duplicate. Results of the experiments were averaged and are summarized in Table 1.

Table 1

	emu-oil treated hand (lands/bites)	untreated hand (lands/bites)
Test 1	0/0	11/11
Test 2	1/0	26/26

These results demonstrate that topically applied emu oil is an effective mosquito repellent. It greatly reduces the number of mosquitoes which land, and completely eliminates biting.

Example 2

The effectiveness of emu oil as a mosquito repellent over time

To determine how long topically applied emu oil maintains efficacy as a mosquito repellent, the treated hand was exposed to a cage of mosquitoes at 15, 30 and 60 minutes after application. The number of lands and bites were compared at each time point with the untreated hand. Results from duplicate experiments were averaged and are presented in Table 2.

Table 2

Time after application	Number of lands on emu-oil treated hand	Number of lands on untreated hand
15	4	18
30	2	12
60	10	12

These results show that emu oil remains an effective mosquito repellent for at least 30 minutes.

Example 3

The effectiveness of diluted emu oil

To determine the effectiveness of diluted emu oil, the emu oil was diluted with ethyl acetate to a fixed percentage, applied to one hand and inserted into a mosquito cage. The number of lands were recorded. The experiments were performed in duplicate at each dilution level. Results are shown in Table 3.

Table 3

Percent emu oil	Number of lands Exp't 1	Number of lands Exp't 2	Average Number of lands
0	10	9	9.5
0.50	10	10	10
1.0	5	5	5
5.0	6	4	5
10.0	4	5	4.5
25.0	2	1	1.5
50.0	1	1	1
75.0	1	1	1
100.0	0	0	0

These results demonstrate that dilute amounts of emu oil effectively repel mosquitoes. At a dilution as low as 1%, emu oil reduces by one-half the number of mosquitoes which land. At 25% emu oil, the number of mosquito lands drops to one-tenth of lands on an untreated hand. Thus, emu oil is an effective insect repellent at a concentration of 1% or higher.

Example 4

Fractionation of emu oil and the effectiveness of the fractions

An 850 mg sample (776) of emu oil was steam-distilled and fractionated using silica flash chromatography (Baker silica gel, 40 μm), essentially as described in Still *et al.* (1978) *J. Organic Chem.* 43:2923. Two major components of the sample were eluted from the column with 100% hexane and 25% ethyl acetate/hexane. Fractions were analyzed by thin layer chromatography (TLC) on silica plates developed with 50% hexane/ethyl acetate. Components on the TLC plates were observed by exposing the plates to UV light (indicating UV chromophores) and spraying plates with vanillin/sulfuric acid (indicating the presence of higher alcohols, sterols, phenols or essential oils). 430 mg of a clear oil, termed F2, was found to be UV active and reactive to vanillin/sulfuric acid. The second component, 380 mg of a pale yellow oil termed F1, was not UV active and did not stain

with vanillin. F1 and F2 fractions were analyzed by ^{1}H NMR (300 MHz, $CDCl_3$) as shown in Figures 3 and 4.

A mosquito repellent bioassay was performed by treating wedges of filter paper with sucrose overlaid with either an aliquot of the crude sample 776, F1 or F2. Filter paper treated with water or sucrose served as controls. Samples of F1 and F2 were tested at full-strength or diluted with corn oil to 50% or 25% of full-strength. At regular time intervals, the number of mosquitoes which landed and fed on the wedges of filter paper was recorded. Results are shown in Figures 1 and 2.

As shown in Figure 1, even diluted to 25% of full-strength, the crude sample (776) greatly reduces the number of mosquitoes landing on the sucrose paper. In addition, Figure 2 shows that both the F1 and F2 fractions of sample 776 at full-strength and diluted to 50% or 25% of full-strength were effective in repelling mosquitoes when compared to the sucrose-treated control.

