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Behavior, Chemical Ecology

Chirality Effects on Repellent Properties of 4-AlkoxyCoumarins Against Asian Tiger Mosquito (Diptera: Culicidae)

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Abstract

The use of semiochemicals as repellents and attractants has been proposed to complement insecticides used for the control of vector mosquito populations. In several studies, the optical purities of the molecules tested have been described as having little or no effect on repellent activity. However, these observations seem difficult to explain because of the chirality effect of molecules on the olfactory system of insects and humans. Thus, the purpose of this study is to assess the effects of chirality on the repellent properties of 4-alkoxy coumarins against *Aedes albopictus* Skuse, mosquito vector of arboviruses. We report here that the racemic (*R/S*)-4-*sec*-butoxycoumarin had the highest repellent effect (Repellent Index = 49.9%) followed by (*R*) enantiomer (Repellent Index = 24.2%) for the dose of 5 mg/mL. Contrary, no significant repellent activity was recorded for *S*-(+)-4-*sec*-butoxycoumarin. This experiment demonstrates the close relationship between the molecules' optical purities and the behavioral response of mosquitoes.

Key words: chirality, repellent, Asian tiger mosquito, 4-alkoxy coumarin, synthesis

Many infectious disease-causing agents are transmitted to humans by hematophagous arthropods, insects in particular. The most dangerous and deadly diseases in the world are due to mosquitoes (Rodhain 1996) such as *Anopheles*, *Culex*, and *Aedes* which are the main vectors of malaria, arboviruses (dengue, Zika, chikungunya), and lymphatic filariasis. In order to protect human and livestock health, mosquito vector populations have been controlled by the use of insecticides for decades (WHO Expert Committee on Insecticides and World Health Organization 1970). This control process is effective, economically beneficial, but has a strong ecological impact (Bolzonella et al. 2019). However, it has been observed that as the number of insecticides of greater effectiveness and selectivity grows, the resistance within the

vector population increases limiting their long-term use (Hemingway et al. 2004). Thus, the development of alternatives for integrated vector management is strongly encouraged by WHO (WHO 2017).

Mosquitoes communicate and interact closely with their environment through volatile compounds called semiochemicals (kairomones, pheromones, and allomones). Therefore, the use of these molecules in monitoring mosquito vector populations seems to be a credible alternative or complement to insecticides (Cook et al. 2007, Watentena and Okoye 2019, Andrianjafy et al. 2020, Wooding et al. 2020). This approach has received relatively little attention from the organic synthesis chemistry community, although it could become an important research field for sustainable development.

For years, several molecules have been widely used as topical mosquito repellents (DEET, Picaridin, etc.) despite the short duration of their effectiveness and their harmful effects (Miller 1982, Roland et al. 1985, Curtis et al. 1987). More recently, attractants have been used for the preparation of traps to attract specifically mosquito vector species (Weinzierl et al. 2005). Some coumarin derivatives were described as selective attractants on malaria vectors such as *Anopheles gambiae* s.l., *Anopheles mascarensis*, and others showed both, either attractant or repellent effects towards *Aedes albopictus* and *Culex quinquefasciatus* depending on their structure (Chauvin and Mentzer 1952, Andrianjafy et al. 2018). Delaveau et al. (2012) have demonstrated that the *R*- and *S*-enantiomers of 4-*sec*-butoxycoumarin showed an effect as a repellent on *Drosophila* as its racemic mixture (Delaveau et al. 2012). Similarly, the four isomers of *p*-menthane-3,8-diols (PMD) behaved in the same way towards *An. gambiae* s.s (Barasa et al. 2002). The four diastereoisomers of picaridin had different repulsion indices values on mosquitoes with a biting proportion of 0.18 for the best (1*R*, 2'*S*) and 0.44 for the worst (1*S*, 2'*S*) compared to those of racemate (0.22) and control (0.72) (Natarajan et al. 2005). Finally, stereo-isomers of dihydronepetalactone presented the same repellent effect on *Anopheles albimanus* (Feaster et al. 2009). The diversity of these results reflects the complexity of the relationship between chirality and the olfactory system of insects, particularly for kairomones (attractants) (Brenna et al. 2003). For example, the *R*-enantiomer of 1-octen-3-ol was proven to be eight times more attractive than the (*S*) enantiomer towards various Culicidae (Harrup et al. 2012).

We report the synthesis of the pure enantiomers of 4-*sec*-butoxycoumarin to assess their repellent effects against *Aedes albopictus* using two kinds of bioassays to determine the dose/response curve for each enantiomer. The first one uses a cage system device requiring a longer duration (24 h) to better reproduce the conditions “*in natura*” (Andrianjafy et al. 2018), the second one is a tunnel olfactometer allowing rapid evaluation with short test duration inspired by wind tunnel (Geier et al. 1999). The results obtained will contribute to understanding the relationship between molecular structure and the behavioral response of mosquitoes in the field of chemical ecology.

Materials and Methods

Chemical Synthesis

All reagents, solvents, and chemicals were used directly as obtained commercially. Proton (¹H) and carbon (¹³C) NMR spectra were recorded on Bruker spectrometer (300 MHz both for ¹H and ¹³C) using deuterated chloroform (CDCl₃). Thin-layer chromatography (TLC) was done on Merck precoated silica gel plates (with fluorescence indicator UV_{254 nm}) using ethyl acetate/n-hexane as solvent system. Column chromatography was carried out on silica gel 60 (230–400 mesh ASTM) with ethyl acetate/n-hexane as solvent at various proportions specified in the corresponding experiment. A Stuart melting point apparatus SMP3 was used to determine the melting point of solid products. Optical rotation was measured by a Bellingham & Stanley ADP 220 polarimeter at 25°C with a 0.5% dilution in EtOH.

General Procedure for The Synthesis of (*R/S*)-4-*sec*-butoxycoumarin and 4-(1-ethylpropoxy)coumarin Called 4-(3-pentoxycoumarin

Phase transfer catalysis (PTC) and acid catalysis (Scheme 1) were used for the synthesis of racemic 4-*sec*-butoxycoumarin and 4-(3-pentoxycoumarin.

(Refer to [supplementary materials](#)) Thin-layer chromatography was done to examine the conversion and the purity of the modified compounds for all reactions and ¹H and ¹³C NMR analyses were performed to determine the structure of the obtained compounds.

Phase Transfer Catalysis

Nine hundred and seventy-two milligrams of 4-hydroxycoumarin (6 mmol) was mixed with 2-bromo-alkane (36 mmol) in 45 mL of toluene. K₂CO₃ (6 equiv) and tetrabutylammonium hydrogen sulfate (0.05 equiv) were added. The reaction medium was refluxed in an oil bath at 120 °C for 6 h. After, the mixture was filtrated and evaporated. Purification on silica gel chromatography (30 g) using AcOEt/Hexane (90/10, v/v) as a solvent, afforded the expected compound.

Acid Catalysis

Seven hundred and forty milligrams of 4-hydroxycoumarin (4.5 mmol, 1 equiv) was dissolved in 4 mL of 2-butanol (6 equiv). Then, 0.5 mL to 2 mL of H₂SO₄ concentrated was added. The mixture was heated at 65 °C to 70°C for 65 h to 144 h before being neutralized with 1 N NaOH and evaporated. Chromatography analysis on silica gel gave the expected compound. (*R/S*)-4-*sec*-butoxycoumarin or (*R/S*)-4-(1-methylpropoxy) coumarin White solid; yield 67% (496 mg, 2.3 mmol), melting point 56°C.

