position. The urine was processed by the described method, and the developed chromatogram was placed on X-ray film for 48 hr. The resulting radioactive spot on the X-ray film had the same shape and position as the white spot on the chromatogram.

CONCLUSIONS

Urine surveillance is an integral part of every successful drug abuse treatment and rehabilitation program. For urine surveillance to be effective, samples must be collected, labeled, and transported under controlled conditions. Urine must be analyzed accurately, and results must be reported promptly. A reliable laboratory method, specific for abuse drugs, is required to avoid both false-positive results, which would damage a patient's confidence and morale, and false-negative data, which could permit the undetected use of abuse drugs to continue.The laboratory method must also be inexpensive and efficient enough to evaluate a high volume of samples rapidly on a continuing basis.

The barbiturate identification procedure developed by the present investigators satisfies these requirements. It is particularly significant that interference from sedatives and tranquilizers has been eliminated. The method described in this paper avoids false-positive results and provides reliable identification of barbiturate metabolites in the urine of patients taking various drugs.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 11, 1973, from the *Clinical Pharmacology Service, Wood Veterans Administration Center, Milwaukee, WI 53193*

Accepted for publication August 7, 1973.

The authors thank Mrs. Terry Ellsworth, Miss Constance Gabriel, and Ms. Elizabeth Hieb for technical assistance, and Dr. James M. Fujimoto and Dr. David L. Roerig for their valuable advice.

▲ To whom inquiries should be directed.

Precursor-Type Insect Repellents: Kinetics of Hydrolysis

DANIEL D. GARNER and LORRIN R. GARSON▲

Abstract □ The rates of ester hydrolysis of representative dihydroxyacetone monoesters were determined by titrimetric analysis. The esters were designed to elicit prolonged insect repellency by gradual hydrolysis in dermal tissue. No correlation was found between the agents' repellent activity and their susceptibility to hydrolysis. The repellency elicited by the esters appears to be due to the intact molecules rather than a hydrolytic product.

Keyphrases □ Dihydroxyacetone monoesters—rates of hydrolysis, effect on insect repellency □ Insect repellents, dihydroxyacetone monoesters—rates of hydrolysis, compared to repellent activity □ Hydrolysis kinetics—dihydroxyacetone monoesters, attempted correlation of hydrolysis rates and repellent activity

In previous articles from these laboratories, the syntheses and insect repellent data of 1,3-dihydroxy-2-propanone (dihydroxyacetone) monoesters were reported (1–4). The activity of these precursor-type repellents was explained, in part, by their ability to hydrolyze, subsequent to topical application, and thereby release the insectifugal acids. The rationale of this approach was previously discussed in detail (2, 5). Compounds I, III, IV, VI, XI, and XIII (Table I) were studied because of their similarities in structure and physical properties and also because of their wide range in repellent activity. Due to the many physical parameters which reportedly play a role in the insect repellency of a compound (6), the objective was to ascertain if the hydrolysis of the dihydroxyacetone esters was responsible for their activity or if some other factors were involved.

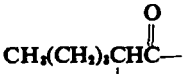
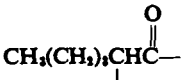
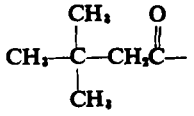
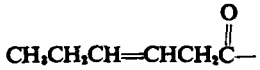
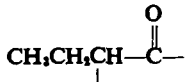
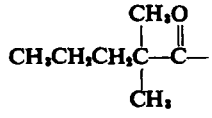
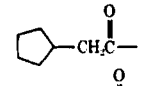
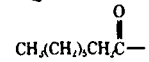
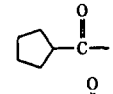
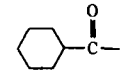
EXPERIMENTAL

Materials—Compounds I, III, IV, VI, XI, and XIII were previously prepared in these labotatories (1–4). However, since these agents can exist in both monomer and dimer (a cyclic hemiketal) forms, the structure and purity of these moieties had to be confirmed. Heating the dimeric dihydroxyacetone monoesters in ethanol converts the dimer to the corresponding monomeric form (7). Solutions of the esters in either acetone or ethanol (the ethanol solution had been previously heated) elicited identical chromatographic behavior, that is, a single spot with the same R_f values[1]. Therefore, in an acetone–water (3:1) solution, the medium selected to conduct the hydrolysis studies, the esters would appear to be exclusively in the monomeric state. IR spectra of the samples were equivalent to those of analytically pure compounds; NMR spectra were consistent with their respective structures[2].