Example 5

The effect of emu oil as a tick repellent

To determine if emu oil was an effective tick repellent, a test subject's hands were treated with emu oil while the fingers of the hand were left untreated. As a positive control, Ultrathon (3M, Minneapolis, MN) was applied to the hand and the fingers were left untreated. An untreated hand was used as a negative control. Unfed nymphal Western Black-legged ticks were placed on the fingers of the hands and observed as they climbed toward the treated or untreated skin of the hand. Ticks crossing onto the treated skin were scored as "crossing." Those not crossing were scored as "repelled." Ticks were removed after a single score was recorded. Repellency is calculated as the proportion of all trials in which a tick is repelled. For example, 8 repels in 10 trials provides a repellency of 80%. In this study, each subject tested a tick at 15 minute intervals for 2 hours and 15 minutes. The results are shown below:

Negative control - untreated skin -	0% repellency
Positive control Ultrathon (3M) -	70% repellency
Emu oil -	40% repellency

There was no indication that the repellency declined over the two hour test period.

Example 6

The effect of a composition of emu oil and citronella on frequency of mosquito bites

To determine if citronella would enhance the mosquito repelling effect of emu oil, the repellent activity of emu oil alone and a composition comprising emu oil and citronella (30 drops of 100% oil of citronella/25 mL of pure emu oil) were compared to a positive control, Ultrathion® (3M, Minneapolis, MN, approximately 31% DEET). Oil of citronella, *Cymbopagon nardus*, was obtained from Aura Cacia, Weaverville, CA.

Testing was conducted in the Florida Keys, namely Big Pine Key and Little Pine Key. During testing, the air temperature was 24–26°C, with clear skies and slight wind. *Aedes taeniorhynchus* accounted for >99% of mosquitoes that approached or attacked subjects.

In the first study, three subjects applied pure emu oil, one subject applied Ultrathon, and two subjects served as negative controls. In the second study, oil of citronella was added to the emu oil. Three subjects applied the composition comprising emu oil and citronella, two subjects applied Ultrathon, and two subjects served as negative controls. Six of the participants were employees of the Monroe County, Florida, Mosquito Control Service. Treatments were evenly divided among leg and arm surfaces. The surface areas of treated surfaces were calculated for each subject in advance of the application. The test materials were applied at dosage rate of approximately 3 mL/650 square centimeters of surface area.

Test subjects counted and recorded bites in a series of 10 minute periods. Counts were recorded on data sheets. In the first study, the testing period was two hours, with 12 consecutive 10 minute recording periods. In the second study, the test was interrupted during two brief windy periods by the need to move to more productive locales (*i.e.*, places with higher biting rates) within the test sites. As a result, the total testing period including the interruption was two hours and twenty-five minutes.

Ambient biting rates were measured throughout the study by the subjects with untreated control limbs. The average ambient biting rates were sufficient for testing in both studies and ranged between 17 and 70 bites per 10 minute interval (mean = 38.5; see Figure 5).

Both emu oil and the positive control, Ultrathon, repelled mosquitoes over the test periods. A composition comprising emu oil and oil of citronella was particularly effective as a repellent (see Figure 5). The repellent properties of emu oil alone reduced the number of bites to an average of 5.7 bites per ten minutes. The composition comprising emu oil and citronella approximated the positive control, Ultrathon, with 0.8 bites per ten minutes for the emu oil/citronella composition compared to 0.2 bites per ten minutes for Ultrathion. Over the testing period, emu oil, a composition comprising emu oil and citronella and Ultrathon substantially reduced biting rates from *Aedes taeniorhynchus*. A composition comprising emu oil and citronella was much more effective than emu oil alone and offers protection comparable to that offered by mosquito repellents registered by the U.S. Environmental Protection Agency. No pattern of diminishing repellency over time was observed for emu oil and citronella.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations and conditions without departing from the spirit and scope of the invention and without undue experimentation. While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows the scope of the appended claim.