General Procedure for The Synthesis of *R*-(-)-4-*sec*-butoxycoumarin and *S*-(+)-4-*sec*-butoxycoumarin

The two enantiomers were obtained by Mitsunobu Reaction and acid catalysis (Scheme 2) (See [supplementary materials](#)). To have quickly the two stereoisomers, we chose the Mitsunobu Reaction which is known for its high efficiency and selectivity among methods leading to a selective inversion of hydroxyl groups stereochemistry (Ahn et al. 2002). But taking into account the price and the ecological impact of the Mitsunobu reagents, we have synthesized the pure enantiomers by acid catalysis. Thin-layer chromatography was done to examine the conversion and the purity of the modified compounds for all reactions and ¹H and ¹³C NMR analyses were performed to determine the structure of the obtained compounds.

Mitsunobu Reaction

Two grams of 4-hydroxycoumarin (12.3 mmol, 1 equiv) and 6.45 g of triphenylphosphine (24.6 mmol 2 equiv) were dissolved in dichloromethane (30 mL). The corresponding alcohol (2.4 mL, 2 equiv) was added. Then, diethylazodicarboxylate (DEAD, 4.2 mL 2.2 equiv) was introduced dropwise at room temperature. The reaction mixture was stirred at room temperature for 24 h, was filtrated, and evaporated. The concentrated filtrate was chromatographed on silica gel using Hexane/AcOEt (90/10, v/v).

Acid Catalysis

Seven hundred and forty milligrams of 4-hydroxycoumarin (4.5 mmol, 1 equiv) was dissolved in 4 mL of chiral alcohol (6 equiv). Then 0.5 mL to 2 mL of H₂SO₄ concentrated was added. The mixture was heated at 65°C to 70°C for 65 h to 144 h before being neutralized with 1 N NaOH and evaporated. Chromatography analysis on silica gel gave the expected compound. When using (*R*) or (*S*) enantiomer, excess of butanol was isolated and its specific rotation was determined and compared to the starting material to study the potential recycling of this product.

Biological Evaluation

Mosquitoes

Larvae and pupae of *Ae. albopictus* were collected from the zoo of Tsimbazaza (S18°55'42.31"; E47°31'38.23") in Antananarivo Madagascar and then brought back to the insectary of the International Associated Laboratory located in the Ampasapito Campus for rearing. The mosquitoes were reared in a controlled insectary at 26°C, ±70% RH, and a 12 h photo phase. Specimens collected with breeding site water were fed every 3 d with dog cookies powder (Tetramin®). After, pupae were collected into cups and placed in a cage for the emergence of adults. Adults of mosquitoes were kept in a gauze cage (35 cm × 35 cm × 35 cm) and provided with a diet of 6% sucrose solution on cotton. Females were all intended for the test and a new collection campaign was done regularly to have wild strains.

Cage System Olfactometer

This device was already described previously (Andrianjafy et al. 2017). Briefly, the test procedures are as follows: 25 female mosquitoes, 4–12 d old, were placed in a release cage and fasted there for 1 h. This cage was then placed inside a test cage called also test cage and the mosquitoes were left there for 1 h so that they could acclimatize to their new environment. Two traps including a control (with a filter paper impregnated with pure ethanol) and a treated (with a filter paper impregnated with the product) were placed inside the test cage, on two opposite sides. These traps are spaced 1.45 m apart. The location of the traps was systematically reversed and the mosquitoes were replaced with new individuals for each testing session. The test was launched by slowly opening the release cage. The

duration of the test was 24 h. After each test, the traps were removed and the number of mosquitoes in each trap was counted. To prevent the build-up of odors, the test room was ventilated intensively for a few hours and the mosquito nets were changed and cleaned. Eight repetitions were carried out for each concentration of products to be tested. These steps are summarized in Fig. 1.

Tunnel Olfactometer

This olfactometer (Fig. 2) consists of a long square glass tube (7 cm side × 1.20 m long). The tube is fitted with three openings: two on either side on both ends, allowing the introduction of impregnated papers (control and treated) and an opening in the middle for the introduction of the specimens to be tested. The two ends are closed with a cover. The olfactometer is divided into three equal compartments: a neutral or non-exposed compartment in the middle, a test compartment at one end with product-impregnated paper, and a control compartment at the other end with control paper. Fifteen nonblood-fed females of *Ae. albopictus*, 5–12 d old, were introduced into the olfactometer in the neutral zone by the opening in the middle. Barriers were first placed to keep mosquitoes in this area for 10 min (adaptation time). Then, after the introduction of product-impregnated filter papers and ethanol, the barriers were opened. The total test time was 20 min. Three repetitions were performed for each product. The results were recorded every 5 min during the 20 min of the test.

Products Tested

The following products: (*R/S*)-4-*sec*-butoxycoumarin, *R*-(-)-4-*sec*-butoxycoumarin, *S*-(+)-4-*sec*-butoxycoumarin and 4-(3-pentoxo) coumarin were tested at 5 different doses: 2.5 mg/mL, 5 mg/mL,

