ethyl acetate (9:1) solvent system, and the residue was crystallized from methanol to yield colorless plates, mp 246–248° [lit. (1) mp 244–248°]. The IR and PMR spectra were identical to the previously reported spectra (1). Mass spectrometry indicated a parent peak at *m/e* 384.

Anal.—Calc. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24; mol. wt., 384. Found: C, 65.52; H, 5.08; *m/e* 384.

Compound II—Methylation of I (25 mg) with ethereal diazomethane in dry dichloromethane for 48 hr at room temperature yielded II, which crystallized from methanol as colorless prisms, mp 168° [lit. (1) mp 167–169°]. The IR spectrum was identical to the previously reported spectrum (1).

REFERENCES

(1) J. Polonsky, J. Moron, and H. Pourrat, *Mem. Present. Soc. Chim.*, **1962**, 1722.

(2) R. I. Geran, N. H. Greenberg, M. N. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, **3**, 17 (1972).

(3) *Ibid.*, **3**, 9 (1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 29, 1976, from the *Division of Pharmaceutical Chemistry, College of Pharmacy, University of Arizona, Tucson, AZ 85721.*

Accepted for publication May 19, 1976.

Supported by Contract NOI-CM-33750 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md., and the Elsa U. Pardee Foundation, Midland, Mich.

[x] To whom inquiries should be directed.

Topical Mosquito Repellents X: 2-Oxazolidones

W. A. SKINNER [x], H. T. CRAWFORD, D. SKIDMORE, and H. I. MAIBACH *

Abstract □ A number of 2-oxazolidones were evaluated for their effectiveness as topical mosquito repellents. Although some compounds approached diethyltoluamide in potency, none was superior.

Keyphrases □ 2-Oxazolidones, various—synthesized, evaluated as topical mosquito repellents □ Repellents, mosquito—various 2-oxazolidones synthesized and evaluated □ Structure–activity relationships—various 2-oxazolidones evaluated as topical mosquito repellents

Several derivatives of 2-oxazolidone have been prepared and evaluated for effectiveness as topical mosquito repellents. The repellency of all of the compounds against female *Aedes aegypti* (yellow fever) mosquitoes was determined by topical application on human subjects as previously described (1).

Since the majority of effective repellents are either amides or esters, it is surprising that carbamates have not been more extensively investigated. The two literature reports (2, 3) of repellent carbamates indicate that simple alkyl derivatives of the type $R_1OC(=O)N(R_2)R_3$ have provided some degree of protection against *A. aegypti* mosquitoes.

The fact that little data were available in a structural area of reported repellent activity, combined with the intriguing similarity between the two most common classes of repellents (esters and amides) and carbamates, encouraged further studies. 2-Oxazolidones were chosen because of practical considerations. Physical properties for a number of 2-oxazolidones were available (proper volatility is an important consideration for a topically applied insect repellent), as were some well-documented synthetic methods for their preparation.

With few exceptions, all compounds tested were prepared *via* published procedures. Table I gives their structures, boiling points, and topical repellency data. Diethyltoluamide was used as the standard repellent in all tests.

EXPERIMENTAL[1]

Preparation of 3-Methoxymethyl-2-oxazolidone (XIX)—Thionyl chloride (9.5 g, 0.08 mole) was placed in a 20-ml flask and stirred at room temperature. 3-Hydroxymethyl-2-oxazolidone (3.2 g, 0.03 mole) was added dropwise over 30 min, and the resulting solution was stirred overnight.

Excess thionyl chloride was removed *in vacuo*, and the residue was distilled. The desired product (bp 100–103°/0.25 mm) was obtained as a colorless oil, 2.9 g; IR (film): 2970, 1740, 1480, 1260, 1031, and 766 cm^{-1}; NMR ($CDCl_3$): δ 5.31 (s, 2H), 4.45 (t, 2H), and 3.74 (t, 2H).

The 3-chloromethyl-2-oxazolidone was not stable at room temperature (loss of hydrogen chloride), but it could be stored for short periods at −20°.

