linearized form of the Michaelis–Menten equation (Eq. 2). These data appeared to be nonlinear for both monkeys (Fig. 3). This nonlinearity is of the type observed in cases involving two saturable pathways with different values of K_m (5). Nonlinear least-squares fitting of data to acquire the best estimates of parameters requires at least one more datum point than the number of parameters to be estimated. In this case, there are only four data points to estimate four parameters; therefore, no estimates of the parameters for the pathway having the lower K_m were attempted. The v_{max} and K_m for the higher capacity pathway could be approximated by fitting the data from the three higher infusion rates to a straight line. The values of v_{max} and K_m were 120 mg/hr and 3.35 mg/liter for monkey 306 and 114 mg/hr and 4.43 mg/liter for monkey 307. A plot of data from the femoral vein infusions according to the linearized form of the Michaelis–Menten equation shows nonlinearity similar to that of the portal vein data (Fig. 4). The estimated values of v_{max} and K_m are 170 mg/hr and 15.6 mg/liter, respectively.

These values of v_{max} and K_m for femoral vein administration may be compared with those obtained for the same monkey dosed *via* the portal vein. The observed difference in v_{max} was not expected. It may be a result of the metabolism of cinromide by more than one enzyme, as indicated by the nonlinearity of the data from all four infusions. Also, the clearance values obtained from the three higher infusion rates into the femoral vein span a narrow range (5.1 to 9.3 liter/hr), which can result in uncertainty in the intercept. According to theory (Eqs. 2 and 4) the value of K_m obtained by femoral vein administration (15.6 mg/liter) should exceed that obtained by portal vein administration (4.4 mg/liter) by the ratio v_{max}/Q_H. This ratio can be roughly estimated if the hepatic blood flow in monkey 307 is calculated using the portal and femoral infusion data and the equation of Wilkinson and Shand (7). Although this equation was developed for a dose-independent intrinsic clearance, it can be applied in the dose-dependent case, provided that the steady-state concentration of drug at the enzyme is equal for the two routes of administration. This is accomplished by the administration of drug by the femoral and portal routes at equal rates. The average value obtained from the four pairs of infusions was 21.0 ± 2.8 liter/hr. Division of the two values of v_{max} from monkey 307 by this value of blood flow yields 5.4 and 8.4 mg/liter, within 50–70% of the difference between the two values of K_m (11.2 mg/liter). This calculation is compatible with the theoretical prediction. Equality between these two estimates of the difference in K_m arising from route of administration is not expected, since the determination of K_m, v_{max}, and Q_H involve error. In fact, the degree of compatibility suggests that the well-stirred model of the liver can be used to explain the effects of administration route on the disposition of cinromide.

The dose-dependent nonlinearity of cinromide, a medium extraction ratio drug, has been demonstrated. Catheterization of the portal vein for chronic drug administration provided a means of evaluating the intrinsic clearance of cinromide independently of hepatic blood flow and, thereby, a means of examining the dose dependence of cinromide. Comparison of values obtained for the whole body Michaelis–Menten constant for the peripheral and portal routes of administration confirmed the theoretically predicted effects of flow limitation on clearance.

REFERENCES

(1) A. J. Sedman and J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **2**, 161 (1974).

2 J. L. Rheingold, R. E. Lindstrom, and P. K. Wilkinson, *J. Pharmacokinet. Biopharm.*, **9**, 261 (1981).

(3) K. S. Pang, M. Rowland, and T. N. Tozer, *Drug. Metab. Dispos.*, **6**, 197 (1978).

(4) E. A. Lane and R. H. Levy, *J. Pharm. Sci.*, **72**, 493 (1983).

(5) A. J. Sedman and J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **2**, 149 (1974).

(6) T. M. Ludden, D. W. Hawkins, J. P. Allen, and F. Hoffman, *Lancet*, **i**, 307 (1976).

(7) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).

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Tick Repellents II: *N*-Substituted Azacyclopentanones and Azacyclopentenones

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Received May 10, 1982, from *Life Sciences Division, SRI International, Menlo Park, CA 94025.* Accepted for publication September 3, 1982. *Present address: Waldstrasse 11, 5600 Wuppertal 1, West Germany.

