DISCUSSION

Circular dichroic spectropolarimetry is a modified form of UV absorption spectrophotometry applicable to compounds which are both optically active and absorb light (2). All of the accepted procedures for UV absorption apply equally well to circular dichroism, and the data obey the simple Beer's Law dependence. Adjusting instrument parameters is not a problem as it is with GC, high-pressure liquid chromatography, or mass spectrometry (8) which reduces the routine analysis time. The time is reduced even further where separation is not a prerequisite to identification.

The detection limit for tetracycline in urine is 1.8 μg/ml. This value easily could be improved with a more modern instrument equipped with computer data handling accessories. A lower limit of detection is also possible if tetracycline is first separated from other species which absorb in the UV because of an improved signal–noise ratio. Based upon known $[\theta]$ values for other drugs such as morphine (5), codeine (5), cocaine (6), and lysergide (7), and comparing these to the value for tetracycline, calculations show that these drugs also can be quantitated, but only at overdose levels with the spectropolarimeter[3].

In general terms the most difficult analytical problem will arise when a mixture of optically active drugs are present (7); then separation will again be necessary. Dissolved sugars or proteins and glucuronide derivatives of extracted metabolites do not absorb for the most part, and they are not interfering.

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Tick Repellents I: Ethylene Glycol Acetamides

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Abstract □ Acetamides derived from ethylene glycol were synthesized and evaluated as repellents for the brown dog tick *Rhipicephalus sanguineus*. Several of these compounds showed repellency equal to the standard repellents, *N,N*-diethyl-*m*-toluamide and butopyranoxyl.

Keyphrases □ Tick repellents—ethylene glycol acetamides, synthesized □ Acetamides—ethylene glycol, synthesized, tick repellents □ Ethylene glycol—synthesized acetamides, tick repellents

A previous study of insect repellents showed that alkyl triethylene glycol monoethers had good mosquito repellency, being superior to *N,N*-diethyl-*m*-toluamide in certain tests against *Aedes aegypti* mosquitoes (1). Amides in general are known to be repellent to mosquitoes and other insects, the most widely used amide repellent being *N,N*-diethyl-*m*-toluamide.

During World War II, an extensive repellent screening program took place at the USDA Laboratories in Orlando, Florida. Compounds were screened for repellency both to yellow fever and malaria mosquitoes, and against fleas and ticks (2, 3). The ticks used for that program were the lone star tick, and about one thousand compounds were evaluated as tick repellents.

Since that time, major emphasis has been on mosquito repellents, largely supported by the U.S. Army Medical Research and Development Command. However, recently more emphasis has developed with regard to other militarily important insects: sand flies and ticks.

A previous study (3) evaluated a series of amides against ticks (*Amblyomma americanum*) and found that the di-*n*-butyl toluamides were best. Another study (4) found that certain amides and esters were effective against hard and soft ticks (*Ixodes persulcatus* P. Sch., *Dermacentor pecitus* Herm., *D. marginatus* Salz., *Hyalomma asiaticum* P. Sch., and *Alectorobius tholozan papillipas* Birula). Evaluated were butylacetanilide, tetrahydroquinoline, a mixture of ethyleneoxide–carbon dioxide (1:9)[1], dibutyl adipate, dimethyl phthalate, *N,N*-diethyl-*m*-toluamide benzimide, isoamyl acetamide, and benzoyl piperidine.

In the present study, a combination of the amide function with the ethylene glycol moiety were examined for repellent activity against ticks.

EXPERIMENTAL[2]

2-(Hydroxyethyoxy)acetamides—2-(2-Hydroxyethoxy) - *N*, *N* - diisopropylacetamide was prepared as follows: Sodium (1.4 g, 0.006 mole) was dissolved in 13 ml (0.24 mole) of ethylene glycol. After cooling to room temperature, 12 g (0.0676 mole) of *N,N*-diisopropyl-chloroacetamide (prepared from chloroacetylchloride and diisopropylamine) was added. The mixture was stirred at 90° for 1 hr. The ethylene glycol was distilled under reduced pressure and the residue taken up in ether, filtered to remove the sodium chloride, evaporated *in vacuo*, and distilled[3] to give 10.6 g of product, 125° air bath temperature/0.8 mm Hg.

2,2′-Ethylenedioxy-bis(*N,N* - dialkylacetamides)—2,2′- Ethylenedioxy-bis(*N,N*-diisopropylacetamide) was prepared as follows: A 150-ml three-necked flask was fitted with a stirrer, a reflux condenser, and a dropping funnel. Sodium (0.78 g, 0.034 mole) was suspended by vigorous stirring in 20 ml of boiling xylene. Ethylene glycol (1.05 g, 0.017 mole) was dropped slowly into the sodium suspension at reflux temperature, the suspension stirred and refluxed for an additional 7 hr, and then 6 g (0.034 mole) of *N,N*-diisopropyl-chloroacetamide in 15 ml of xylene was dropped into the stirred suspension at reflux temperature during 1 hr. The reaction mixture was refluxed and stirred for an additional hour.

[1] Carboxide.

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

[3] Distilled with a Kugelrohr.

