Dermophilic Insect Repellents with Perdurable Efficacy

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Abstract □ The synthesis of the monohexanoate, monopropionate, and monobenzoate esters of dihydroxyacetone is reported; the compounds were designed to provide long-lasting insect-repellent efficacy. The insectifugal properties of these compounds and those of the corresponding monoundecanoate ester are also described. While several of the compounds exerted significant repellency, the perdurable effect of dihydroxyacetone monohexanoate (II) was particularly noteworthy.

Keyphrases □ Dihydroxyacetone—synthesis of monohexanoate, -propionate, -benzoate esters □ Insect repellents, dermophilic, long-lasting—dihydroxyacetone monohexanoate, -propionate, -benzoate, -undecanoate esters, evaluation □ Synthesis—dihydroxyacetone monohexanoate, -propionate, -benzoate esters, insect-repellent activity

This approach to the development of dermophilic[1] insect repellents with prolonged activity involves the synthesis of compounds, designed for topical application, incorporating an insect-repellent component along with a component capable of anchoring to the skin; the respective entities are linked together by chemical bonds, constituting the so-called "precursor molecule" (1–4). The specifics of the rationale have been discussed in detail relatively recently (3). While the precursor molecule may be an effective repellent *per se*, it is the gradual breakdown of the precursor molecule, anchored to the epidermis, that is expected to provide long-lasting protection by means of the sustained release of the repellent component.

In two preceding communications (3, 4), the authors reported the preparation of dihydroxyacetone monoundecanoate (I). Based upon the effects elicited by I and upon the observations of Drake and Melamed (5), it was felt that structural designs inherent in the monohexanoate, monopropionate, and monobenzoate esters of dihydroxyacetone (II, III, and IV, respectively) should contribute substantially to the elucidation of relationships between chemical constitution and insect repellency (6). These compounds, like I, derive their dermophilic properties from the α-hydroxymethyl ketone moiety (7), retaining the hydroxyacetone ester function known to enhance susceptibility substantially to the hydrolytic process (8). In addition to the synthesis and the insectifugal efficacies of Compounds II, III, and IV, the insect repellency of I is reported here for the first time.

Compounds II–IV were prepared by procedures patterned after the direct acylation employed in the synthesis of I (4). Their properties are summarized in Table I. Compound II was also prepared by the method utilizing the diazoketone intermediate (3); however, the former method of preparation turned out to be a much more feasible one. The conditions associated with monomer and dimer formation in a monoester of dihydroxyacetone were discussed earlier (4); the physicochemical characteristics of Compounds II–IV parallel those observed in the referenced communication. Since the formulation used in the insect-repellent tests assured conversion to the sought monomeric state in each instance (4), circumstances did not warrant characterization of the monomers and dimers of the three new compounds.

The repellency of Compounds I–IV against *Aedes aegypti* mosquitoes is summarized in Table II. Compound II completely prevented biting at the 4- and 8-hr. test intervals and effected a dramatic reduction in biting at the 26-hr. test interval compared to the corresponding control. It is important to note the similarity in effects produced by Compounds I and II at the 22- and 26-hr. intervals; in both cases there was a resurgence of repellent effects from the 22-hr. test to that at 26 hr.

The test 22 hr. after application (after the overnight period) tends to coincide with the point following a period of limited exertion and limited perspiration, while that at 26 hr. appears to coincide with a 4-hr. active period following the overnight hours. If these premises are accepted, the results may be interpreted in terms of enhanced hydrolytic release of the repellent component prior to the 4-, 8-, and 26-hr. testing points. The repellency level of Compounds I and II with respect to the USDA standard deet, at the 26-hr. interval, is also noteworthy, particularly that of Compound II.

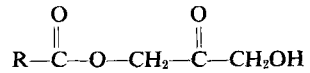
In addition to the tests summarized in Table II, Compound II was subsequently evaluated at additional time intervals; the treated subjects remained in a warm, humid room for a 3-hr. period immediately prior to the evaluation. Under these conditions, Compound II provided essentially complete protection 16 and 20 hr. after application to the skin [biting at 16 hr., 1.9% (control 64%, $LSD_{0.05}$ 10.8); biting at 20 hr., 9.7% (control 64%, $LSD_{0.05}$ 10.8)].

The repellent efficacy of Compound III is also apparent; the latter provided almost complete protection 4 and 8 hr. after application. The fact that it exerted no repellency at the subsequent test intervals can be interpreted in terms of a comparatively increased rate of hydrolysis normally associated with propionic acid esters with respect to those of substantially larger aliphatic acids. The relatively higher volatility of the *released* propionic acid with respect to the larger homologs also could be a contributing factor.

The specific contributions of the precursor molecules' intrinsic repellency and of the released acid-components have not been ascertained. It is apparent, however, that hydrolytic release of the latter is associated with maximum protection.

[1] Dermophilic properties include all those chemical and physical characteristics of an organic molecule that contribute to its affinity for the skin.