A solution of sodium methoxide in methanol was prepared by the addition of sodium (0.345 g, 0.015 mole) to a stirred solution of dry methanol under nitrogen. 3-Chloromethyl-2-oxazolidone (2.0 g, 0.015 mole) was then added dropwise over 30 min. After stirring for 1 hr at room temperature, TLC indicated no remaining starting material.

The crude reaction mixture was filtered, and methanol was removed under reduced pressure. Distillation (bp 76°/0.20 mm) afforded XIX as a colorless oil, 1.2 g; IR (film): 2930, 1740, 1421, 1259, and 1080 cm^{-1}; NMR ($CDCl_3$): δ 4.70 (s, 2H), 4.45 (t, 2H), 3.71 (t, 2H), and 3.38 (s, 3H).

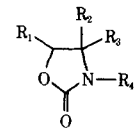
Anal.—Calc. for $C_5H_9NO_3$: C, 45.79; H, 6.92; N, 10.68. Found: C, 45.82; H, 6.81; N, 10.95.

Preparation of 3-Acetoxymethyl-2-oxazolidone (XX)—Acetic anhydride (2.04 g, 0.02 mole) was added dropwise to a stirred solution of 3-hydroxymethyl-2-oxazolidone (2.0 g, 0.017 mole) in dry pyridine (5 ml). The colorless solution was then stirred overnight at room temperature.

The crude reaction mixture was concentrated *in vacuo;* the residue was dissolved in methylene chloride, washed with saturated sodium chloride, dried, and reconcentrated *in vacuo*. Distillation (100–104°/0.1 mm) afforded XX as a colorless oil, 1.9 g; IR (film): 2950, 1760, 1740, 1420, 1210, 1020, and 940 cm^{-1}; NMR ($CDCl_3$): δ 5.37 (s, 2H), 4.40 (t, 2H), 3.76 (t, 2H), and 2.07 (s, 3H).

[1] Melting points were determined on a capillary melting-point apparatus and are uncorrected. Boiling points were determined using a short path distillation apparatus and also are uncorrected. IR and NMR spectra were taken of all compounds and were consistent with the assigned structures. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

Table I—Physical Properties of 2-Oxazolidones



Compound	R_1	R_2	R_3	R_4	Boiling Point (mm) or Melting Point	Repellency, hr[a] (0.35 mg/cm²)	Reference
I	H	CH_3	CH_3	H	55°	1.0 (2)	4
II	CH_3	H	H	H	111° (0.5)	0.3 (2)	5
III	H	H	C_2H_5	H	141° (0.5)	1.0 (2)	4
IV	H	H	H	CH_3	82° (0.5)	1.3 (2)	4
V	H	H	H	C_2H_5	88° (0.5)	1.0 (2)	4
VI	H	H	H	$C(CH_3)_3$	78° (0.5)	1.3 (2)	6
VII	H	H	H	$CH_2CH(CH_3)_2$	112° (0.5)	1.8 (2)	—[b]
VIII	H	H	H	C_5H_{11}	120° (0.5)	5.5 (5)	7
IX	H	H	H	C_6H_{13}	131° (0.5)	5.3 (7)	—[c]
X	H	H	H	C_7H_{15}	138° (0.5)	4.8 (4)	—[d]
XI	H	H	H	C_8H_{17}	143° (0.5)	3.8 (2)	—[e]
XII	H	H	H	Cyclohexyl	134° (0.5)	4.8 (4)	—[f]
XIII	H	H	H	o-Tolyl	131° (0.5)	1.0 (2)	6
XIV	H	H	H	Furfuryl	142° (0.5)	1.0 (2)	—[g]
XV	H	H	H	$CH_2N(C_2H_5)_2$	140° (0.5)	1.3 (2)	8
XVI	H	H	H	*N*-Methylene-pyrrolidino	137° (0.5)	1.0 (2)	8
XVII	H	H	H	$CH_2N(C_4H_9)_2$	145° (0.5)	2.0 (2)	8
XVIII	H	H	H	CH_2OH	58°	1.0 (2)	9
XIX	H	H	H	CH_2OCH_3	91° (0.5)	1.0 (2)	—[h]
XX	H	H	H	CH_2OCOCH_3	124° (0.5)	1.5 (2)	—[h]
XXI	H	H	H	$(CH_2)_3Cl$	124° (0.5)	1.0 (2)	10
Diethyltoluamide					100° (0.5)	6.5	