Abstract □ Several *N*-substituted azacyclopentanones and azacyclopentenones were synthesized and evaluated as repellents for the brown dog tick *Rhipicephalus sanguineus*. Several of these compounds were more effective in our test system than were the standard repellents, *N,N*-diethyl-*m*-toluamide and butopyranoxyl.

Keyphrases □ *N*-Substituted azacyclopentanones—synthesis, structure–activity relationships, evaluation as tick repellents □ *N*-Substituted azacyclopentenones—synthesis, structure–activity relationships, evaluation as tick repellents □ Tick repellents—potential, *N*-substituted azacyclopentanones and azacyclopentenones, synthesis

Tick-borne diseases still represent a problem and the need for a safe means of controlling ticks exists. Compounds useful for repelling mosquitoes are not necessarily those that are the most effective for ticks. Screening of repellents for ticks required the development of a rapid, simple assay system, which has been completed in our laboratories. This method, which was described previously (1), involved the use of a plastic vial containing the ticks with a filter paper cap impregnated with the test substance. The common behavior of ticks to travel upward is used to compare the control behavior with that in a vial treated at the top with repellent.

Most repellents reported for ticks have been amides or esters (2). We decided to explore azacyclopentanones and azacyclopentenones as a group of cyclic amides for their ability to repel the brown dog tick, *Rhipicephalus sanguineus*.

Compounds reported in Tables I–III were prepared by the methods described in *Experimental* for selected compounds.

EXPERIMENTAL[1]

Preparation of 1-Decyl-azacyclopentane-2-one (Ib)—A mixture of 22.1 g of 1-bromodecane (0.1 mole), 8.5 g (0.1 mole) of 2-pyrrolidone,

[1] Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

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Table I—1-Alkyl-azacyclopentane-2-ones

Compound	R	Boiling Point/ 0.5 mm Hg[a]	Yield, %	I.R. (C=O), cm^{-1}	Formula	Analysis, % Calc.	Analysis, % Found	Tick Repellency at 0.44 mg/cm², %
Ia	C_8H_{17}	110°	85	1690	$C_{12}H_{23}NO$	C 73.04 H 11.75 N 7.10	72.86 11.64 7.11	78[b]
Ib	$C_{10}H_{21}$	122°	78	1690	$C_{14}H_{27}NO$	C 74.61 H 12.08 N 6.21	74.49 11.94 6.21	25
Ic	$C_{12}H_{15}$	150°	91	1690	$C_{16}H_{31}NO$	C 75.83 H 12.33 N 5.53	75.96 12.59 5.46	20
N,N-diethyl-*m*-toluamide								32
Butopyranoxyl								48
Solvent control								7%
Nontreated control								9%

[a] Boiling points normalized to 0.5 mm Hg for direct comparison. [b] 25% at 0.29 mg/cm² and 20% at 0.19 mg/cm².

and 11.2 g (0.1 mole) of potassium *tert*-butoxide was stirred in 100 ml of anhydrous dimethyl sulfoxide for 1 hr. The dimethyl sulfoxide was removed under reduced pressure, the residue dissolved in 100 ml of ether, and washed with water (2 times). After drying the organic phase with anhydrous magnesium sulfate and removing the solvent under reduced pressure, the oily residue was distilled *in vacuo*. The first fraction, (bp 140–147°/1.2 mm Hg), was discarded. The second fraction gave 17.4 g of product, bp 148°/1.2 mm Hg.

1-Ethyl-5-octyl-azacyclopent-4-ene-2-one (IIe)—A solution of octylmagnesium bromide was prepared by adding in a dropwise manner, 34.2 g (0.177 mole) of 1-bromooctane in 10 ml of ether to 6.5 g (0.268 g-atom) of magnesium in 10 ml of ether and then heating at reflux for 3 hr. The Grignard reagent was added over a 2-hr period to a solution of 15 g (0.118 mole) of *N*-ethyl-succinimide in 50 ml of anhydrous tetrahydrofuran. The mixture was allowed to stand at room temperature for 2 days, and then was poured into a mixture of ice and 10% sulfuric acid. After separation of the organic layer, the aqueous phase was extracted twice with 50 ml of ether. The combined organic phases were washed with saturated sodium hydrogen carbonate and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was evaporatively distilled to give 10 g of a yellow oil, 120° air bath temperature/0.9 mm Hg. A second fractional distillation using a Vigreux column yielded 6.6 g of product, bp 130°/1.0 mm Hg.