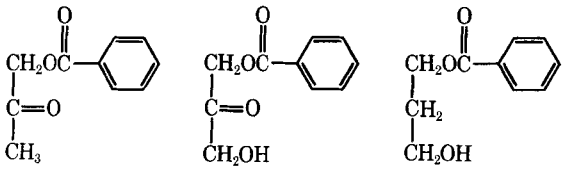
Table I—Esters of Dihydroxyacetone



No.	R	B.p. (mm.)	M.p.	Yield, %	Formula	Anal., % Calcd.	Found
II	n-C_5H_{11}	114–116°(0.25)	...	52.0	$C_9H_{16}O_4$	C, 57.43 H, 8.57	C, 57.57 H, 8.43
III	C_2H_5	80–82°(0.10)	...	21.2	$C_6H_{10}O_4$	C, 49.31 H, 6.90	C, 49.52 H, 6.92
IV	C_6H_5	...	86.7–89.2°[a]	25.4	$C_{10}H_{10}O_4$	C, 61.85 H, 5.19	C, 61.86 H, 5.18

[a] The analytical sample was obtained by recrystallization from ethanol after the more insoluble dihydroxyacetone dibenzoate (V) had been removed by crystallization from this solvent. The latter was obtained in 6% (crude) yield; the analytical sample (*Anal.*—Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.34; H, 4.68.) melted at 123.6–124.1°; Romo (9) reports m.p. 118–119°.

The lack of insectifugal activity observed with Compound IV is somewhat surprising when one considers that the two closely related analogs, 1-hydroxy-2-propanone benzoate (VI) and 1,3-propanediol monobenzoate (VII), are effective repellents (10).



VI IV VII

These findings constitute the results of exploratory work involving structural designs, with gradual changes in chemical constitution or physical properties or both, which enabled correlations between molecular configuration and biological response in terms of concepts reasonably well established in contemporary theoretical chemistry. The work currently in progress leads to the anticipation of even more encouraging results; some of these are expected to be reported in the authors' next communication.

EXPERIMENTAL

Synthetic Work[2]—The esters of dihydroxyacetone, listed in Table I, were prepared by the procedure reported in the authors' preceding communication (4). The hexanoyloxyacetic acid (VIII) used in the earlier cited alternate method (3) for the preparation of dihydroxyacetone monohexanoate (II) distilled at 130–132° (1.8 mm.), m.p. 30.7–33.9°, $\nu_{max.}^{CHCl_3}$ 1745 cm.$^{-1}$ (carbonyl), and was obtained in a 46.0% (59.6 g.) yield.

Anal.—Calcd. for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 55.37; H, 8.04.

The latter (VIII) was converted through 1-hexanoyloxy-3-diazoacetone (IX) to Compound II, b.p. 100–102° (0.1 mm.).

Anal.—Calcd. for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found: C, 57.52; H, 8.69.

Evaluation of Insect-Repellent Activity—Female *Aedes aegypti* mosquitoes, 7–8 days old, were confined in small cylindrical cages (4 × 12 cm.). The sides of the cages were clear plastic; one end was covered with gauze and the other end was fitted with a plastic slide closure. Mosquitoes in stock cages were immobilized by exposure to a low temperature, and six females were placed in each small cage. The cages were then held in a warm room for at least 1 hr. to permit the mosquitoes to recover before tests were begun. Three squares, each 25 cm.^{2}, were outlined on the skin of each forearm of three subjects, and each square was treated with 20 mg./cm.^{2} of the repellent. The latter was applied as an ethanol solution heated on a steam bath for 30 min. prior to application. Tests were made by placing the end of the cage equipped with the slide in contact with a treated area on a human arm and by opening the slide to give the mosquitoes direct access to the treated skin for a period of 1 min. In each test period, cages of mosquitoes were exposed to untreated areas of the skin to provide checks on the percentage biting. Three tests on each of three subjects were employed in determining average values.

In the tests reported in Table II, the treated subjects remained in a room maintained at 27.7° (82°F.) and 78–80% relative humidity during the 8-hr. workday. In the subsequent evaluations conducted on Compound II, the subjects remained in the temperature- and humidity-controlled room only for 3 hr. immediately prior to the repellency test.

Table II—Percent of Mosquitoes (*Aedes aegypti*) Biting Forearms of Human Volunteers at Various Intervals after Topical Application

Repellent[a]	*LSD*[b]	Percent Biting at Hours Indicated[c] 4	8	22	26	30
Compd. I	14.4	24.2	22.2	52.4	13.1	28.3
Control	(14.4)	(69.7)	(59.2)	(40.8)	(40.8)	(59.2)
Compd. II	9.4	0[d]	0	61.2	8.2	53.8
Control	(9.4)	(79.7)	(68.4)	(57.4)	(57.4)	(76.8)
Compd. III	11.6	5.5	2.8	73.4	81.6	77.3
Control	(11.6)	(62.9)	(62.9)	(75.1)	(75.1)	(62.9)
Compd. IV	9.4	57.3	53.2	63.1	68.2	83.3
Control	(9.4)	(79.7)	(68.4)	(57.4)	(57.4)	(76.8)
Deet[e]	12.1		0		55.7	
Control	(12.1)		(69.5)		(68.7)	

[a] Application rate 20 mg./cm.^{2}, applied in ethanol solution. [b] Least significant difference at the 0.05 level. [c] Average of three tests on each of three subjects with six mosquitoes per test. [d] In addition, one subject received no bites when exposed to a stock cage (containing 1000–1500 mosquitoes) for 3 min. [e] *N,N*-Diethyl-*m*-toluamide; application rate 3 mg./cm.^{2}.