[a] Number of determinations is given in parentheses; for more than one determination, reproducibility was ±0.5 hr. [b] *Anal.*—Calc. for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.31; H, 9.21; N, 9.32. [c] *Anal.*—Calc. for $C_9H_{17}NO_2$: C, 63.12; H, 10.01; N, 8.18. Found: C, 62.95; H, 10.25; N, 7.91. [d] *Anal.*—Calc. for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 65.07; H, 10.50; N, 7.60. [e] *Anal.*—Calc. for $C_{11}H_{21}NO_2$: C, 66.29; H, 10.62; N, 7.03. Found: C, 65.88; H, 10.69; N, 7.04. [f] *Anal.*—Calc. for $C_9H_{15}NO_2$: C, 63.88; H, 8.94; N, 8.28. Found: C, 63.74; H, 9.15; N, 8.14. [g] *Anal.*—Calc. for $C_8H_9NO_3$: C, 57.49; H, 5.43; N, 8.39. Found: C, 57.43; H, 5.49; N, 8.31. [h] See *Experimental*.

Anal.—Calc. for $C_6H_9NO_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.22; H, 5.83; N, 8.59.

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

RESULTS AND DISCUSSION

As discussed previously (11), the utility of a particular compound as a topical insect repellent is determined by its volatility and its inherent intrinsic repellency. Analysis of the data presented in Table I allows structural characteristics resulting in maximum intrinsic repellency as well as a range of volatilities appropriate to the 2-oxazolidones (resulting in maximum duration of repellency) to be elucidated.

Of the 2-oxazolidones examined (Table I), the 3-alkyl derivatives were the best repellents. Compounds containing an unsubstituted nitrogen or those having electronegative atoms or an aromatic substituent in the side chain attached at position 3 all afforded less protection. Since some compounds have boiling points similar to the more repellent 3-alkyl derivatives, the differences in protection time probably reflect differences in intrinsic repellencies or skin absorption differences. The apparent decrease in intrinsic repellency with the introduction of an aromatic substituent (XIII and XIV) was surprising and contradictory to results obtained earlier in the isoquinoline system (11).

Another interesting observation concerns the relationship between steric bulk and repellency. Wright (12) recently suggested that repellents function by blocking moisture-sensitive pores in the cuticle of the mosquito's sensory hairs, thus preventing the sensors from responding normally to the raised humidity levels of a host. The argument is that steric bulkiness is a desirable characteristic of an effective repellent (pore blocker).

The bulky, somewhat spherically shaped, repellents dimethyl phthalate and 2-ethyl-1,3-hexanediol were compared with their essentially long and flat isomers, dimethyl terephthalate and 1,6-hexanediol, both poor repellents. The facts that dimethyl phthalate has a boiling point of 250° while dimethyl terephthalate melts at 142° and that 2-ethyl-1,3-hexanediol and 1,6-hexanediol are not isomers at all were overlooked. In addition, just how the lipophilic repellent dimethyl phthalate is to be attracted to, and held in, the presumably hydrophilic environment of a pore designed to be a water receptor was not discussed.

Data obtained with the 3-alkyl-2-oxazolidones indicate that proper volatility (not steric bulkiness) is the major factor in determining duration of repellency and that there are no great differences in intrinsic repellency among the various 3-alkyl-2-oxazolidones. The sterically bulky 3-*tert*-butyl-2-oxazolidone (VI) had a repellency nearly identical to the much less bulky 3-methyl (IV) and 3-ethyl (V) derivatives. All have similar boiling points. The same is true of the 3-hexyl (IX) and 3-cyclohexyl (XII) compounds. In the alkyl-substituted 2-oxazolidone series, steric bulk and molecular shape did not appear to be major factors determining repellency.

