TABLE I—VIABLE MICROORGANISMS PER GRAM FOUND IN NONSTERILE ANTIBIOTICS

Antibiotic Powders	Bacteria/Gm.	Molds/Gm.
Antifungal		
Antibiotic A	6,700	None detected
Antibiotic B	400	None detected
Antibiotic C	200	None detected
Antibiotic D	80	15
Antibiotic F (old process)	9,400,000	2
Antibiotic F (old process)	1,000,000	None detected
Antibiotic F (new process)	3,000	None detected
Antibiotic F (new process)	2,000	None detected
Antibacterial		
Nafcillin	None detected	None detected
Bacitracin	None detected	None detected
Neomycin	18	None detected
Zinc bacitracin	2	38
Penicillin	12	None detected
Tyrothricin	None detected	3

No other counts approached those of Agent F. As shown in Table I, the highest counts were found in samples of the antifungal antibiotics, indicating that they may present more of a problem than the antibacterial antibiotics.

The methods described here can be applied to bacterial counting of most antibiotics. However, the solvent systems should be modified according to the solubility characteristics of the antibiotic or antibiotic formulation under test. Any organic solvent used should, of course, be nontoxic to the contaminating microorganisms. Since the problem encountered with Agent F was gross bacterial contamination of a spore-forming organism, the tests were developed specifically for spore-forming bacteria. The techniques described may also be used for fungal contamination by using Sabouraud's agar in place of nutrient agar, incubating at 25° instead of 32°, and extending the incubation time from 2 to 5 days.

Antibiotic drugs, even those that are represented as being nonsterile, should be relatively free of microorganisms. Gross contamination is an indication of poor manufacturing practices. In addition, Curtis *et al.* (6) have stated that any organism, under certain conditions, can be considered pathogenic.

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Keyphrases

Antibiotic preparations—bacterial contamination

Antifungal preparations—bacterial contamination

Agar overlay—test procedure

Synthesis of Imidazolthiones from 3,4-Diphenyl-4-oxazolin-2-thione

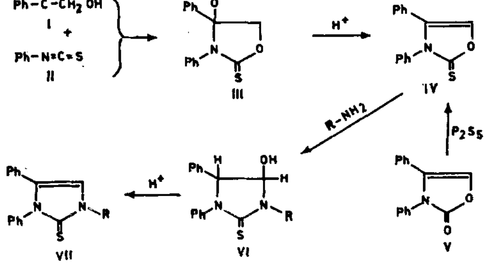
By S. CARBONI, E. GROTHE, and M. F. SAETTONE

The synthesis of some 4-hydroxyimidazolidin-2-thiones (VI) and 4-imidazolin-2-thiones (VII) by a method involving the reaction of 3,4-diphenyl-4-oxazolin-2-thione (IV) with primary amines is described.

A METHOD FOR THE preparation of imidazolones, based on the reaction of 5-unsubstituted 4-oxazolin-2-ones (such as V, Scheme I) with primary amines, was recently reported (1). In view of the interesting bacteriostatic (2, 3), fungistatic (3, 4), and CNS-depressant (5) activities reported for some 4-imidazolin- and imidazolidin-2-thiones, the authors have applied their method to the preparation of imidazolthiones from 4-oxazolin-2-thiones; some preliminary results are reported herein.

3,4-Diphenyl-4-oxazolin-2-thione (IV), prepared either from phenacyl alcohol (I) and phenyl isothiocyanate (II) through the intermediate 3,4-diphenyl-4-hydroxyoxazolidin-2-thione (III), or from V and P_2S_5, was chosen as a model compound for this study.

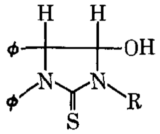
Treatment of IV with some primary amines gave good yields of 3,4-diphenyl-5-hydroxyimidazolidin-2-thiones (VI, see Table I). These could be easily dehydrated to afford the corresponding 4-imidazolin-2-thiones VII (Table II). In order to secure a structure proof for compounds of type VII, 1-



Scheme I

Received January 3, 1968, from the Institute of Pharmaceutical Chemistry, University of Pisa, 56100 Pisa, Italy.
Accepted for publication February 23, 1968.
The cooperation of Mr. Alessandro Simonini in the experimental work is acknowledged.