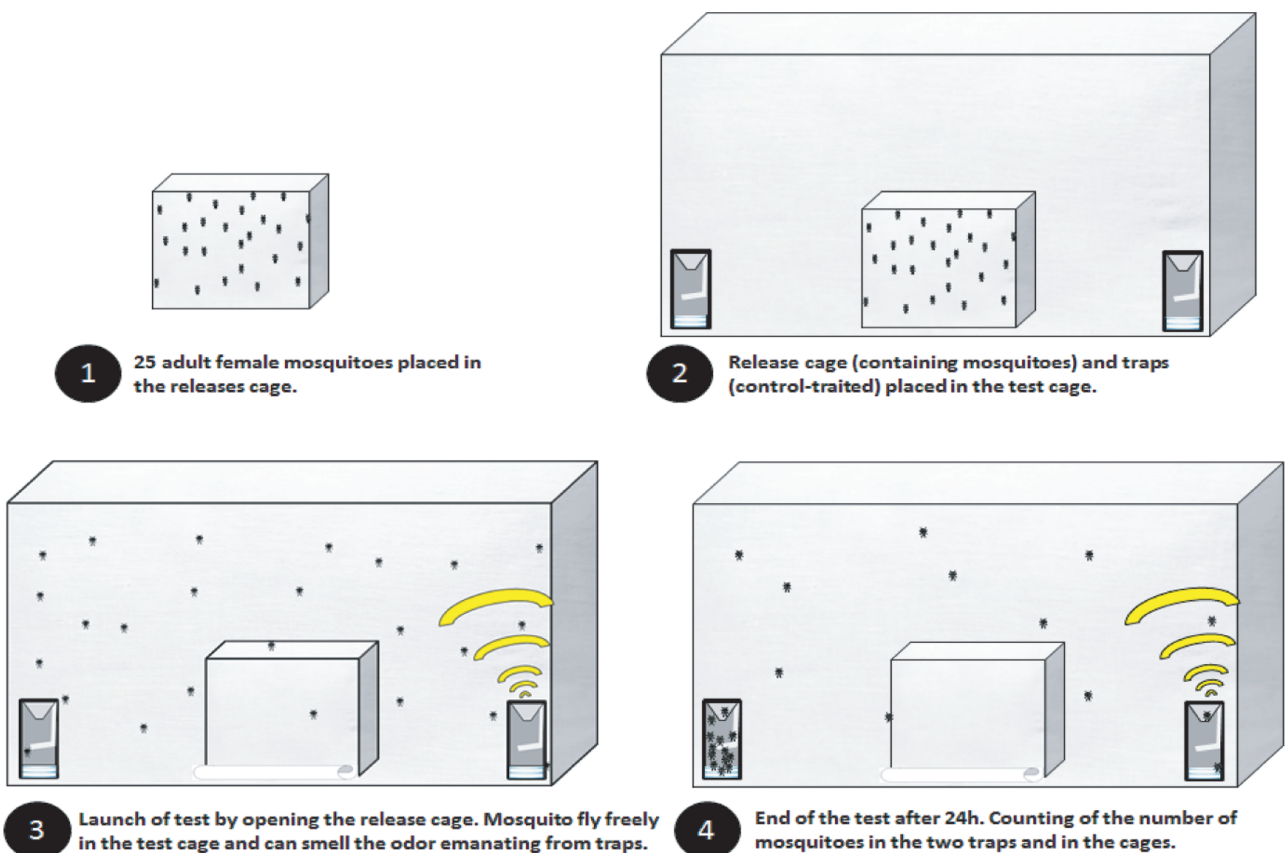


Fig. 1. Testing process of the system cage olfactometer.

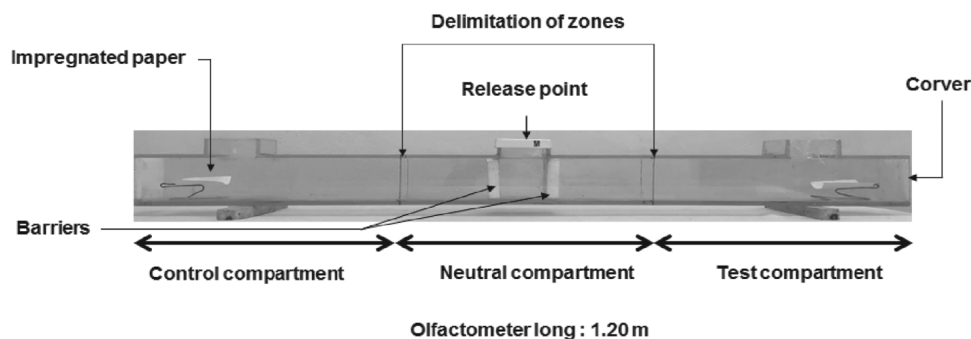


Fig. 2. The tunnel olfactometer.

10 mg/mL, 30 mg/mL and 60 mg/mL. N,N-Diethyl-3-methylbenzamide (DEET) known as leader repellent were tested for reference.

Statistical Analysis

The highlighted values are the activity index (AI) and the repellent index (RI). The activity index is the percentage of mosquitoes trapped in the two trap bottles compared to all mosquitoes used in the test. The results are not taken into account if the value of this index is less than 30% because these values indicate very low activity of the selected mosquitoes and are generally due to daily weather changes (heavy rain, storm, etc.) which could affect mosquito behavior in the insectary.

$$AI (\%) = \left(\frac{\text{Number of mosquitoes in the control trap} + \text{Number of mosquitoes in the baited trap}}{\text{Total number of mosquitoes}} \right) \times 100$$

The repellent index (RI) is the percentage of the difference of mosquitoes in the control trap (T) and the baited trap (P) divided by the total number of mosquitoes in both traps. When the repellent index is negative the product is an attractant and has a kairomone effect.

$$RI (\%) = \left(\frac{\text{Number of mosquitoes in the control trap} - \text{Number of mosquitoes in the baited trap}}{\text{Total number of mosquitoes in both traps}} \right) \times 100$$

AI: Activity Index

RI: Repellent Index

Analysis of data and figures were done by using GraphPad Prism 8.4.2 Software, LLC. This program made it possible to analyze by *t-test* the means of two groups (treatment and control), for paired samples. Analysis of variance by ANOVA was carried out to assess the variation in behavioral responses of mosquitoes between the different doses of products. The confidence interval was chosen at 95%. The evaluation of the reliability of the interpretations was also carried out by studying respectively the standard errors (\pm SE) and the standard deviations (\pm SD) illustrated in the graphs and the tables.

Results

Chemistry

Three synthesis pathways: phase transfer catalysis, acid catalysis and Mitsunobu Reaction were used giving a good yield of coumarins. The reaction with PTC produced a better yield with 72%

of (*R/S*)-4-*sec*-butoxycoumarin with spectra NMR ^1H (300 MHz, CDCl_3) δ 7.76 (*d*, 1H), 7.47 (*t*, 1H), 7.23 (*d*, 1H), 7.19 (*t*, 1H), 5.58 (*s*, 1H), 4.42 (*m*, 1H), 1.70 (*m*, 2H), 1.35 (*d*, 3H), 0.95 (*t*, 3H); NMR ^{13}C (300 MHz, CDCl_3) δ 164.55, 163.28, 153.48, 132.27, 123.77, 123.24, 116.79, 116.2, 90.64, 77.07, 28.8, 18.64, 9.57 ppm and 68% of 4-(3-pentoxycoumarin with spectra NMR ^1H (300 MHz, CDCl_3) δ 7.84 (*d*, 1H), 7.55 (*t*, 1H), 7.28 (*d*, 1H), 7.28 (*t*, 1H), 5.65 (*s*, 1H), 4.32 (*m*, 1H), 1.67 (*m*, 2H), 1.67 (*m*, 2H), 0.99 (*t*, 3H), 0.99 (*t*, 3H); NMR ^{13}C (300 MHz, CDCl_3) δ 165.14, 163.29, 153.48, 132.26, 123.81, 123.12, 116.722, 116.25, 90.6, 82.09, 25.68, 25.06, 9.44, 9.42 ppm (See [supplementary materials](#)). The amount of (*R/S*)-4-*sec*-butoxycoumarin formed by acid catalysis was obtained with 67% yield (Table 1).

The two enantiomers (*R*)- and (*S*)-4-*sec*-butoxycoumarin with spectra identical to that of racemate were synthesized using two different methods: Mitsunobu Reaction and acid catalysis. The conversion rates of enantiomers from racemic 4-*sec*-butoxycoumarin were different. The yields of (*R*)- and (*S*)-4-*sec*-butoxycoumarin isolated by Mitsunobu were 51% and 53%, respectively. Acid catalysis gave 29% of *R*-(-)-4-*sec*-butoxycoumarin and 19% of *S*-(+)-4-*sec*-butoxycoumarin with an $[\alpha]_D^{25} = -40$ for (*R*) enantiomer and $[\alpha]_D^{25} = +40$ for (*S*) enantiomer (Table 2).

Biological Evaluation

In general, mosquito activity is defined as the percentage of the number of individuals in both traps (control and treated) divided by the total number of mosquitoes tested ranged from 51% to 70%.

From the Cage System Olfactometer

(*R/S*)-4-*sec*-butoxycoumarin

Statistically, significant differences ($P < 0.05$) were observed between the average number of *Ae. albopictus* recorded in the control and baited trap for the different doses of (*R/S*)-4-*sec*-butoxycoumarin. The average number of mosquitoes recorded in the baited trap was significantly lower than that recorded in the control trap. Analysis of the variance showed a significant difference for the repellent effect on the mosquito for the different doses of the (*R/S*)-4-*sec*-butoxycoumarin tested ($F = 9.192$, $P < 0.05$). The repellent effect increased with the amount used (of the order of RI = 39% to 89.5%). For 5 and 10 mg/mL; RI was about 48%. The effect observed for the 2.5 mg/mL dose was the lowest with RI = 39%. The dose of 60 mg/mL was the most repellent (RI = 89.5%); at this dose, the activity index (AI = 57.9%) is high enough to take into account the results obtained. These results are summarized in Table 3.