Of interest, however, in this series is the lower protection time attained by the electronegatively substituted compounds (XV–XVII, XX, and XXI) when compared with saturated alkyl derivatives having comparable boiling points.

None of the compounds evaluated here offers a significant advantage in terms of increased protection times over topical repellents currently available. However, interesting synthetic possibilities in the area of the 3-alkyl-2-oxazolidones remain to be explored and will be the subject of future work wherein a membrane *in vitro* system will be used for initial screening rather than human subjects.

REFERENCES

(1) H. L. Johnson, W. A. Skinner, D. Skidmore, and H. I. Maibach, *J. Med. Chem.*, **11**, 1256 (1968).

(2) Y. Naumov, A. Mentus, V. Kazhadan, and I. Dunaeva, *Khim. Sredstva Zashch. Rast.*, **2**, 269 (1972); through *Chem. Abstr.*, **81**, 135422g (1974).

(3) Section of Insect Repellent Research (Institute of Zoology, Academy of Science, Peoples Republic of China), *K'un Ch'ung Hseueh Pao*, **16**, 39 (1973); through *Chem. Abstr.*, **82**, 150466p (1975).

(4) P. G. Sears and H. L. Huffman, *J. Solution Chem.*, **1**, 187 (1972).

(5) J. B. Bell and J. D. Malkemus (to Jefferson Chemical Co.), U.S. pat. 2,755,286, July 17, 1956; through *Chem. Abstr.*, **51**, P2871i (1957).

(6) R. Oda, M. Miyanoki, and M. Okano, *Bull. Chem. Soc. Jpn.*, **35**, 1309 (1962).

(7) A. Boucherle, G. Carraz, and J. Bonnin, *Bull. Soc. Chim. Fr.*, **1958**, 231.

(8) Badische Anilin and Soda Fabrik Akt. Ges., British pat. 728,699, Apr. 27, 1955; through *Chem. Abstr.*, **50**, P6521b (1956).

(9) S. Okamara and T. Endo (to Seitesy Chem. Ind. Co.), Japanese pat. 69 32,048 (C1. 16E 341), Dec. 20, 1969; through *Chem. Abstr.*, **72**, P79016c (1970).

(10) R. Delaby, R. Damiens, and G. d'Huyteza, *Ann. Pharm. Fr.*, **13**, 565 (1955).

(11) W. A. Skinner, H. T. Crawford, H. Tong, D. Skidmore, and H. I. Maibach, *J. Pharm. Sci.*, **65**, 1404 (1976).

(12) R. H. Wright, *Sci. Am.*, **233**, 104 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 16, 1976, from the *Life Sciences Division, Stanford Research Institute, Menlo Park, CA 94025.*

Accepted for publication May 28, 1976.

Supported by U.S. Army Medical Research and Development Command Contract DADA 17-70-C-0112 (W. A. Skinner) and DADA 70-C-0190 (H. I. Maibach).

* Present address: Division of Dermatology, School of Medicine, University of California, San Francisco Medical Center, San Francisco, Calif.

[x] To whom inquiries should be directed.

GLC Determination of Methenamine in Tablets

J. G. STROM, Jr., and H. W. JUN [x]

Abstract □ A rapid and sensitive GLC method was developed for the quantitative determination of methenamine in tablets. The method was shown to possess several advantages over the official NF assay. After dissolution of the whole tablet in absolute ethanol and addition of an internal standard (pentylenetetrazol), an aliquot was injected into the gas chromatograph for analysis. The sample was chromatographed using a stainless steel column packed with 10% OV-17 on Chromosorb W-HP. Quantitation was achieved by measuring peak heights. The simplicity, directness, extreme rapidity, and accuracy of the method represent an improvement over the official method and the other proposed assays.

