Topical Mosquito Repellents IX: Quinolines, Isoquinolines, and Quinoxalines

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Abstract □ Various quinoxalines, quinolines, and isoquinolines were evaluated for their effectiveness as topical mosquito repellents. Several tetrahydroquinolines and isoquinolines also were included. None of the compounds tested was superior to diethyltoluamide.

Keyphrases □ Quinolines, substituted—synthesized, effectiveness as topical mosquito repellents evaluated □ Quinoxalines, substituted—synthesized, effectiveness as topical mosquito repellents evaluated □ Isoquinolines, substituted—synthesized, effectiveness as topical mosquito repellents evaluated □ Repellents, mosquito—substituted quinolines, quinoxalines, and isoquinolines synthesized and evaluated □ Structure–activity relationships—substituted quinolines, quinoxalines, and isoquinolines synthesized and evaluated for effectiveness as topical mosquito repellents

To formulate a more effective topical mosquito repellent, heterocyclic compounds in the quinoline, isoquinoline, and quinoxaline series were prepared and evaluated. The repellency of these compounds against *Aedes aegypti* was determined by topical application on human subjects as previously described (1).

In one study, 8-hydroxyquinoline was reported to be more effective against *A. aegypti* than the standard repellent, dimethyl phthalate (2). Following this report, other derivatives, chiefly in the quinoline series, were evaluated, including esters and amides of cinchoninic acid and alkyl-substituted cinchoninic acids (3, 4). Some *N*-acyl tetrahydroquinolines and isoquinolines also were reported to be effective repellents (5, 6). These reports were regarded as sufficiently promising to justify a more extensive investigation of derivatives in this series.

With few exceptions, all of the compounds tested (Tables I–IV) were either available commercially or were prepared *via* published procedures. Diethyltoluamide was used as the reference in all tests.

EXPERIMENTAL[1]

1,6-Dimethyl-8-hydroxymethyl-1,2,3,4-tetrahydroquinoline (Compound 51)—A 50-ml flask was charged with formic acid (0.1 *M*, 5.12 g of a 90% solution), aqueous formaldehyde (0.05 *M*, 4.5 g of a 37% solution), and 6-methyl-1,2,3,4-tetrahydroquinoline (0.015 *M*, 2.0 g). The reaction mixture was heated on a steam bath for 8 hr, cooled to room temperature, made basic with 10% NaOH, and then extracted with ether.

The ether layers were combined, washed with saturated sodium chloride, dried, and stripped. Distillation afforded the desired material (114°/0.20 mm) as a slightly yellow oil, 1.6 g; IR (film): 2.96, 3.41, 6.78, 8.30, and 9.62 μm; NMR ($CDCl_3$): δ 6.81 (s, 2H), 4.80 [s (sharp), 2H], 3.08 (m, 2H), 2.80 (m, 2H), 2.71 [s (sharp), 3H], 2.23 [s (sharp), 3H], and 1.86 (m, 2H).

Anal.—Calc. for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.63; H, 9.24; N, 7.58.

Tests on Skin—Compounds were uniformly applied in ethanol to an exposed area of the forearm of a human subject as previously described (1). Repellency was evaluated utilizing female *A. aegypti*.

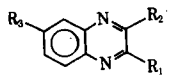


Table I—Physical Properties of Quinoxalines

Compound	R_1	R_2	R_3	Boiling Point (mm) or Melting Point	Repellency, hr[a] (0.35 mg/cm²)	Reference
1	H	H	H	31°	1.0 (1)	—[b]
2	H	H	OCH_3	57°	2.0 (1)	7
3	H	H	Cl	55°	1.0 (1)	7
4	CH_3	H	H	64° (0.5)	0.3 (1)	—[b]
5	OCH_3	H	H	33°	0.3 (1)	8
6	Cl	CH_3	H	80°	0.3 (1)	—[b]
7	$N(CH_2)_5$	H	H	62°	2.0 (1)	9
8	SCH_3	H	H	39°	2.0 (1)	9
9	$N(CH_2)_4$	H	H	89°	1.0 (1)	—[c]
10	$N(CH_2)_4$	H	H	90°	1.0 (1)	—[d]
11	2,4-$(CH_3)_2C_6H_3$	H	H	56°	1.0 (1)	10
12	OC_2H_5	OC_2H_5	H	78°	0.9 (3)	11
13	OC_4H_9	OC_4H_9	H	46°	1.1 (3)	11
14	OC_5H_{11}	OC_5H_{11}	H	154° (0.5)	2.0 (1)	11
15	CH_3	H	CH_3	100° (0.5)	1.0 (1)	12
16	$COOC_2H_5$	CH_3	H	72°	1.0 (1)	13
17	C_6H_5	CH_3	H	165° (0.5)	1.0 (1)	14
18	C_3H_7	CH_3	H	62°	0.3 (2)	15
Diethyltoluamide				100° (0.5)	6.0–7.0	