Table 1. Yields of coumarins ether synthesis by phase transfer and acid catalysis with physical characteristics of synthesized products

Entry catalysis	Alkylating agent	Product	Isolated yield	Physical characteristics
1 PTC	2-bromobutane	<p style="text-align: center;">Phase transfer catalysis</p> <p style="text-align: center;">4-alkoxycoumarin</p>	72%	58°C White solid
2 PTC	3-bromopentane	<p style="text-align: center;">Acid Catalysis Mitsunobu Reaction</p> <p style="text-align: center;">R(-)-4-sec-butoxycoumarin</p>	68%	Pale yellow oil
3 Acid catalysis	2-butanol	<p style="text-align: center;">Acid Catalysis Phase transfer catalysis</p> <p style="text-align: center;">4-alkoxycoumarin</p>	67%	58°C White solid

Table 2. Yields of pure enantiomer of 4-sec-butoxycoumarin synthesis by Mitsunobu and acid catalysis with physical characteristics of synthesized products

Entry method	Entry alcohol	Product	Isolated yield	Physical characteristics	Specific rotation
Mitsunobu reaction	4 (S)-butan-2-ol	R(-)-4-sec-butoxycoumarin	51%	51°C White solid	-40
	5 (R)-butan-2-ol	S-(+)-4-sec-butoxycoumarin	53%	51°C White solid	+40
Acid catalysis	6 (R)-butan-2-ol	R(-)-4-sec-butoxycoumarin	29%	51°C White solid	-40
	7 (S)-butan-2-ol	S-(+)-4-sec-butoxycoumarin	19%	51°C White solid	+40

R(-)-4-sec-butoxycoumarin

Significant differences were observed between the average number of individuals in control and baited traps ($P < 0.05$). The number of mosquitoes trapped in the treatments was lower than that in the controls. Analysis by ANOVA showed also a significant difference for the repellent effect on the mosquito for the different doses of the product ($F = 14.373$, $P < 0.05$). The activity index observed during the tests varied around 60% so the results obtained are valid. At a dose of 2.5 and 5 mg/mL of R(-)-4-sec-butoxycoumarin, the repellent effect was

lower compared to that of racemic with RI = 10.7% and 24.9%, respectively. From the 10 mg/mL dose, an increase in the effect was observed. The 60 mg/mL dose was the most repellent ($P < 0.0001$) with a repellent index equal to 51.6%. These results are summarized in Table 4.

S-(+)-4-sec-butoxycoumarin

The test results showed an activity index value of more than 50%, except for the 10 mg/mL dose (45.3%). A significant difference between the average number of mosquitoes in baited and control traps

Table 3. Comparison by *t*-test of the average number of individuals in control and baited traps at different doses of racemic (*R/S*)-4-*sec*-butoxycoumarin in cage system olfactometer

Dose	Baited (\pm SD)	Control (\pm SD)	t	<i>P</i> -value*	<i>Df</i>	AI (%)	RI (%)
2.5 mg/mL	4.5 \pm 0.5	10.3 \pm 0.4	-15.06	<0.0001	6	53.6	39
5 mg/mL	3.3 \pm 0.8	9.5 \pm 1	-7.77	0.00023	6	51	49.9
10 mg/mL	4.3 \pm 1.1	12 \pm 1.3	-9.71	< 0.0001	14	64	47.5
30 mg/mL	2.3 \pm 0.8	13 \pm 2	-7.49	0.00023	6	61	70.2
60 mg/mL	0.8 \pm 0.8	14 \pm 1	-13.99	< 0.0001	6	57.9	89.5

**P*-value at confidence interval $P < 0.05$ by *t*-test with number of replicates, $n = 4$ and the total number of mosquitoes tested $N = 100$
SD, standard deviation; *Df*, degree of freedom; AI, Activity index of mosquitoes; RI, repellent index

Table 4. Comparison by *t*-test of the average number of individuals in control and baited traps at different doses of *R*(-)-4-*sec*-butoxycoumarin in cage system olfactometer

Dose	Baited (\pm SD)	Control (\pm SD)	t	<i>P</i> -value*	<i>Df</i>	AI (%)	RI (%)
2.5 mg/mL	7.5 \pm 2.2	14 \pm 2	-1.86	0.0084	14	54	10.7
5 mg/mL	5.9 \pm 1.6	9.4 \pm 1.5	-3.42	0.004	14	61	24.2
10 mg/mL	4.5 \pm 0.5	12.5 \pm 0.8	-16.56	< 0.0001	10	67	46.4
30 mg/mL	3.8 \pm 1	11 \pm 1.3	-9.11	< 0.0001	14	59	50.2
60 mg/mL	3.8 \pm 1	11 \pm 1.8	-8.43	< 0.0001	14	56	51.6

**P*-value at confidence interval $P < 0.05$ by *t*-test with number of replicates, $n = 8$ and the total number of mosquitoes tested $N = 200$
SD, standard deviation; *Df*, degree of freedom; AI, Activity index of mosquitoes; RI, repellent index

was observed for the 2.5 and 10 mg/mL doses ($P < 0.05$, $Df = 10$). Although, for the other doses (5 mg/mL, 30 mg/mL, 60 mg/mL), there were no noticeable variations between the two traps ($P > 0.05$, $Df = 14$). The same results were obtained by comparing effects at different doses ($F = 5.408$, $P > 0.05$) using ANOVA. At low doses (2.5 mg/mL and 5 mg/mL), the repellent index of *S*(+)-4-*sec*-Butoxycoumarin was equal to 6%. The highest value was recorded at 10 mg/mL (RI = 18.4 %). A non-constant variation of the effects or even contradictory effect were observed at high doses; a minor attractant effect with RI = -0.2% at 30 mg/mL dose and slight repellent effect (RI = 11%) at 60 mg/mL dose. In summary, the (*S*) enantiomer was inactive against *Ae. albopictus* at the different doses tested (Table 5).

The curves in Fig. 1 relate the effects of each enantiomer compared to the racemic mixture of 4-*sec*-butoxycoumarin. At the same doses, the racemic is the most repellent. The effect can be termed "dose-dependent" because it increases with the dose for the racemic and (*R*) enantiomer unlike the (*S*) enantiomer which has no significant effect against *Ae. albopictus* (Fig. 3). This meets our objective to study the effect of chirality on mosquito behavior.

Bioassays using cage system olfactometer lasting 24 h and traps baited by the two enantiomers (*R*) and (*S*), at 5 mg/mL dose each, on separate supports were carried out to assess an eventual synergistic effect. Statistically, a significant difference was found comparing the variance of products repellent effect against mosquitoes ($F = 6.414$, $P > 0.012$) (Table 6). A weaker effect (RI = 36%) than that of racemic (10 mg/mL) on single support (RI = 48.8%) was recorded for the (*R*) and (*S*) enantiomers (5 mg/mL dose of each).