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Table I—2-(2-Hydroxyethoxy)acetamides [a]

Compound	R	Boiling point, 0.5 mm Hg	Yield, %	IR, cm^{-1}	Formula	Calc.	Found
I-1	C_2H_5, C_2H_5	135°	46	OH, 3400 Amide, 1650	$C_8H_{17}NO_3$	C 54.84 H 9.78 N 7.99	54.80 9.99 7.80
I-2	CH_3, H	136° mp 47	47	OH, 3300 Amide I, 1650 Amide II, 1550	$C_5H_{11}NO_3$	C 45.10 H 8.33 N 10.52	44.95 8.08 10.27
I-3	$CH(CH_3)_2$, $CH(CH_3)_2$	130°	86	OH, 3300 Amide, 1650	$C_{10}H_{21}NO_3$	C 59.09 H 10.41 N 6.89	58.97 10.55 6.70
I-4	Cyclohexyl, Cyclohexyl	170° mp 65	77	OH, 3300 Amide, 1650	$C_{16}H_{29}NO_3$	C 67.81 H 10.31 N 4.94	67.87 10.67 4.76

[a] $HO—CH_2CH_2OCH_2CONR_2$.

Table II—2,2′-Ethylenedioxy-bis-(*N,N*-dialkylacetamides) [a]

Compound	R	Boiling point, 0.5 mm Hg	Yield, %	IR, cm^{-1}	Formula	Calc.	Found
II-1	C_2H_5	160°	42	Amide, 1650	$C_{14}H_{28}N_2O_4$	C 58.31 H 9.79 N 9.71	58.44 9.91 9.79
II-2	$CH(CH_3)_2$	160°	56	Amide, 1650	$C_{18}H_{36}N_2O_4$	C 62.76 H 10.53 N 8.13	62.81 10.61 7.81

[a] $R_2N—COCH_2OCH_2CH_2OCH_2CONR_2$.

Table III—*N,N*-Dialkyl-2(2-alkyloxyethoxy)acetamides [a]

Compound	R	R′	Boiling Point, 0.5 mm Hg	Yield, %	IR, cm^{-1}	Formula	Calc.	Found
III-1	C_2H_5	C_8H_{17}	135°	55	Amide, 1650	$C_{16}H_{35}NO_3$	C 66.86 H 11.57 N 4.87	67.04 11.72 4.87
III-2	C_2H_5	C_6H_{13}	118°	59	Amide, 1650	$C_{14}H_{31}NO_3$	C 64.83 H 11.72 N 5.40	64.84 10.98 5.38
III-3	Cyclo- hexyl	CH_3	142°	95	Amide, 1650	$C_{17}H_{33}NO_3$	C 68.65 H 10.51 N 4.71	68.68 10.49 4.84
III-4	C_2H_5	$C_2H_5OCH_2CH_2$	120°	45	Amide, 1650	$C_{12}H_{27}NO_4$	C 58.27 H 10.19 N 5.66	58.34 9.99 5.61
III-5	C_2H_5	C_4H_9	104°	50	Amide, 1650	$C_{12}H_{27}NO_3$	C 62.30 H 10.89 N 6.05	62.35 10.93 5.96
III-6	$CH(CH_3)_2$	C_4H_9	112°	62	Amide, 1650	$C_{14}H_{31}NO_3$	C 64.83 H 11.27 N 5.40	64.77 11.26 5.32

[a] R′—O—$CH_2CH_2OCH_2CONR_2$.

Table IV—*N,N,N′,N′*-Tetraalkyl-2,2′-oxydiacetamides [a]

Compound	R	Boiling point, 0.5 mm Hg	Yield, %	IR, cm^{-1}	Formula	Calc.	Found
IV-1	C_2H_5	145°	91	Amide, 1650	$C_{12}H_{24}N_2O_3$	C 58.99 H 9.90 N 11.47	59.00 10.04 11.29
IV-2	C_3H_7	155°	91	Amide, 1650	$C_{16}H_{32}N_2O_3$	C 63.96 H 10.74 N 9.32	64.17 10.90 9.40
IV-3	C_4H_9	175°	96	Amide, 1650	$C_{20}H_{40}N_2O_3$	C 67.37 H 11.31 N 7.86	67.61 11.48 7.80

[a] $R_2NCOCH_2OCH_2CONR_2$.

After cooling to room temperature and filtering off the sodium chloride, the xylene was removed under reduced pressure. The residue was distilled with a spin evaporator to give 3.3 g of product, 160° air bath temperature/0.8 mm Hg.

***N,N*-Dialkyl-2(2-alkyloxyethoxy)acetamides**—*N,N*-Diethyl-2-(2-hexyloxyethoxy)acetamide was prepared by dropping 2-hexyloxyethanol (6 g, 0.041 mole) into a stirred suspension of 0.945 g (0.041 mole) of sodium in 20 ml of xylene. After refluxing for 1 hr, 6.14 g (0.041 mole) of *N,N*-diethylchloroacetamide was dropped into the alcoholate suspension. After refluxing for an additional 3 hr, the xylene was removed under reduced pressure. The residue was distilled with a spin evaporator to give 7.4 g of liquid, 125° air bath temperature/0.8 mm Hg. A second distillation gave 6.3 g of pure product, bp 134°/1.1 mm Hg.