CLAIMS

What is claimed is:

1. A method for repelling biting insects comprising the step of topically applying emu oil to the skin of a subject.

2. A method for repelling biting insects comprising the step of topically applying to the skin of a subject a fraction of emu oil obtained by flash chromatography having the nuclear magnetic resonance (NMR) spectrum of Figure 3 and that is ultra violet light active, reactive to vanillin/sulfuric acid.

3. The method of claim 2 wherein the emu oil fraction is diluted.

4. A method for repelling biting insects comprising the step of topically applying to the skin of a subject a fraction of emu oil obtained by flash chromatography having the nuclear magnetic resonance (NMR) spectrum of Figure 4 and that is not ultra violet light active and non reactive with vanillin/sulfuric acid.

5. The method of claim 4 wherein the emu oil fraction is diluted.

6. A compound consisting of a flash chromatography fraction of emu oil having the NMR spectrum of Figure 3, that is ultra violet light active and that is reactive to vanillin/sulfuric acid.

7. A compound consisting of a flash chromatography fraction of emu oil having the NMR spectrum of Figure 4, that is not ultra violet light active and that is not reactive to vanillin/sulfuric acid.

8. A composition for repelling biting insect comprising emu oil and citronella.

9. The composition of claim 8, wherein the composition comprises about 30 drops of 100% oil of citronella per 25 mL of pure emu oil.

10. A method for repelling biting insects comprising the step of topically applying a composition comprising emu oil and citronella to the skin of a subject.