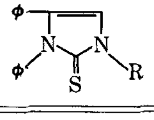
TABLE I—3,4-DIPHENYL-5-HYDROXYIMIDAZOLIDIN-2-THIONES



No.	R	M.p., °C.[a]	Yield, %	Formula	Anal., % Calcd. C	H	N	S	Found C	H	N	S
1	Benzyl	169–171	70	$C_{22}H_{20}N_2OS$	73.31	5.59	7.77	8.88	73.05	5.70	7.71	8.78
2	2-Diethylaminoethyl	119–121	68	$C_{21}H_{27}N_3OS$	68.27	7.37	11.37	8.66	68.15	7.30	11.44	8.87
3	2-Morpholinoethyl	183–185	87	$C_{21}H_{25}N_3O_2S$	65.78	6.57	10.96	8.34	65.57	6.73	11.02	8.45
4	2-Pyrrolidinoethyl	165–167	83	$C_{21}H_{25}N_3OS$	68.64	6.86	11.44	8.70	68.37	6.89	11.66	8.90

[a] Uncorrected.

TABLE II—3,4-DIPHENYL-4-IMIDAZOLIN-2-THIONES



No.	R	M.p., °C.[a]	Yield, %	Formula	Anal., % Calcd. C	H	N	S	Found C	H	N	S
5	Benzyl	177–178	92	$C_{22}H_{18}N_2S$	77.17	5.30	8.18	9.35	76.96	5.57	8.32	9.10
6	2-Diethylaminoethyl	108–109	95	$C_{21}H_{25}N_3S$	71.77	7.17	11.96	9.10	71.61	7.09	11.93	9.27
7	2-Diethylaminoethyl HCl	197–199	45	$C_{21}H_{26}ClN_3S$	65.01	6.75	10.83		64.98	6.98	10.96	
8	2-Morpholinoethyl	132–135	87	$C_{21}H_{23}N_3OS$	69.02	6.34	11.50	8.76	68.98	6.49	11.73	8.84
9	2-Morpholinoethyl HCl	206–209	64	$C_{21}H_{24}ClN_3OS$	62.76	6.01	10.45		62.93	6.24	10.38	
10	2-Pyrrolidinoethyl	129–131	94	$C_{21}H_{23}N_3S$	72.18	6.63	12.03	9.16	72.03	6.49	12.27	9.02
11	2-Pyrrolidinoethyl HCl	227–230	36	$C_{21}H_{24}ClN_3S$	65.35	6.26	10.89		65.62	6.57	11.00	

[a] Uncorrected.

benzyl-3,4-diphenyl-4-imidazolin-2-thione (Compound 5) was independently synthesized (Scheme II) from 2-benzylaminoacetophenone (VIII) and phenyl isothiocyanate (II), following a known synthetic method (6) for 4-oxazolin-2-thiones.

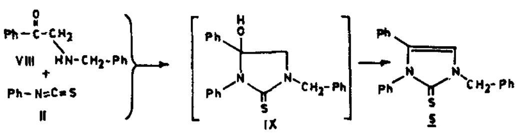
In the course of the work, compounds of type VII were observed to differ noticeably in reactivity from their oxygenated analogs, 4-imidazolin-2-ones. A chemical investigation on these compounds is now in progress and will be the object of a further communication.

All new compounds described herein were submitted to a primary biological screening[1] aimed at detecting CNS, autonomic, antifungal, antibacterial, and antiviral activity. Compound 2 exhibited weak antiviral activity *in vitro*, and compound 10 showed smooth muscle relaxant activity *in vitro* (minimal effective dose 10 mcg./ml.). Both levels of activity were considered too weak to consider further study.