The comparison of (*R/S*)-4-*sec*-butoxycoumarin and DEET effect showed that (*R/S*)-4-*sec*-butoxycoumarin was less repulsive than DEET with RI values equal to 39% and 62% respectively at 2.5 mg dose and to 48% versus 71% at 10 mg dose. But at a high dose (60 mg), the repellent index of (*R/S*)-4-*sec*-butoxycoumarin increases to RI = 89% whereas that of DEET remains stable with RI = 72% (Fig. 4).

4-(3-pentoxo)coumarin

The average number of mosquitoes in control and baited traps by different doses of 4-(3-pentoxo)coumarin was measured. Statistically, significant differences were observed except for 5 mg/mL dose ($P = 1$). At 1 and 2.5 mg/mL doses, mosquitoes in control trap were remarkably low compared to those in baited trap. Conversely, at 10 to 60 mg/mL doses, the value obtained in control trap became higher than in baited trap (Table 7).

Repellent indexes of 4-(3-pentoxo)coumarin at different doses were measured. A significant difference was detected ($F = 131.083$, $P < 0.0001$). We observed a similar S curve for the dose-response relationship (Fig. 5). At low 1 and 2.5 mg/mL doses, an attractant effect was recorded with RI = -58.5 % and -46%, respectively. Then, the 5 mg/mL dose exhibited no effect (RI = 0.5 %). The remaining doses (10 to 60 mg/mL) showed a repellent effect with RI > 55%. The maximum repellency index (69.1%) which was relatively important was attributed to the 60 mg/mL dose. So, we can deduce 4-(3-pentoxo)coumarin, in particular, can be both a potential repellent at high concentration and an effective kairomone (attractant) at low concentration.

From The Tunnel Olfactometer

Repellent effects of racemic and pure enantiomers of 4-*sec*-butoxycoumarin were evaluated at the dose of 5 mg/mL using tunnel olfactometer. High activity indexes of mosquitoes were obtained during the experiment (over 80 %). Statistical studies showed a significant difference between the repellent indices of the three products ($F = 30.07$; $P = 0.001$) (Table 8). The racemic and the *R*(-)-4-*sec*-butoxycoumarin had almost similar repellent index values (around RI = 48 %). The *S*(+)-4-*sec*-butoxycoumarin exhibited a lower repellent index with RI = 6.9%.

Discussion

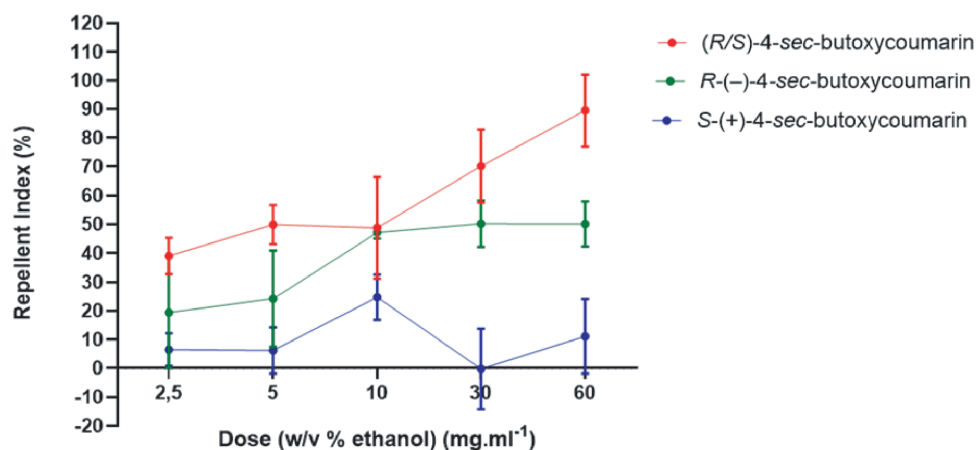
More than 600 pharmaceutical or chemical processes use phase transfer catalysis. These commercial applications are justified by

Table 5. Comparison by *t*-test of the average number of individuals in control and baited traps at different doses of *S*-(+)-4-*sec*-butoxycoumarin in cage system olfactometer

Dose	Baited (\pm SD)	Control (\pm SD)	t	<i>P</i> -value*	Df	AI (%)	RI (%)
2.5 mg/mL	7.5 \pm 2.2	14 \pm 2	-2.183	0.047	14	68.5	6.5
5 mg/mL	5.9 \pm 1.6	9.4 \pm 1.5	-1.821	NS	14	70.5	6.2
10 mg/mL	4.5 \pm 0.5	12.5 \pm 0.8	-2.924	0.015	10	45.3	18.4
30 mg/mL	3.8 \pm 1	11 \pm 1.3	0	NS	14	67	-0.2
60 mg/mL	3.8 \pm 1	11 \pm 1.8	0	NS	14	58.5	11.1

**P*-value at confidence interval $P < 0.05$ by *t*-test with number of replicates, $n = 8$ and the total number of mosquitoes tested $N = 20$

NS indicates no significant difference; SD, standard deviation; Df, degree of freedom; AI, Activity index of mosquitoes; RI, repellent index

**Fig. 3.** Repellent effect of (R/S), S-(+) and R-(-)-4-*sec*-butoxycoumarin against *Aedes albopictus* at different doses (Repellent Index \pm SE; test at the 95% confidence interval).

the overall efficiency of the method in terms of simplicity and reaction rate (Freedman 1986, Albanese et al. 1999, Makosza 2000, Tan and Yasuda 2015). By the same technique, we synthesized some compounds and tested them for their attractant and repellent properties against the widespread mosquito vector of human and animal disease, *Ae. albopictus*. The (R/S)-4-(1-methylpropoxy) coumarin known as (R/S)-4-*sec*-butoxycoumarin in common name and 4-(1-ethylpropoxy)coumarin cited as 4-(3-pentoxycoumarin in this paper were obtained using acid catalysis by substituting the hydroxyl group on electron-rich aromatic ring. This reaction is described for a very long time (Davis 1900) and was re-evaluated a few years ago (Cazorla et al. 2011) for its efficiency and selectivity in many cases. The additional advantage of this method lies in its ecological impact: a better atom economy. Indeed, a molecule of water is produced as a by-product; the excess alcohol and catalyst can be separated and recycled. In the case of (R/S)-4-*sec*-butoxycoumarin, we have also observed the formation of 2-hydroxyacetophenone due to the decarboxylation of the starting product. Optimization of product yield was carried out by controlling temperature, reaction time, and acid concentration. The best condition limited the formation of 2-hydroxyacetophenone to less than 10% and gave 67% of pure (R/S)-4-*sec*-butoxycoumarin, similar to that obtained with PTC (Table 1).