[1] The TLC procedure utilized Mallinckrodt SilicAR TLC-7GF as the sorbent and acetone–water (3:1) as the solvent system. The compounds were applied to the plates and, after development, the spots were visualized with iodine vapor.

[2] Spectra (IR and NMR) were obtained with the Beckman model IR-33 and the Hitachi Perkin-Elmer model R-24 spectrophotometers, respectively.

Table I—1,3-Dihydroxy-2-propanone Esters

Compound	R	Repellency Index[a]	k_2 (SD)[b]	$t_{1/2}$, min.
I		0.62	2.66 (0.05)	188
II		0.54	—	—
III		0.37	1.87 (0.04)	267
IV		0.30	1.13 (0.03)	442
V		0.26	—	—
VI		0.19	1.43 (0.04)	350
VII		0.19	—	—
VIII		0.15	—	—
IX		0.13	—	—
X		0.13	—	—
XI		0.11	2.53 (0.05)[c]	198
XII		0.11	—	—
XIII		0.08	2.11 (0.04)	237

[a] The repellency index is the average protection time effected by the dihydroxyacetone monoester divided by the average protection time effected by the standard, diethyltoluamide. Repellency tests were conducted on the forearms of human volunteers as previously described (1–4). [b] Units of k_2 are liters mole^{-1} minute^{-1}. [c] The reproducibility of the technique was evaluated using Compound XI and found to be ±4.59%.

The two aqueous potassium hydroxide solutions[3] employed were standardized against potassium acid phthalate and found to be 0.3905 and 0.004684 *N*. These solutions are designated Solutions 1 and 2, respectively.

Kinetic Measurements—The kinetics of the base-catalyzed reaction were recorded[4] at constant pH (pH 12). The procedure employed for Compounds I, III, IV, VI, XI, and XIII is illustrated by the following example for Compound I.

A 5.00×10^{-2}-mmole sample of I [25 ml. of a 2.00×10^{-3} *M* solution in acetone–water (3:1)] was pipeted into the reaction vessel. After equilibration at 25 ± 0.2°, 0.128 ml. (5.00×10^{-2} mmole) of Solution 1 was pipeted[5] into the solution. The disappearance of base was then followed as a function of time by the continual addition of Solution 2 so that a pH of 12 was maintained[6].

To confirm that the reaction rate being studied was indeed ester hydrolysis, 1.30 g. (6.00 mmoles) of XI was subjected to conditions identical to those already described [3000 ml. acetone–water (3:1), 15 ml. (6.00 mmoles) of Solution 1]. After 30.5 hr., the acetone was removed from the mixture by distillation *in vacuo*, affording a

[3] Vaughn, Inc., Memphis, Tenn.

[4] The components consisted of a pH meter (PHM28), an automatic titrator (TTT11), a titrigraph (SBR2c), and a syringe buret assembly (SBU1). A saturated calomel electrode (Radiometer type K401) and a glass electrode (Radiometer type G202C) were employed, and stirring was accomplished by means of a mechanical electrically driven motor, as provided by the Radiometer Co. The jacketed glass reaction cell was thermostated at 25 ± 0.2° by circulating water from a Heto (Denmark) Ultrathermostat.

[5] This was accomplished with a 250-μl. syringe with a 10.16-cm. (4-in.) needle (Hamilton Co., Inc.).

[6] A more detailed description of the instrumental procedure was given by Stanley (8).

residual aqueous solution which was brought to pH > 12 with Solution 1 and extracted with chloroform (6 × 50 ml.). The aqueous phase was acidified with dilute hydrochloric acid and extracted with chloroform (8 × 50 ml.). The chloroform extracts of the acidic aqueous solution were combined, dried, and distilled *in vacuo*, affording a residual liquid. Distillation *in vacuo* of the oily residue yielded pure octanoic acid (0.39 g., 45%), b.p. 63–64° (0.05 mm.); IR (carbon tetrachloride): 3400–2500 (—OH) and 1705 (C=O) $cm.^{-1}$; NMR ($CDCl_3$): δ 11.82 (s, 1, —COOH), 2.36 (m, 2, $—CH_2CO—$), 1.33 [m, 10, $CH_3(CH_2)_5CH_2—$], and 0.88 (m, 3, $CH_3—$) p.p.m.