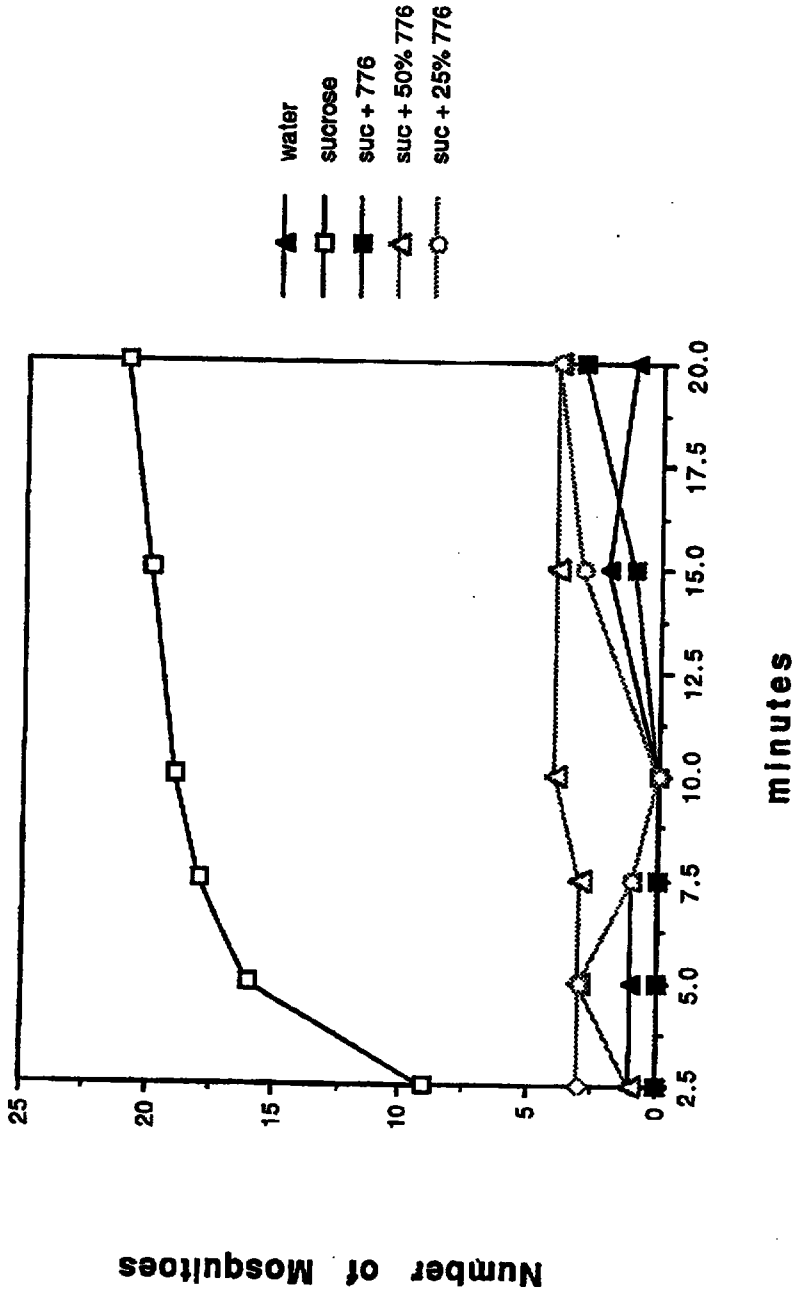

water
sucrose
suc + 776
suc + 50% 776
suc + 25% 776
Number of Mosquitoes
0
5
10
15
20
25
minutes
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