[a] Number of determinations is given in parentheses; for more than one determination, reproducibility was ±0.3 hr. [b] Commercially available compound. [c] See Ref. 9. (*Anal.*—Calc. for $C_{12}H_{13}N_3$: C, 72.33; H, 6.57; N, 21.09. Found: C, 72.08; H, 6.32; N, 21.17.) [d] See Ref. 9. (*Anal.*—Calc. for $C_{14}H_{17}N_3$: C, 73.97; H, 7.54; N, 18.49. Found: C, 73.84; H, 7.60; N, 18.21.)

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Boiling points were determined using a Bantamware short path distillation apparatus and also are uncorrected. IR (Perkin-Elmer 735 B) and NMR (Varian Associates T-60) spectra were taken of all compounds and were completely consistent with the assigned structures. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

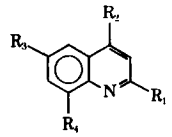


Table II—Physical Properties of Quinolines

Compound	R_1	R_2	R_3	R_4	Boiling Point (mm) or Melting Point	Repellency, hr[a] (0.35 mg/cm²)	Reference
19	Cl	H	H	H	35°	1.0	—[b]
20	CH_3	CH_3	H	H	78° (0.5)	1.0	—[b]
21	CH_3	H	CH_3	H	59°	0.3	—[b]
22	$COOC_2H_5$	H	H	H	151° (0.5)	0.4 (2)	16
23	H	H	H	$COCH_3$	111° (0.5)	3.5 (1)	16
24	$COCH_3$	H	H	H	47°	1.0 (1)	17
25	H	$COCH_3$	H	H	105° (0.5)	2.0 (1)	18
26	H	$COOC_2H_5$	H	H	113° (0.5)	2.0 (1)	18
27	H	$COOCH_3$	H	H	114° (0.5)	1.0 (1)	19
28	H	$COOC_3H_5$	H	H	156° (0.5)	Irritant	—[c]
29	H	H	COC_6H_5	H	61°	1.0 (1)	20
30	H	COC_6H_5	H	H	154° (0.5)	0.3 (2)	21
31	C_6H_5	$COOCH_3$	H	H	55°	0.5 (1)	22
32	H	$COOC_2H_5$	OCH_3	H	65°	0.3 (2)	—[d]
33	H	Cl	H	H	71° (0.5)	Irritant	—[b]
34	CH_3	H	H	H	65° (0.5)	1.3 (2)	—[b]
35	C_6H_5	$COOC_3H_5$	H	H	32°	0.5 (1)	23
36	OC_2H_5	H	H	H	82° (0.5)	0.3 (2)	24
37	H	OC_2H_5	H	H	116° (0.5)	1.5 (1)	25
38	OC_3H_7	H	H	H	110° (0.5)	0.6 (2)	26
39	$OCH(CH_3)_2$	H	H	H	100° (0.5)	0.5 (3)	—[e]
40	OC_3H_5	H	H	H	106° (0.5)	0.3 (2)	26
41	H	OC_3H_5	H	H	135° (0.5)	1.8 (2)	27
42	CH_3	CH_3	CH_3	H	36°	1.8 (4)	—[b]
43	CH_3	H	OCH_3	H	62°	2.3 (2)	—[b]
Diethyltoluamide					100° (0.5)	6.0–7.0	

[a]Number of determinations is given in parentheses; for more than one determination, reproducibility was ±0.3 hr. [b]Commercially available compound. [c]*Anal.*—Calc. for $C_{13}H_{11}NO_2$: C, 73.22; H, 5.20; N, 6.57. Found: C, 73.05; H, 5.43; N, 6.47. [d]*Anal.*—Calc. for $C_{13}H_{13}NO_3$: C, 67.51; H, 5.74; N, 6.14. Found: C, 67.58; H, 5.74; N, 6.09. [e]*Anal.*—Calc. for $C_{12}H_{12}NO$: C, 77.38; H, 6.50; N, 7.52. Found: C, 77.28; H, 6.72; N, 7.93.