The two enantiomers (R) and (S) of 4-*sec*-butoxycoumarin were obtained either by Mitsunobu Reaction (with inversion of the asymmetric carbon) or by acid catalysis (with retention of configuration) and had the same physicochemical properties. Yields and reaction rate obtained with the former are higher than those of the latter. However, transformation by acid catalysis gave pure enantiomers with a little amount of by-product and needed cheap and less toxic

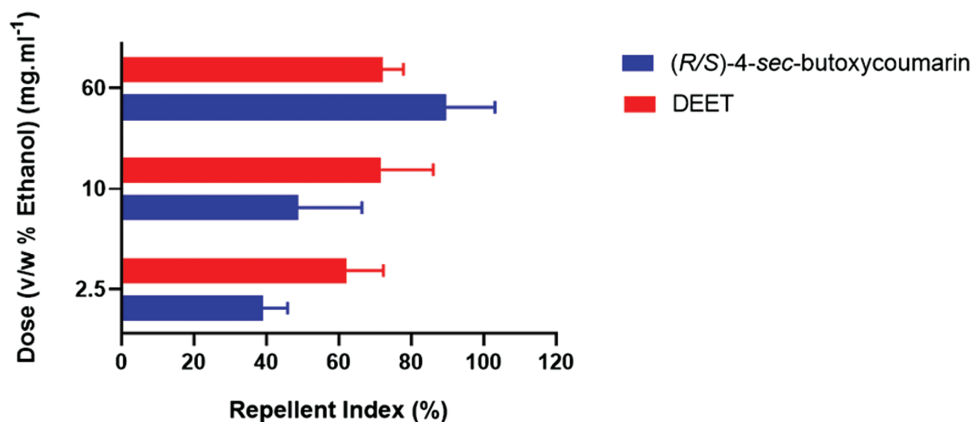
reagents (butanol as solvent versus methylene chloride). Moreover, the excess of alcohol could be recovered. Synthesis reaction using chiral *sec*-butanol had a better stereoselectivity (little or no racemization). Regarding the ecological impact of the two processes, synthesis of (R) and (S) of 4-*sec*-butoxycoumarin by Mitsunobu Reaction exhibited a low atom economy (32.9%) compared with that of acid catalysis (92.4%). Even if we consider the yield difference; it is better with acid catalysis (18.5 %) and Mitsunobu (10.5%). After recycling and analyzing the excess reagent from acid catalysis reaction, we obtained (R)-butan-2-ol and (S)-butan-2-ol with optical rotation values of -12 and $+12$, respectively. They are similar to those of the starting material ($[\alpha]_D^{25}$ neat = -12.5 and $+12.5$). This indicates a performant recovering of the reagent. Until now, we have demonstrated that acid catalysis is a useful and ecological method to synthesize aromatic ether from electron-rich substrates but less efficient in terms of reaction rate. We are looking for another activation process to improve reaction efficiency.

The results obtained that the repellent effect of the racemic and the (R) enantiomer increased with the dose of the tested product. But this is not the case for the (S) enantiomer. The (R/S)-4-*sec*-butoxycoumarin was the most repulsive against *Ae. albopictus*, followed by the R-(-)-4-*sec*-butoxycoumarin. The S-(+)-4-*sec*-butoxycoumarin had no significant effect compared to the other two products. The higher repellent effect of the racemic compared to the most efficient enantiomer is difficult to explain. However, the difference in racemic and (R) enantiomer activities was mainly due to the vaporization rate of the two compounds during the 24 h test. It means that racemic mixture and (R) enantiomer effects are similar when there is little or no evaporation (almost no air circulation in the tunnel test) and when the test duration is sufficiently short.

Table 6. Comparison of the repellent indexes of racemic (*R/S*)-4-*sec*-butoxycoumarin and (*R*), (*S*) enantiomers (5 mg/mL each) on separate supports in cage system olfactometer using one trap

Products	AI (%)	RI (\pm SD)	F	Df	<i>P</i> -value*
<i>R</i> -(-)-4- <i>sec</i> -butoxycoumarin (10 mg/mL)	68	47.2 \pm 1.9	6.414	15	0.012
(<i>R/S</i>)-4- <i>sec</i> -butoxycoumarin (10 mg/mL)	64.5	48.8 \pm 6.2			
<i>R</i> -(-)-4- <i>sec</i> -butoxycoumarin (5 mg/mL)+ <i>S</i> -(+)-4- <i>sec</i> -butoxycoumarin (5 mg/mL)	72	36 \pm 5.7			

**P*-value at confidence interval $P < 0.05$ by ANOVA with number of replicates $n = 8$ and the total number of mosquitoes tested $N = 200$
SD, standard deviation; Df, degree of freedom; AI, Activity index of mosquitoes; RI, repellent index

**Fig. 4.** Comparison of DEET and (*R/S*)-4-*sec*-butoxycoumarin repellent effect on *Aedes albopictus* by system cage olfactometer, eight replicates, $N = 200$ mosquitoes tested for each dose of product.

On the contrary, the racemic mixture evaporates slowly and has a stronger effect on longer tests. Indeed, Thermogravimetric analysis of these molecules (See [supplementary materials](#)) showed that at 100°C (in isothermal mode), the racemate mass loss is lower than that of pure enantiomers: less than 5% after 200 min for the racemic mixture and approximately 30% for pure (*R*) or (*S*) enantiomers, under the same conditions as shown in [Fig. 6](#).

Repellency or attractant property of a product depends on many factors. Interaction with insect olfactory system is the main parameter, but the concentration of product vaporized in the air around the impregnated surface also plays an important role ([Klun et al. 2006](#)). This is for this reason that Repellent Indexes (RI) increase with the racemic and (*R*) enantiomer doses. The plateau observed on the (*R*) enantiomer curve (30 mg/mL to 60 mg/mL, [Fig. 3](#)) can be due to the atmosphere saturation surrounding the baited traps. Racemate and enantiomers have markedly different melting points (58°C for the racemic and 51°C for (*R*) and (*S*)). Logically, vaporization of pure enantiomers is faster than that of the racemic for 24 h tests. It is possible to ascribe the lower repellent effect of the pure (*R*) enantiomer by faster elimination of the pure enantiomers compared to the racemic. Finally, our experiments show that there is no synergistic effect between the pure (*R*) and (*S*) enantiomers. Thus, these results are very consistent with high time efficiency of *R/S* due to slow evaporation duration. So, repellent with slow evaporation rate can be used if long-term efficiency is required.

To have more reliable results, we carried out another bioassay, tunnel olfactometer, to evaluate attraction and repellent properties. There are different kinds of olfactometer to study host-seeking principles in mosquitoes. While some are simple, others are rather complex. Whatever the system, there is a common part of the tunnel with a minimum of two choices in which a mosquito recognizes and responds to an odor. These responses could be an attraction, a repellent,

or indifference ([Geier and Boeckh 1999](#), [Omran et al. 2010](#)). This device is generally used for quick screening of compound property. In our case, tests give an equal repellent index value (RI = 48%) for (*R/S*)-4-*sec*-butoxycoumarin and *R*-(-)-4-*sec*-butoxycoumarin due to insufficient air circulation, while *S*-(+)-4-*sec*-butoxycoumarin has a low RI = 6.9%. These results confirm those obtained from cage system olfactometer assay.

Compared to 4-*sec*-butoxycoumarin, 4-(3-pentoxycoumarin has no asymmetric center and a small structural difference. It was demonstrated to have high repellent effect on *Drosophila* ([Delaveau et al. 2012](#)). And in this work, we tested it on *Ae. albopictus* mosquitoes. Assays were carried out under the same conditions as those used for (*R/S*)-4-*sec*-butoxycoumarin in cage system olfactometer during 24 h. 4-(3-pentoxycoumarin exhibited an attractant effect at low doses (1 to 5 mg/mL). Kairomone dose-dependent effect has been observed previously for octenol and lactic acid. These two molecules are attractant at low concentrations and have no effect at high concentrations ([Kline et al. 1990](#)). To our knowledge, no reverse effect has been described until now, some publications highlighted their repellent activity at very high doses but nothing about the attractant effect on mosquitoes ([Shirai et al. 2001](#), [Hoel et al. 2007](#), [Andrianjafy et al. 2017](#)). This new result is very important in so far as substance exhibiting two contradictory effects depending on concentration cannot be used as a repellent. Indeed, it can protect against mosquitoes at the beginning of the application and becomes attractive over time because it evaporates and decreases in concentration. Conversely, this may have a potential interest in specific traps preparation.