RESULTS AND DISCUSSION

The rates of hydrolysis of the compounds followed second-order kinetics, as determined by obedience of the data to the criteria established for second-order reactions. The mean and standard deviation of the rates were computed for each compound and are tabulated in Table I along with the calculated half-lives. The kinetic rates were calculated employing Eq. 1, which requires the concentration of the ester and base to be equivalent:

$$k_2 = \frac{1}{at}\left[\frac{x}{(a-x)}\right] \qquad \text{(Eq. 1)}$$

where k_2 is the second-order rate constant, a is the initial concentration of the ester or base, t is time, and x is the concentration of the ester that has been hydrolyzed at time t.

Under the experimental conditions, the concentration of base present in the reaction mixture is slowly increased, relative to the ester, as the standard base is titrimetrically added. However, the expected increase in kinetic rate was not observed until approximately 10% of the initial ester had been hydrolyzed; that is, the data fit Eq. 1 prior to reaching this point. In calculating the reported rates, only values obtained prior to less than 8% ester hydrolysis were employed.

The order of the rates follow what would be expected from consideration of steric factors (9). For example, introduction of alkyl substituents at the α- or β-carbon atoms markedly diminishes the rate of hydrolysis (compare I and XI with III, IV, and VI). As expected, XIII is more susceptible to hydrolysis than III, XIII being less sterically hindered about the ester carbonyl.

The stability of these agents to *in vitro* acid-catalyzed hydrolysis prompted an examination of the relationship between hydrolysis and biological response. Although the dermal environment is slightly acidic and, therefore, an *in vitro* acid-catalyzed study would be expected to approximate more closely *in vivo* conditions, the determination of acid-catalyzed rates was not experimentally feasible. In a preliminary experiment employing XI (0.02 *N*) in 0.05 *N* sulfuric acid [in acetone–water (3:1)], no detectable hydrolysis was observed within 48 hr. However, according to Newman (9) regarding the structure of the acyl component, the *ranking* of the rates of hydrolysis is probably the same whether carried out under acidic or basic conditions—even though the rate for a specific ester is generally faster in saponification (10).

CONCLUSIONS

It is possible to ascertain whether hydrolysis (acid or base catalyzed) is the major factor regulating repellency of the dihydroxyacetone monoesters. From the data given in Table I, it is readily apparent that there is no correlation between insectifugal activity of the compounds and their ease of hydrolysis. The order of repellent activity of the compounds studied is I > III > IV > VI > XI > XIII, whereas the order of their hydrolytic rates is I > XI > XIII > III > VI > IV. The difference in insect repellency of the corresponding free acids does not account for the lack of correlation; whereas Compounds I (repellency index 0.62) and XI (repellency index 0.11) have similar rate constants, their corresponding acids (hexanoic acid and octanoic acid, respectively) have widely different repellent activity, with octanoic acid being a much better repellent than hexanoic acid[7]. Accordingly, if chemical hydrolysis were a controlling factor, XI would be expected to elicit the greater biological response.

These kinetic studies do not preclude the possibility of hydrolysis of the esters by enzymes in the skin; cholinesterase, the lipases, and probably other esterases are known to be present in dermal tissue (12). Enzymes catalyzing the hydrolysis of esters are generally nonspecific; for example, it has been shown that ethyl hexanoate and ethyl octanoate are hydrolyzed by horse liver carboxylesterase at relative rates of 0.6 and 0.8, respectively, with ethyl butyrate taken as unity (13). Again comparing I and XI, enzymatic cleavage of I would only diminish insect repellent activity whereas scission of XI could be expected to increase repellency. One can only conclude that the insect repellency observed for these esters is due to the intact molecule.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 11, 1973, from the *Department of Medicinal Chemistry, College of Pharmacy, University of Tennessee Medical Units, Memphis, TN 38103*

Accepted for publication August 7, 1973.

Abstracted in part from a dissertation to be submitted by D. D. Garner (American Foundation for Pharmaceutical Education Fellow, 1972–1973) to the University of Tennessee in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by Research Contract DA-49-193-MD-2636 from the U. S. Army Medical Research and Development Command, Washington, D. C.

The authors gratefully acknowledge the technical advice of Dr. James W. Stanley of the National Center for Toxicological Research, Jefferson, Ark.

▲ To whom inquiries should be directed.

[7] Hexanoic acid is completely ineffective as an insect repellent against *Aedes aegypti* L. mosquitoes. Under the same testing conditions, octanoic acid is effective for more than 10 days when applied to cloth (11).