RESULTS AND DISCUSSION

For topically applied repellents, an optimum volatility, which results in maximum duration of repellency, exists for each particular series of structural analogs. The variation in optimum volatility from one series of compounds to another reflects differences in intrinsic repellency for each particular series. The optimum volatility is that which results in the maintenance of the minimum effective concentration (MEC) of the repellent vapor (directly determined by the intrinsic repellency of a particular compound) for the maximum period. The optimum volatility for most compounds appears to lie within the range of 100–150°/0.5 mm.

The series of quinoxalines examined in Table I is, without exception, disappointing. The intrinsic repellency for this series of compounds appears to be far too low.

While the quinoline derivatives evaluated (Table II) are considerably more attractive than the quinoxalines in terms of the range of volatilities attainable, their apparent low level of intrinsic repellency limits their utility as repellents for *A. aegypti*.

An interesting observation concerning polarization and duration of repellency is apparent by comparing 2-(ethoxy)- and 2-(allyloxy)-quinolines (Compounds 36 and 40) with their respective 4-substituted isomers (Compounds 37 and 41). That the 4-(alkoxy)quinolines are considerably more polar than the 2-(alkoxy)quinolines is evident from the substantial increase in boiling point. This difference in polarity can affect the duration of repellency in a number of ways.

An increase in intrinsic repellency or, perhaps, simply an optimization of the boiling point as a result of the increased polarity of the 4-(alkoxy)quinolines may be responsible for the observed differences. Another possibility is that the increase in polarity results in a reduced rate of percutaneous absorption, thereby reducing the amount of repellent dissipated *via* this pathway (32). In view of the complex relationship between repellency and molecular structure, it is unlikely that any of the possibilities mentioned is solely responsible for the observed increase in the duration of repellency with increased molecular polarity. It does, however, seem reasonable that these variables are among those responsible for the observed effect.

None of the compounds included in Table III or IV is exceptional. It is interesting that the reduction of an aromatic ring (Compound 54) to the corresponding 1,4-diene (Compound 55) results in a con-

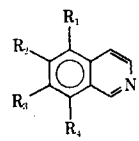
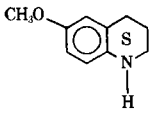
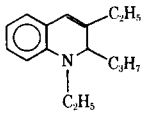
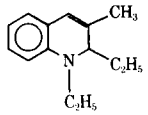
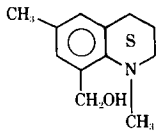
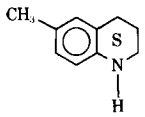
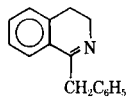
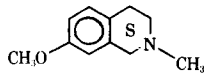
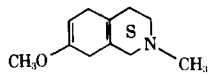


Table III—Physical Properties of Isoquinolines

Compound	R_1	R_2	R_3	R_4	Boiling Point (mm) or Melting Point	Repellency, hr[a] (0.35 mg/cm²)	Reference
43	H	Br	H	H	39°	1.5	—[b]
44	H	H	OCH_3	OCH_3	128° (0.5)	3.5 (2)	28
45	OCH_3	OCH_3	OCH_3	H	160° (0.5)	0.5 (1)	28
46	H	OCH_3	OCH_3	OCH_3	165° (0.5)	0.3 (1)	28
47	H	H	OCH_3	OCH_3	134° (0.5)	2.0 (1)	28
Diethyltoluamide					100° (0.5)	6.0–7.0	

[a]Number of determinations is given in parentheses; for more than one determination, reproducibility was ±0.3 hr. [b]Commercially available compound.

Table IV—Physical Properties of Dihydro- and Tetrahydroquinolines and Isoquinolines

Compound	Structure	Boiling Point (mm) or Melting Point	Repellency, hr[a] (0.35 mg/cm²)	Reference
48		41°	1.5 (1)	—[b]
49		121° (0.5)	2.5 (1)	29
50		110° (0.5)	0.6 (2)	29
51		130° (0.5)	2.5 (2)	—[c]
52		86° (0.5)	1.5 (2)	—[b]
53		131° (0.5)	1.5 (1)	30
54		86° (0.5)	1.5 (2)	31
55		87° (0.5)	0.8 (2)	31
Diethyltoluamide		100° (0.5)	6.0–7.0	

[a] Number of determinations is given in parentheses; for more than one determination, reproducibility was ±0.3hr. [b] Commercially available compound. [c] See *Experimental.*

siderable decrease in protection time, even though the boiling points are essentially the same.

From the data summarized in Tables I–IV, it appears that none of the compounds evaluated offers a significant advantage in terms of increased protection time over topical repellents currently available.