1-Ethyl-5-octyl-azacyclopentane-2-one (IIIb)—1-Ethyl-5-octyl-azacyclopent-4-ene-2-one, (5.3 g, 0.0238 mole) in 25 ml of ethanol was hydrogenated under atmospheric pressure with 0.5 g of 10% Pd-C. After the uptake of hydrogen was completed, the catalyst was removed by filtration, the solvent evaporated, and the residue distilled to give 5 g of product, bp 130°/0.9 mm Hg.

The tick repellency assay was as previously described (1).

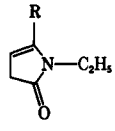
RESULTS AND DISCUSSION

A comparison of the tick repellency of the azacyclopentanones with *N,N*-diethyl-*m*-toluamide and butopyranoxyl at 0.44 mg/cm² shows that a number of these substances are considerably more repellent than the standard compounds (Tables I–III). In the 1-alkyl-azacyclopentane-2-one series, the *n*-octyl derivative is outstanding. The boiling point of 110°/0.5 mm Hg corresponds closely to that of *N,N*-diethyl-*m*-toluamide, whereas the *n*-decyl and dodecyl compounds are considerably higher boiling derivatives and are probably too nonvolatile for optimum repellency.

In the 1-ethyl-5-alkyl-azacyclopentane-4-ene-2-one series, the *n*-pentyl, -hexyl, -heptyl, -octyl, and -decyl derivatives are all outstanding as tick repellents. The maximum activity is reached with the *n*-hexyl derivative (bp 110°/0.5 mm Hg). However, a comparison of the *n*-hexyl derivative with the *n*-octyl derivative in the 1-alkyl-azacyclopentane-2-one series, where the boiling points are identical, reveals that the unsaturation in the ring leads to enhanced repellency.

In the last series explored, 1-ethyl-5-alkyl-azacyclopentane-2-ones, the *n*-octyl and *n*-decyl derivatives have improved repellency over *N,N*-diethyl-*m*-toluamide with the *n*-octyl derivative, boiling point of 120°/0.5 mm Hg, being the best. Comparison of this series with the aza-

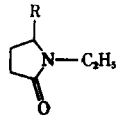
Table II—1-Ethyl-5-alkyl-azacyclopent-4-ene-2-one



Compound	R	Boiling Point/ 0.5 mm Hg	Yield, %	I.R., cm^{-1}	Formula	Analysis, % Calc.	Analysis, % Found	Tick Repellency at 0.44 mg/cm², %
IIa	C_4H_9	86°	36.5	(C=O) 1720 (C=C) 1670	$C_{10}H_{17}NO$	C 71.81 H 10.25 N 8.37	C 71.42 H 10.16 N 8.38	25
IIb	C_5H_{11}	94°	39.0	(C=O) 1720 (C=C) 1670	$C_{11}H_{19}NO$	C 72.89 H 10.56 N 7.73	C 73.15 H 10.74 N 7.43	60
IIc	C_6H_{13}	110°	31.0	(C=O) 1720 (C=C) 1670	$C_{12}H_{21}NO$	C 73.80 H 10.84 N 7.17	C 73.66 H 10.69 N 7.14	100[a]
IId	C_7H_{15}	118°	36.0	(C=O) 1720 (C=C) 1670	$C_{13}H_{23}NO$	C 74.59 H 11.07 N 6.69	C 74.41 H 11.04 N 6.68	95
IIe	C_8H_{17}	125°	25.0	(C=O) 1720 (C=C) 1670	$C_{14}H_{25}NO$	C 75.28 H 11.28 N 6.27	C 75.34 H 11.33 N 6.18	98[b]
IIf	$C_{10}H_{21}$	142°	20.0	(C=O) 1720 (C=C) 1620	$C_{16}H_{29}NO$	C 76.44 H 11.63 N 5.57	C 76.25 H 11.42 N 5.29	98[c]

[a] 93% at 0.29 mg/cm² and 63% at 0.19 mg/cm². [b] 82% at 0.29 mg/cm² and 47% at 0.19 mg/cm². [c] 77% at 0.29 mg/cm² and 20% at 0.19 mg/cm².