***N,N,N′,N′*-Tetraalkyl-2,2′-oxydiacetamides**—*N,N,N′,N′*-Tetrapropyl-2,2′-oxydiacetamide was prepared by dropping diglycoloyl chloride (15 g, 0.0877 mole) in 10 ml of methylene chloride into a stirred

Table V—Tick Repellency Mean Percent Repellency at Test Level, mg/cm² [a]

Compound	1.0	0.66	0.44	0.29
I-1	15	—	—	—
I-2	17	—	—	—
I-3	40	—	—	—
I-4	30	—	—	—
II-1	—	—	5	—
II-2	45	—	15	—
III-1	85	40	10	—
III-2	90	30	—	—
III-3	100	75	45	5
III-4	13	—	—	—
III-5	50	20	—	—
III-6	100	45	15	—
IV-1	15	—	—	—
IV-2	40	—	—	—
IV-3	45	—	—	—
N,*N*-Diethyl-*m*-toluamide	80	58	30	17
Butopyranoxyl	95	—	30	—
Solvent Control	15	—	—	—
Nontreated Control	12	—	—	—

[a] Average of three separate tests.

solution of 48 ml (0.35 mole) of dipropylamine in 100 ml of methylene chloride at −40° during 30 min. The mixture was stirred at room temperature for an additional 10 hr. The methylene chloride was evaporated and the residue taken up in 300 ml of ether and filtered to remove the hydrochloride salt of the dipropylamine. The filtrate was extracted with concentrated potassium hydroxide solution. After drying with anhydrous magnesium sulfate and removal of ether *in vacuo*, the residue was distilled with a spin evaporator to yield 24 g of product, 165° air bath temperature/1.0 mm Hg.

Biological Testing—*Rhipicephalus sanguineus*, the brown dog tick, was the test arthropod. The assay was designed to take advantage of the natural inclination of unfed ticks to climb upward.

Test materials were weighed, dissolved in 95% ethanol, and 0.15 ml of the solution was applied to a disk. The disks were cut from filter paper[4] and were 2.9 cm in diameter. One disk was used per compound per treatment level. Treated disks were kept under a hood and allowed to dry for 24 hr before use.

Disks were then inserted in drilled-out vial caps so that the treated sides faced down when the caps were placed on the test chamber. The test chamber was a polystyrene vial (25 × 52 mm) with an untreated disk glued on the drilled out bottom. Fifteen holes were punched in both disks on the chamber with a 23-gauge needle.

Twenty unfed adult brown dog ticks (10 male and 10 female) sorted 24 hr before use, were placed in each test chamber. The chambers were held with the treated end upright under a hood and were slightly elevated on tongue depressors. After four hours, when the ticks had ceased wandering, the chamber was observed and the number of ticks that settled on the treated surface were counted. The results were expressed in the percentage of ticks repelled from the treated surface. The maximum test level was 1.0 mg/cm² and the dose increments were 0.18 log intervals. Nontreated, standard-treated, and solvent-treated disks were included in each test.

The minimum concentration for the standard *N*,*N*-diethyl-*m*-toluamide that gave consistent significant (>80%) repellency was found to be 1.0 mg/cm². Test materials active at levels lower than 1.0 mg/cm² were considered to be more repellent than *N*,*N*-diethyl-*m*-toluamide.

[4] Number 3 Whatman.

RESULTS AND DISCUSSION

For this study, it was necessary to develop a suitable tick repellency assay, and the brown dog tick, *R. sanguineus*, was chosen as the test species. A common behavior of ticks is to crawl upward and this was taken advantage of in the assay used. The apparatus used consisted of a plastic vial with a filter paper impregnated with the test substance in the top. A measurement was made of the number of ticks climbing upward after a set period of time, with varying concentrations of the test chemical on the filter paper.

Chemical data on the compounds synthesized are presented in Tables I–IV.

Repellency data are presented in Table V. With the tick assay used here, *N*,*N*-diethyl-*m*-toluamide and butopyranoxyl both exhibited good repellency at 1 mg/cm² concentration. Since it was the purpose of this study to determine whether these new compounds were better repellents than these standards, they were tested initially at 1-mg/cm² concentration except for Compound II-1.

Compounds described in Table I having a free hydroxy group were poor repellents, as were the compounds from Table II. The volatility of the bis-compounds in Table II probably was too low (bp = 160°/0.5 mm Hg). Also, two of the compounds in Table I were solids.

Compounds from Table III were more repellent, some of them being equivalent to *N*,*N*-diethyl-*m*-toluamide and butopyranoxyl at 1 mg/cm² concentrations. Compound III-3 seems the best of all, with a repellency of 45% at 0.44-mg/cm² concentration, compared to 30% for *N*,*N*-diethyl-*m*-toluamide and butopyranoxyl at that concentration.

Compounds described in Table IV were not very repellent, probably due to their higher boiling points and reduced volatility.

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