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[2] Boiling points are uncorrected. Melting points are corrected; they were determined with a Büchi melting-point apparatus. IR spectra were obtained with a Perkin-Elmer model 137B spectrophotometer. Analyses were performed by Dr. G. Weiler and Dr. F. B. Strauss, Oxford, England, and by Galbraith Laboratories, Inc., Knoxville, Tenn.

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Interactions of Drugs with Proteins I: Binding of Tricyclic Thymoleptics to Human and Bovine Plasma Proteins

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Abstract □ Data on the binding of imipramine, desipramine, and 3-chlorodesmethylimipramine to plasma proteins have been obtained over a wide range of ligand concentration using a modified equilibrium dialysis technique. Plasma proteins other than albumin do not appreciably contribute to complex formation with the drugs studied. Fifty-nine percent imipramine is bound to albumin in the plasma level range reached under therapeutic conditions. However, the association constants of the complex is low. Species differences in the binding capacity of albumin were observed. Apparently, atypical binding behavior was disclosed for desipramine and 3-chlorodesmethylimipramine. A binding model is discussed, and values of binding parameters are given.

Keyphrases □ Thymoleptics, tricyclic—binding to human, bovine plasma proteins □ Proteins, human, bovine plasma—imipramine, desipramine, 3-chlorodesmethylimipramine, interaction, binding, model

These studies on the binding of drugs and other compounds to plasma proteins are aimed at gaining insight into the following problems:

1. Possible influence on the pharmacokinetics of a drug by its interactions with proteins.
2. Mechanism of interactions between drugs or model compounds and proteins and physicochemical interpretation.
3. Critique of methods used in the study of drug–protein interactions.

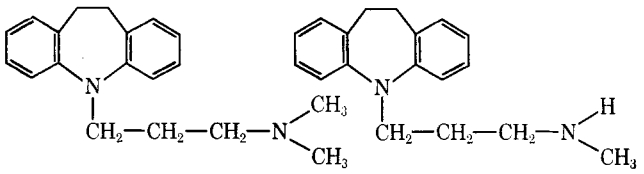
Part I contains data on the complex formation of imipramine, its active metabolite desipramine (desmethylimipramine), and 3-chlorodesmethylimipramine with bovine albumin and human albumin, γ-globulin, plasma, and serum in concentration ranges including the one met with under therapeutic conditions. The first two drugs are used clinically; the latter is a metabolite of the clinically used 3-chloroimipramine accumulating in rats (1) and human (2).

Imipramine (IP) is one of the few drugs about which much of the metabolic and pharmacokinetic data is known (3–8). In addition, physicochemical data of all its major metabolites have been reported (9). Sensitive methods exist for determining imipramine, desipramine, and 3-chlorodesmethylimipramine (5, 6, 10, 11).

Data on IP binding to plasma proteins were published by Tinao and Gomez-Guillen (12) in 1963. Data and species differences in the binding of desipramine to plasma proteins have recently been reported by Borgå *et al.* (13). Earlier, Gillette (14, 15) reported on the binding of imipramine to liver microsomes, which presumably is the reason for the high concentrations of imipramine and related drugs in lung, liver, or kidney tissue in the rat *in vivo* (5, 8). Beside many reports on interactions between thymoleptics and membranes [reviewed by Glowinski and Baldessarini (16)], an interaction between imipramine and the outer membrane of blood platelets has also been reported (17).

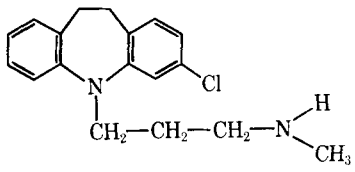
EXPERIMENTAL

Materials—The hydrochlorides of imipramine,[1] desipramine[1] (DMI), and 3-chlorodesmethylimipramine[1] (CDMI) were used.



imipramine
I

desipramine
II



3-chlorodesmethylimipramine
III

Also used were 10,11-^{14}C-imipramine hydrochloride[2] (8.05 mc./mmole) and ^{3}H-acetic anhydride[2] (in benzene, 500 mc./mmole). Gas chromatographic (11) and spectrophotometric tests showed

[1] Supplied by Geigy Ltd., Basel, Switzerland.
[2] Acquired from Radiochemical Centre, Amersham, England.