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Tumor Inhibitory Agents from *Vauquelinia corymbosa* (Rosaceae)

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Abstract □ The chloroform extract of *Vauquelinia corymbosa* Correa has shown activity against the P-388 lymphocytic leukemia test system. The constituents responsible for this activity were identified as uvaol, ursolic acid, and betulinic acid. Their identity was proven by melting point; mixed melting point; elemental analysis; IR, PMR, and mass spectra; and preparation of derivatives.

Keyphrases □ *Vauquelinia corymbosa*—chloroform extract of leaves and stems, uvaol, ursolic acid, and betulinic acid isolated, screened for antitumor activity □ Antitumor agents, potential—uvaol, ursolic acid, and betulinic acid isolated from leaves and stems of *Vauquelinia corymbosa*, screened

As a result of the continuing search for plants having tumor-inhibiting constituents, the ethanol extract of the leaves and stems of *Vauquelinia corymbosa* Correa (Rosaceae)[1] was found to have inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2].

DISCUSSION

The chloroform extract, obtained from an ethanol extract by partition between chloroform and water, was subjected to column chromotography and yielded three pure components. These were identified as uvaol, betulinic acid, and ursolic acid by means of their melting points; mixed melting points; elemental analysis; mass, PMR, and IR spectra; and preparation of derivatives.

In the 3PS test system, uvaol demonstrated an activity of 125% test/control (T/C) at both 100 and 200 mg/kg, betulinic acid demonstrated an activity of 135% T/C at 100 mg/kg and 140% T/C at 50 mg/kg, and ursolic acid demonstrated an activity of 125% T/C at 50 mg/kg. Activity in the 3PS test system is defined as an increase in the survival of treated animals over that of controls, resulting in a test/control value greater than or equal to 125% (1).

EXPERIMENTAL[3]

The dried leaves and stems (6 kg) of *V. corymbosa* were ground and exhaustively extracted in a Lloyd-type extractor with petroleum ether. The marc was air dried and extracted similarly with ethanol. After removal of the solvent in air, the residue (750 g) was partitioned between chloroform and water (1:1), using 1 liter of each phase for each 125 g of alcohol residue.

The chloroform phases were combined and the solvent was removed in air. The residue (207 g) was chromatographed over neutral alumina (5.3 kg) (Brockmann activity grade III), eluting with solvents of increasing polarity. Three crystalline fractions were obtained: uvaol, betulinic acid, and ursolic acid (in order of elution).

Ursolic Acid—Elution with benzene–chloroform (1:1) gave 42.5 g of a semicrystalline solid. Two recrystallizations from ethanol provided pure material, mp 288–291°, $[\alpha]_D^{25}$ + 60°. An authentic specimen[4] of ursolic acid had a melting point of 285–287° and an $[\alpha]_D^{25}$ of + 62°; a mixture of the two samples had an undepressed melting point. PMR, IR, and mass spectra were identical. The methyl ester had a melting point of 168–170° [lit. (2) mp 166–168°]; the acetate had a melting point of 288–293° [lit. (3) mp 288–290°]; and the methyl ester acetate had a melting point of 247–250° [lit. (4) mp 246–247°].

Anal.—Calc. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.57. Found: C, 78.57; H, 10.61.

Betulinic Acid—Elution with petroleum ether–benzene (1:1) afforded 5.8 g of semicrystalline material, 4.1 g of which was decolorized and rechromatographed over silica gel. The latter gave mostly ursolic acid along with 0.25 g of another crystalline material. Recrystallization from methanol gave needles, mp 284–286°, which was undepressed upon admixture with an authentic specimen[5] of betulinic acid. The PMR, IR, and mass spectra of the two samples were identical.

Anal.—Calc. for $C_{30}H_{48}O_3 \cdot CH_3OH$: C, 76.18; H, 10.72. Found: C, 75.86; H, 10.67.

Uvaol—The first eluate of the alumina column with petroleum ether–benzene (1:1) gave 12.5 g of a solid residue. Crystallization from acetone and recrystallization from chloroform–ethanol afforded pure material, mp 224–225°, whose PMR, IR, and mass spectra were sug-

[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resources Laboratory, Agricultural Research Center, Beltsville, Md. A reference specimen was deposited in that herbarium. The plant was collected in Coahuila, Mexico, in July 1970.

[2] Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

[4] From this laboratory.

[5] The authors are grateful to Dr. R. P. Rastogi, Central Drug Research Institute, Lucknow, India, for providing this sample.