Keyphrases □ Methenamine—GLC analysis, commercial tablets □ GLC—analysis, methenamine, commercial tablets □ Antibacterials, urinary—methenamine, GLC analysis, commercial tablets

Methenamine is a urinary antibacterial useful in the long-term therapy of chronic urinary tract infections. The method most often used for the determination of methenamine is based on the hydrolysis of methenamine to formaldehyde and ammonia in a strong acid solution. The formaldehyde may be volatilized by continued heating and the excess acid may be back-titrated, or the liberated formaldehyde may be determined by colorimetry. However, these assays do not differentiate between decomposed and unhydrolyzed methenamine. The NF XIII (1) method involves the back-titration procedure and may be subject to errors due to the uncertainty of complete hydrolysis.

The official NF XIV (2) method and other commonly used techniques (3–5) for the determination of methenamine are based on reaction of formaldehyde with chromotropic acid. Since the decomposition product, formaldehyde, and not the intact methenamine is the analytically available moiety, any formaldehyde released by decomposition and trapped in the tablet is determined with the drug.

Other proposed methods include complexation (6), fluorometry (7), NMR spectroscopy (8), and a 2-hydrazinobenzothiazole reaction (9). This report presents a direct method of analysis of the intact methenamine by GLC. The procedure is rapid, simple, and accurate.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a dual flame-ionization detector was used. The chromatographic column was a 3-mm (i.d.) stainless steel coiled column, 1.83 m in length, packed with 10% OV-17 on 80–100-mesh Chromosorb W-HP. The operating temperatures were 250° for the injection port, 190° (isothermal) for the column oven, and 250° for the detector. Nitrogen, with a flow rate of 60 ml/min, was the carrier gas. The flow rates of hydrogen and compressed air were adjusted to optimum sensitivity. The electrometer range was 10^{-10} amp/mv.

Materials and Reagents—Methenamine[2] and pentylenetetrazol[3] were used as received. Methenamine tablets[4,5] from two different companies were purchased in a local pharmacy. Absolute alcohol[6] USP was also used.

Analytical Calibration Curve—Six samples of methenamine[2], 105.0, 210.0, 420.0, 634.0, 845.0, and 1680.0 mg, were weighed. Each sample was placed into a 100-ml volumetric flask, and 10.0 ml of pentylenetetrazol internal standard alcoholic solution (50 mg/ml) was added and diluted to volume with absolute alcohol. Three microliters of each solution was injected into the chromatograph. The calibration curve was then obtained by plotting the known concentrations of methenamine against the corresponding peak height ratios (methenamine–pentylenetetrazol).

Tablet Analysis—Randomly selected tablets were individually wrapped inside glassine weighing paper and crushed. The powder was transferred to a 25-ml volumetric flask containing about 10 ml of absolute alcohol, shaken until dissolved, and diluted to 25 ml with absolute alcohol. Methenamine tablets contain no insoluble excipients and are totally soluble in the alcohol. Aliquots of 4.0 ml each were removed, placed into 10-ml volumetric flasks containing 1.0 ml of pentylenetetrazol internal standard solution (50 mg/ml), and diluted to 10 ml with absolute alcohol. Three-microliter samples of each tablet solution were injected into the chromatograph.

Calculations—The ratio of the peak height of the sample to the internal standard for each tablet solution was determined. The amount of methenamine per tablet was calculated from:

$$\text{mg/tablet} = \frac{R_{sa}}{R_{st}} \times \text{standard weight} \times \text{dilution factor} \quad \text{(Eq. 1)}$$

where R_{sa} is the ratio of the methenamine peak height to the internal standard peak height for the sample solution, and R_{st} is the methenamine–pentylenetetrazol peak height ratio for the known standard solution (5 mg/ml in absolute alcohol).

[1] Varian Aerograph model 1830.
[2] Riker Laboratories, Northridge, CA 91324.
[3] Metrazol, Knoll Pharmaceutical Co., Whippany, NJ 07981.
[4] Eli Lilly and Co., Indianapolis, IN 46206.
[5] Uritone, Parke-Davis and Co., Detroit, MI 48232.
[6] U.S. Industrial Chemicals Co., New York, N.Y.