Compared to DEET by system cage olfactometer, (*R/S*)-4-*sec*-butoxycoumarin had a slight repellent effect at low dose (2.5 mg/mL). By increasing the dose, (*R/S*)-4-*sec*-butoxycoumarin activity rises (60 mg/mL, RI = 85%) while that of DEET remains more or less stable. These encouraging results must be confirmed by *in vivo*

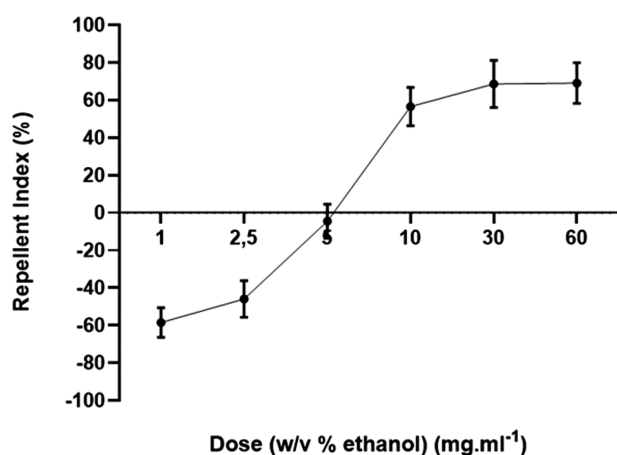
Table 7. Comparison by *t*-test of the average number of individuals in control and baited traps at different doses of 4-(3-pentoxycoumarin in cage system olfactometer

Dose	Baited (± SD)	Control (± SD)	t	<i>P</i> -value*	AI (%)	RI (%)
1 mg/mL	2.5 ± 0.6	9.5 ± 0.6	17.1	0.0001	48	-58.5
2.5 mg/mL	3 ± 0.8	8 ± 0.8	8.7	0.0001	44	-46
5 mg/mL	5.7 ± 0.9	5.7 ± 1.2	0	NS	45.6	0.5
10 mg/mL	10.7 ± 1.2	3 ± 0.8	-10.3	0.0001	54.8	56.5
30 mg/mL	10.7 ± 0.9	2 ± 0.8	-13.9	0.0001	50.8	68.5
60 mg/mL	10.7 ± 0.5	2 ± 0.8	-18.3	0.0001	50.8	69.1

**P*-value at confidence interval $P < 0.05$ by *t*-test with number of replicates, $n = 8$ and the total number of mosquitoes tested $N = 200$

NS indicates no significant difference; SD, standard deviation; Df, degree of freedom; AI, Activity index of mosquitoes; RI, repellent index

Number in bold indicates an attractant effect

**Fig. 5.** Effects of 4-(3-pentoxycoumarin against *Aedes albopictus* (Repellent Index ± SE), 95 % confidence interval, eight replicates.

tests to evaluate the real effectiveness of (R/S)-4-sec-butoxycoumarin on the human skin.

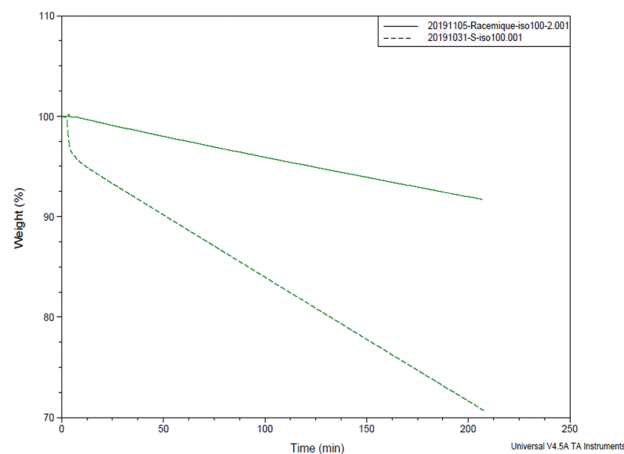
In conclusion, acid catalysis is the best process to synthesize alcoxycoumarin with good yields and to have pure enantiomers. Chirality greatly influences molecule repellent property against *Ae. albopictus*. Racemic mixture of 4-sec-butoxycoumarin exhibited high repellency compared to (R) enantiomer although (S) enantiomer was almost inactive. The higher effect of racemate was due to the low evaporation rate toward pure enantiomers during 24 h test. We reported no synergistic effect between the two enantiomers. (R/S) 4-sec-butoxycoumarin was more active than DEET the best mosquito repellent used actually (with RI = 89% from the dose of 60 mg). 4-(3-pentoxycoumarin was selected for its contradictory responses to the test: high repellency effect at high doses but a strong attractive effect at low doses. To our knowledge, this phenomenon has never been observed before although attractive effect-concentration dependence is known. This last property can be important for specific traps preparation to control insect population. For that, it is essential to highlight the importance of dose-response relationship to

Table 8. Comparison of the repellent indexes of (R/S)-4-sec-butoxycoumarin, (R)- enantiomer and (S)- enantiomer at 5 mg/mL dose using tunnel olfactometer

Products	AI (%)	RI (± SD)	F	Df	<i>P</i> -value*
(R/S)-4-sec-butoxycoumarin	87.2	49.1 ± 3.7	30.073	8	0.001
R(-)-4-sec-butoxycoumarin	82.2	47.5 ± 3.1			
S-(+)-4-sec-butoxycoumarin	93.9	6.9 ± 8.4			

**P*-value at confidence interval $P < 0.05$ by ANOVA with number of replicates $n = 3$ and the total number of mosquitoes tested $N = 45$

SD, standard deviation; Df, degree of freedom; AI, Activity index of mosquitoes; RI, repellent index

**Fig. 6.** Superposition of graphs from thermogravimetric analysis of (R/S)-4-sec-butoxycoumarin and R(-)-4-sec-butoxycoumarin or S-(+)-4-sec-butoxycoumarin.

choose a repellent or a kairomone. More generally, organic synthesis, including asymmetric synthesis associated with physical chemistry and entomology, can be a powerful tool to improve scientist knowledge in insect population control and are of medical, veterinary, or agricultural significance.

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References Cited

- Ahn, C., R. Correia, and P. DeShong. 2002. Mechanistic study of the Mitsunobu reaction. *J. Org. Chem.* 67: 1751–1753.
- Albanese, D., D. Landini, A. Maia, and M. Penso. 1999. Phase transfer catalysis: some recent applications in organic synthesis. In *J. Mol. Catal. A Chem.* 150: 113–131.
- Andrianjafy, T. M., L. Ravaomanarivo, M. Rakotondramanga, V. Ramanandraibe, P. Mavingui, and M. Lemaire. 2017. New bioassay to evaluate repellency and attractively of chemical products against adults mosquitoes *Aedes albopictus* and *Culex quinquefasciatus*. *Ann. Community Med. Pract.* 3: 1020.
- Andrianjafy, T. M., L. H. Ravaomanarivo, V. V. Ramanandraibe, M. F. Rakotondramanga, P. Mavingui, and M. Lemaire. 2018. Synthesis, bioassays and field evaluation of hydroxycoumarins and their alkyl derivatives as repellents or kairomones for *Aedes albopictus* Skuse (Diptera: Culicidae). *J. Chem. Ecol.* 44: 299–311.