FIGURE 1

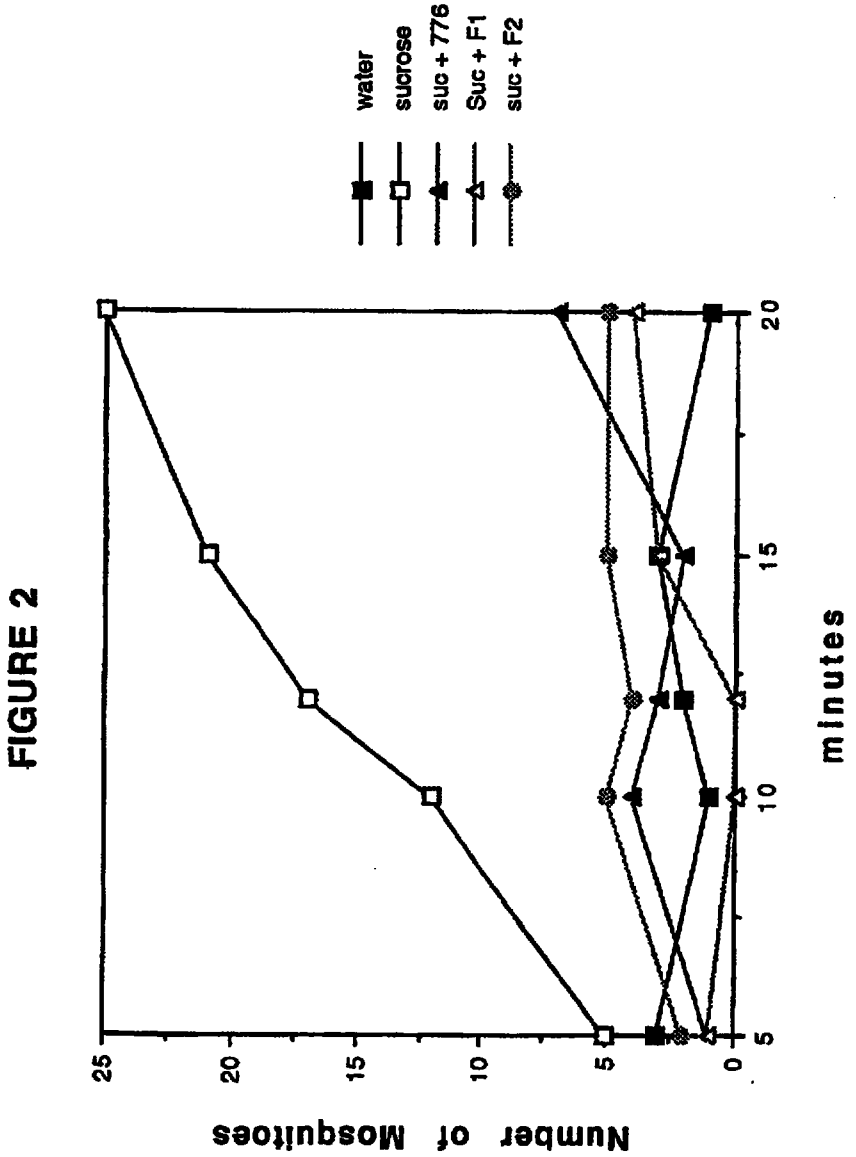

water
sucrose
suc + 776
Suc + F1
suc + F2
Number of Mosquitoes
0
5
10
15
20
25
minutes
5
10
15
20