Table III—1-Ethyl-5-alkyl-azacyclopentane-2-ones



Compound	R	Boiling Point/ 0.5 mm Hg	Yield, %	I.R. (C=O), cm^{-1}	Formula	Analysis, % Calc.	Analysis, % Found	Tick Repellency at 0.44 mg/cm^2, %
IIIa	C_6H_{13}	110°	89	1690	$C_{12}H_{23}NO$	C 73.04 H 11.75 N 7.10	C 73.26 H 11.97 N 6.99	25
IIIb	C_8H_{17}	120°	94	1690	$C_{14}H_{27}NO$	C 74.61 H 12.08 N 6.21	C 74.94 H 12.09 N 6.11	75[a]
IIIc	$C_{10}H_{21}$	154°	90	1690	$C_{16}H_{31}NO$	C 75.83 H 12.33 N 5.33	C 76.07 H 12.44 N 5.41	40

[a] 55% at 0.29 mg/cm^2.

cyclopentene series again indicates the importance of unsaturation as an enhancer of repellency toward the tick in this series of compounds.

REFERENCES

(1) W. A. Skinner, U. Rosentreter, and T. Elward, *J. Pharm. Sci.*, 71, 837 (1982).

(2) W. A. Skinner and H. L. Johnson, in "Drug Design," Vol. X, H. J. Ariens, Ed., Academic, New York, N.Y., 1980, p. 278.

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Synthesis and Antileukemic Activity of 2-(2-Methylthio-2-aminovinyl)-1-methylquinolinium Iodides

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Abstract □ Reaction of 2-bis(2-methylthio)vinyl-1-methylquinolinium iodide with several heterocyclic aliphatic amines at 30–70° resulted in replacement of one methylthio group to give the title compounds. Reaction with pyrrolidine gave an unidentified product lacking sulfur. Antileukemic screening against P-388 lymphocytic leukemia showed positive activity only with the 6-methyl-morpholino derivative, whereas the 6-unsubstituted morpholino derivative was inactive. This result is in contrast to previous testing results with the 2-bis(2-methylthio)vinyl compounds where both 6-substituted and 6-unsubstituted derivatives showed activity.

Keyphrases □ 2-(2-Methyl-2-aminovinyl)-1-methylquinolinium iodides—synthesis, antileukemic activity in mice □ Synthesis—2-(2-methyl-2-aminovinyl)-1-methylquinolinium iodides, antileukemic activity in mice □ Antileukemia agents—potential, 2-(2-methyl-2-aminovinyl)-1-methylquinolinium iodides, synthesis, P-388 screen in mice

A series of 6-substituted-1-methylquinolinium-2-dithioacetic acid zwitterions (I) (1, 2) has shown appreciable antileukemic activity against P-388 lymphocytic leukemia in mice. An attempt to find compounds having better solubilities, in both water and organic solvents, led to the synthesis of the derived 2-bis(2-methylthio)vinyl-1-methylquinolinium iodides (II) (3). The latter compounds had comparable antileukemic activity, but at lower dose levels than found for the zwitterions. The 6-unsubstituted derivative, however, had activity equal to or better than that of the 6-substituted compounds. Compounds with electron-attracting and electron-releasing 6-substituents were equally active in this series.

Another type of derivative of the dithioacetic acid zwitterion structure (I), which should show improved solubility properties, is the 2-methylthio-2-aminovinyl compound (III) (3). Accordingly, a series of these compounds, using several heterocyclic amines, was prepared for anticancer screening. With the exception of the previously prepared morpholino derivative where a 6-methyl substituent was included, the 6-position was left unsubstituted.

Leukemia cell culture studies were carried out with the 6-methyl derivative of II, but no effects on cell cycle traverse were observed (4). This indicated that a metabolic conversion of the bis(methylthio)vinyl compounds, and most likely their dithioacetic acid precursors as well, is required for antileukemic activity. A study of DNA-binding specificity and RNA polymerase-inhibitory activity showed that the bis(methylthio)vinyl compounds (II), as well as the 2-methylthio-2-morpholinovinyl derivative (III, R = CH_3), had DNA-binding ability involving

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