- Andrianjafy, T. M., V. V. Ramanandraibe, E. T. Andrianarijaona, N. H. Ramarosandratana, L. H. Ravaomanarivo, P. Mavingui, and M. Lemaire. 2020. Field assessment of 4-hydroxycoumarin as an attractant for anthropophilic *Anopheles* spp. vectors of malaria in Madagascar. *Sci. Rep.* 10: 3048.
- Barasa, S. S., I. O. Ndiege, W. Lwande, and A. Hassanali. 2002. Repellent activities of stereoisomers of p-menthane-3,8-diols against *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.* 39: 736–741.
- Bolzonnella, C., M. Lucchetta, G. Teo, V. Boatto, and A. Zanella. 2019. Is there a way to rate insecticides that is less detrimental to human and environmental health? *Glob. Ecol. Conserv.* 20: e00699.
- Brenna, E., C. Fuganti, and S. Serra. 2003. Enantioselective perception of chiral odorants. *Tetrahedron Asymmetry.* 14: 1–42.
- Cazorla, C., É. Pfordt, M. C. Duclos, E. Métay, and M. Lemaire. 2011. O-Alkylation of phenol derivatives via a nucleophilic substitution. *Green Chem.* 13: 2482–2488.
- Chauvin, R. A. J., and C. Mentzer. 1952. Procédé de protection des végétaux contre les acridiens. assignée 1952. Patent 1.003.646.
- Cook, S. M., Z. R. Khan, and J. A. Pickett. 2007. The use of push-pull strategies in integrated pest management. *Annu. Rev. Entomol.* 52: 375–400.
- Curtis, C. F., J. D. Lines, J. Ijumba, A. Callaghan, N. Hill, and M. A. Karimzad. 1987. The relative efficacy of repellents against mosquito vectors of disease. *Med. Vet. Entomol.* 1: 109–119.
- Davis, W. A. 1900. IV. Etherification of derivatives of β -naphthol. *J. Chem. Soc. Trans.* 77: 33–45. doi:10.1039/ct9007700033
- Delaveau, J., E. Vialle, M. Lemaire, S. Pellet-Rostaing, and B. Andrioletti. 2012. Novel insect repellent coumarin derivatives, syntheses, and methods of use. U.S. Patent Application No. 13/528,944. <https://patentimages.storage.googleapis.com/67/7c/6f/d6a2f15eae89e6/WO2013003168A1.pdf>
- Feaster, J. E., M. A. Scialdone, R. G. Todd, Y. I. Gonzalez, J. P. Foster, and D. L. Hallahan. 2009. Dihydronepetalactones deter feeding activity by mosquitoes, stable flies, and deer ticks. *J. Med. Entomol.* 46: 832–840.
- Freedman, H. H. 1986. Industrial applications of phase transfer catalysis (Ptc): past, present and future. *Pure Appl. Chem.* 58: 857–868.
- Geier, M., and J. Boeckh. 1999. A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. *Entomol. Exp. Appl.* 92: 9–19.
- Geier, M., O. J. Bosch, and J. Boeckh. 1999. Influence of odour plume structure on upwind flight of mosquitoes towards hosts. *J. Exp. Biol.* 202 (Pt 12): 1639–1648.
- Harrup, L. E., J. G. Logan, J. I. Cook, N. Golding, M. A. Birkett, J. A. Pickett, C. Sanders, J. Barber, D. J. Rogers, P. S. Mellor, et al. 2012. Collection of *Culicoides* (Diptera: Ceratopogonidae) using CO₂ and enantiomers of 1-octen-3-ol in the United Kingdom. *J. Med. Entomol.* 49: 112–121.
- Hemingway, J., N. J. Hawkes, L. McCarroll, and H. Ranson. 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem. Mol. Biol.* 34: 653–665.
- Hoel, D. F., D. L. Kline, S. A. Allan, and A. Grant. 2007. Evaluation of carbon dioxide, 1-octen-3-ol, and lactic acid as baits in mosquito magnet™ Pro traps for *Aedes albopictus* in north central Florida. *J. Am. Mosq. Control Assoc.* 23: 11–17.
- Kline, D. L., W. Takken, J. R. Wood, and D. A. Carlson. 1990. Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. *Med. Vet. Entomol.* 4: 383–391.
- Klun, J. A., A. Khirman, and M. Deboun. 2006. Repellent and deterrent effects of SS220, Picaridin, and Deet suppress human blood feeding by *Aedes aegypti*, *Anopheles stephensi*, and *Phlebotomus papatasi*. *J. Med. Entomol.* 43: 34–39.
- Makosza, M. 2000. Phase-transfer catalysis. A general green methodology in organic synthesis. *Pure Appl. Chem.* 72: 1399–1403.
- Miller, J. D. 1982. Anaphylaxis associated with insect repellent. *N. Engl. J. Med.* 307: 1341–1342.
- Natarajan, R., S. C. Basak, A. T. Balaban, J. A. Klun, and W. F. Schmidt. 2005. Chirality index, molecular overlay and biological activity of diastereoisomeric mosquito repellents. *Pest Manag. Sci.* 61: 1193–1201.
- Omrani, S. M., H. Vatandoost, M. A. Oshaghi, F. Shokri, P. M. Guerin, M. R. Yaghoobi Ershadi, Y. Rassi, and S. Tirgari. 2010. Fabrication of an olfactometer for mosquito behavioural studies. *J. Vector Borne Dis.* 47: 17–25.
- Rodhain, F. 1996. Problems posed by the spread of *Aedes albopictus*. *Bull. Soc. Pathol. Exot.* 89: 131–137.
- Roland, E. H., J. E. Jan, and J. M. Rigg. 1985. Toxic encephalopathy in a child after brief exposure to insect repellents. *Can. Med. Assoc. J.* 132: 155–156.
- Shirai, Y., K. Kamimura, T. Seki, and M. Morohashi. 2001. L-lactic acid as a mosquito (Diptera: Culicidae) repellent on human and mouse skin. *J. Med. Entomol.* 38: 51–54.
- Tan, J., and N. Yasuda. 2015. Contemporary asymmetric phase transfer catalysis: large-scale industrial applications. *Org. Process Res. Dev.* 19: 1731–1746.
- Watentena, A., and C. I. Okoye. 2019. The untapped potential of mosquito lures for malaria vector surveillance and mass trapping of mosquitoes: a review. *Int. J. Mosq. Res.* 6: 132–137.
- Weinzierl, R., T. Henn, P. G. Koehler, and C. L. Tucker. 2005. Insect attractants and traps. ENY277. Available: <http://ufdcimages.uflib.ufl.edu/IR/00/00/27/94/00001/IN08000.pdf>
- WHO. 2017. Action mondiale pour lutter contre les vecteurs 2017-2030 (version 5.4). 58 pp. http://www.who.int/malaria/areas/vector_control/Draft-WHO-GVCR-2017-2030-fre.pdf?ua=1
- WHO Expert Committee on Insecticides, and World Health Organization. 1970. Résistance aux insecticides et lutte antivectorielle: dix-septième rapport du Comité OMS d'experts des insecticides [réuni à Genève du 19 au 25 novembre 1968].
- Wooding, M., Y. Naudé, E. Rohwer, and M. Bouwer. 2020. Controlling mosquitoes with semiochemicals: a review. *Parasit. Vectors.* 13: 1–20.