FIGURE 2

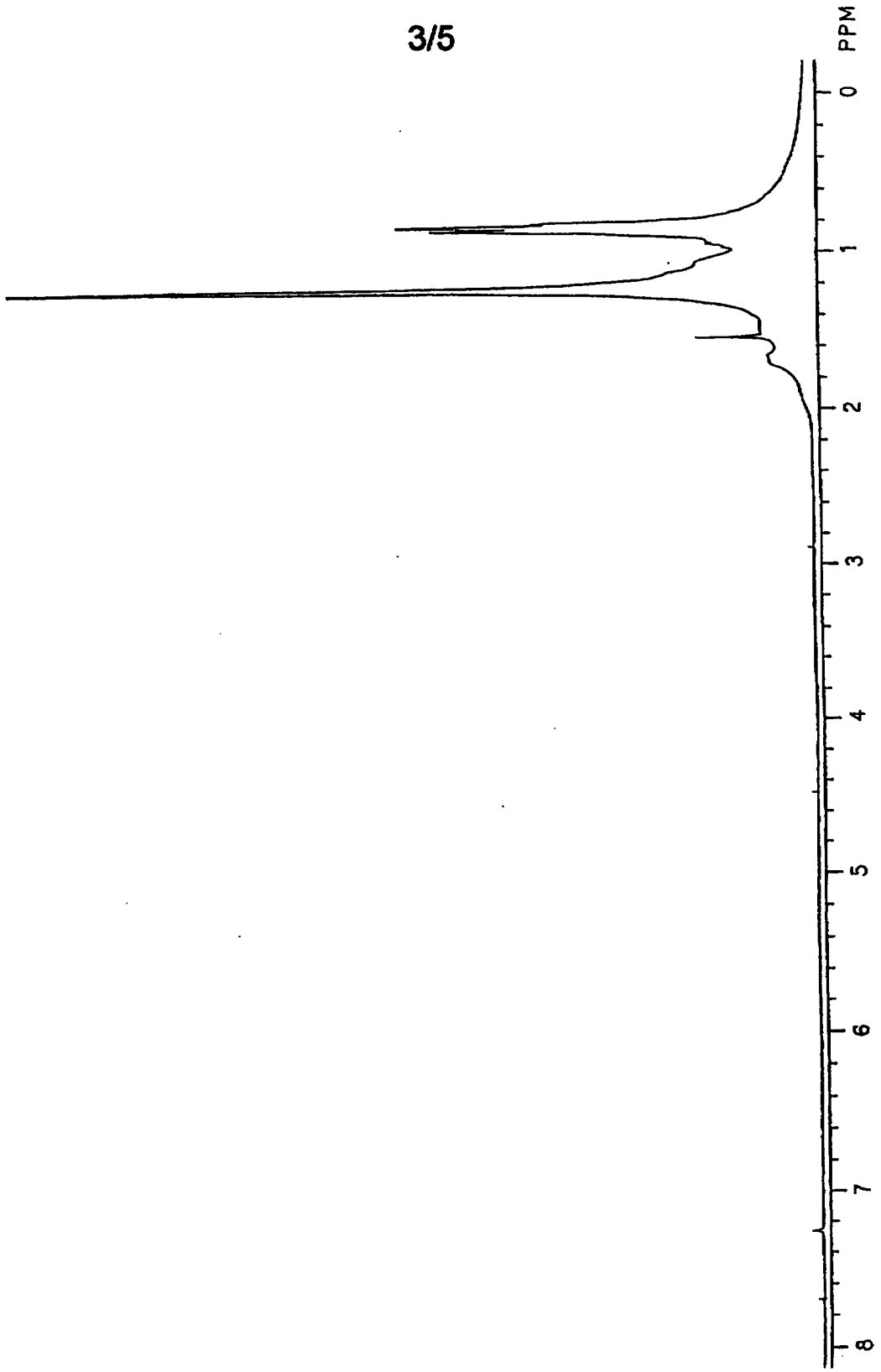


FIGURE 3. ^{1}H NMR spectrum of 776 F1

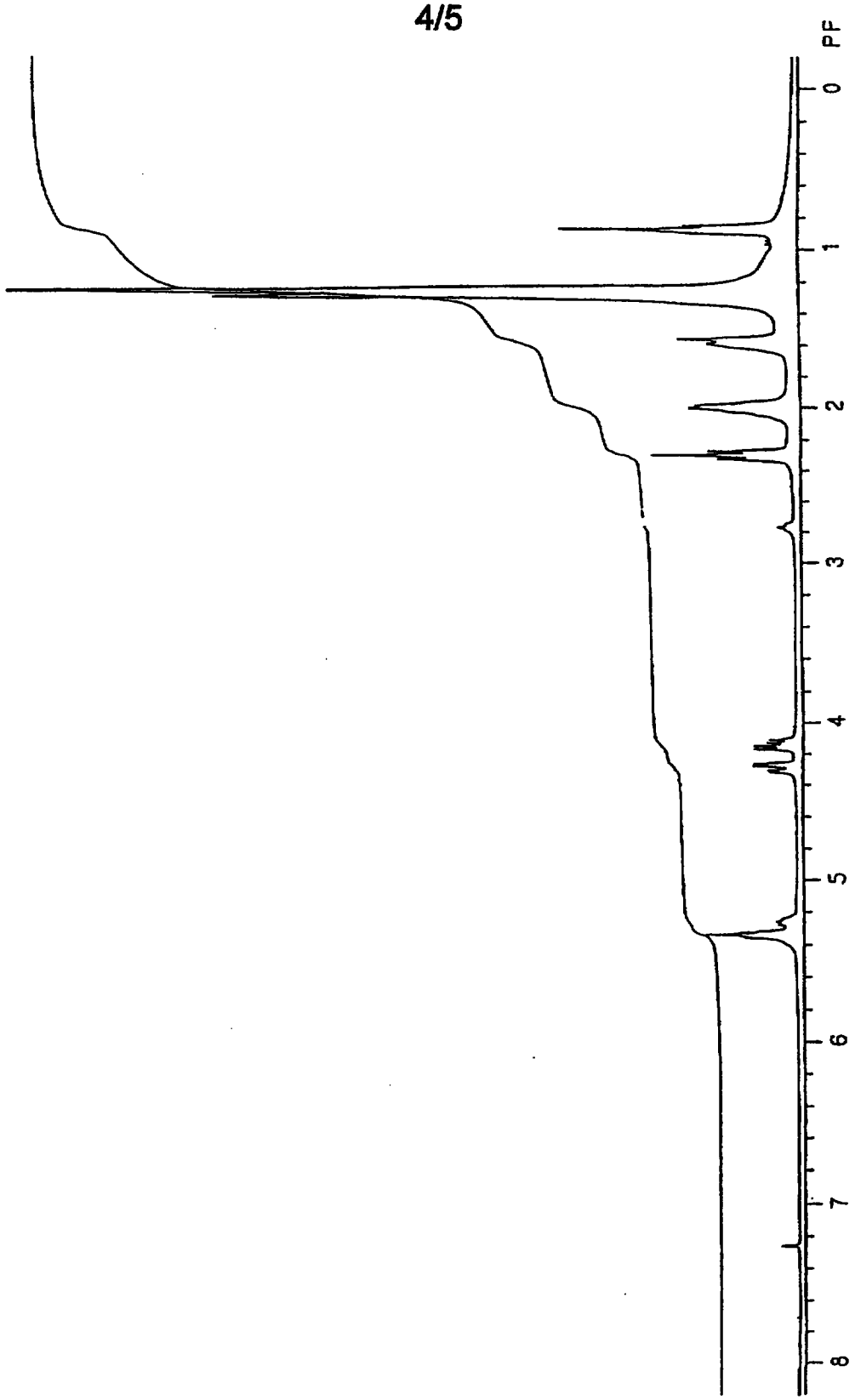


FIGURE 4. ^{1}H NMR spectrum of 776 F2

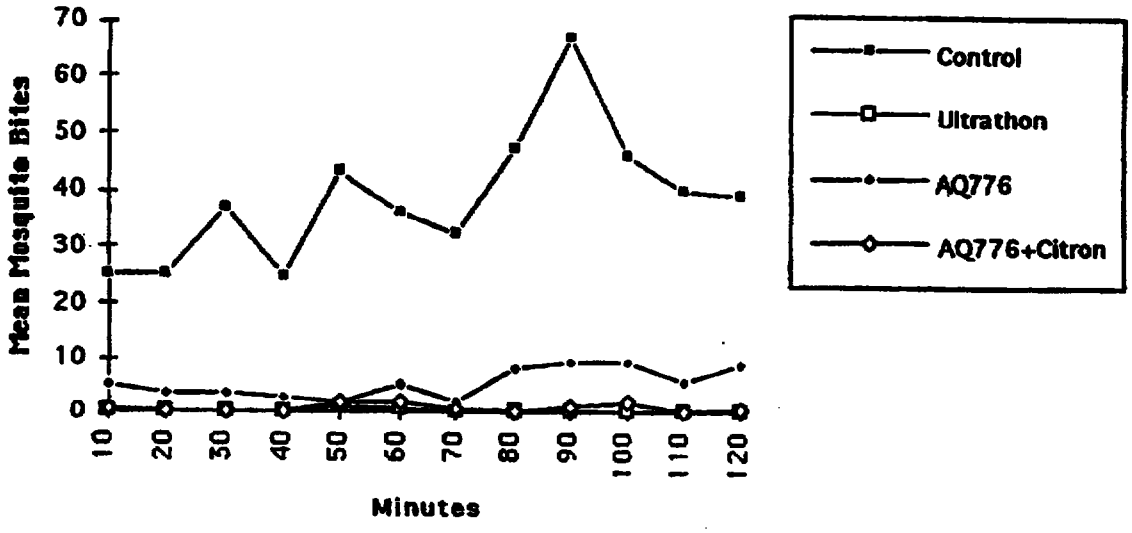

Mean Mosquito Bites
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
Minutes
Control
Ultrathon
AQ776
AQ776+Citron

Figure 5

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(57) Abstract

This invention provides a method for repelling biting insects such as mosquitoes by topically applying to the skin of a subject emu oil, fractions of emu oil obtained by flash chromatography or a composition comprising emu oil and citronella. Also provided are methods for repelling biting insects by topically applying diluted fractions of emu oil. The invention also provides a compound useful in repelling biting insects having the NMR spectrum of Figure 3, that is ultraviolet light sensitive and is reactive to vanillin/sulfuric acid. Also provided are compounds useful in repelling biting insects having the NMR spectrum of Figure 4, that is not ultraviolet light sensitive and is not reactive to vanillin/sulfuric acid. A composition for repelling biting insects comprising emu oil and citronella is further provided.