EXPERIMENTAL[2]

3,4-Diphenyl-4-oxazolin-2-thione (IV)—*From V*—A solution of 3,4-diphenyl-4-oxazolin-2-one (1) (V, 5.0 Gm., 0.021 mole) in xylene (150 ml.) was treated with P_2S_5 (10 Gm.) according to the procedure described by Gompper (7) for the preparation of 4,5-diphenyl-4-oxazolin-2-thione. The solution, after a 10-hr. reflux, afforded 2.85 Gm. (53%) of IV as colorless prisms, m.p. 159–161° after recrystallization from benzene, IR $\lambda_{max.}$ (nujol) 6.70, 6.91, 7.25, 7.42, 7.70, 8.81, 10.28, 10.61, 12.90, 13.31, 14.24 μ.

From I and II—A mixture of phenacyl alcohol (I, 13.6 Gm., 0.1 mole), phenyl isothiocyanate (II, 13.5 Gm., 0.1 mole), and pyridine (0.3 ml.) was heated at 120° for 4 hr., then allowed to cool. Trituration of the semisolid reaction mixture with benzene afforded 8.8 Gm. of an impure solid, which was fractionally crystallized from ethanol to afford 5.1 Gm. (18%) of a compound, m.p. 161–163° after recrystallization from benzene, IR $\lambda_{max.}$ (nujol) 3.17, 6.71, 6.94, 7.36, 7.68, 8.21, 9.41, 10.25, 13.02, 14.19 μ, which was identified as 3,4-diphenyl-4-hydroxyoxazolidin-2-thione[3] (III).



Scheme II

Anal.—Calcd. for $C_{15}H_{13}NO_2S$: C, 66.41; H, 4.83; N, 5.16. Found: C, 66.23; H, 4.63; N, 5.32.

Concentration of the mother liquor from III afforded unreacted phenacyl alcohol (2.0 Gm.), then a small amount (0.5 Gm.) of a compound with m.p. 173–175°, identified as 1,3,4-triphenyl-4-imidazolin-2-thione by comparison with an authentic sample[4].

A solution of III (8.13 Gm., 0.03 mole) in glacial acetic acid (25 ml.) was refluxed for 1 hr. After evaporation of the solvent under reduced pressure,

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[1] Carried out by Bristol Laboratories, Syracuse, N. Y.

[2] Melting points were determined on a Kofler hot stage and are uncorrected.

[3] In no case phenacyl thiocarbanilate, open-chain tautomer of III, could be isolated. It should be pointed out that the oxygenated analog of III could never be obtained: phenacyl carbanilate, formed in the reaction between phenacyl alcohol and phenyl isocyanate, resisted all attempts at tautomerization to 3,4-diphenyl-4-hydroxyoxazolidin-2-one (*cf.* 1).

[4] Prepared according to McCombie and Scarborough (*cf.* 6). The compound originates probably in the reaction between *N,N′*-diphenylthiourea (formed as a secondary product in the reaction mixture) and phenacyl alcohol. Indeed, when equimolar amounts of these compounds were heated together at 160° for 4 hr., 1,3,4-triphenyl-4-imidazolin-2-thione was obtained in 67% yield.

the residue was crystallized from ethanol to afford 7.0 Gm. (92%) of IV, identical with the previously obtained material. The analytical sample was crystallized from ethanol and melted at 162–163°.

Anal.—Calcd. for $C_{15}H_{11}NOS$: C, 71.14; H, 4.37; N, 5.53. Found: C, 70.94; H, 4.24; N, 5.75.

Procedure for the Preparation of the 5-Hydroxyimidazolidin-2-thiones VI (Table I)—Solutions of IV (2.53 Gm., 0.01 mole) in the appropriate amine (benzylamine, 2-diethylaminoethylamine, 2-morpholinoethylamine or 2-pyrrolidinoethylamine, 15 Gm. *ca.*) were heated at 100° for 8–9 hr. The mixtures were then poured into ice cold water (1000 ml.); the product which separated was collected and crystallized from aqueous ethanol. A further crystallization from ethanol or from benzene–petroleum ether afforded the pure products in the yields reported in Table I.

Procedures for the Preparation of the 4-Imidazolin-2-thiones VII (Table II)—Compound 5 was prepared as follows: a solution of 1 (3.6 Gm., 0.01 mole) in ethanol (70 ml.) was treated with 1.0 ml. of concentrated H_2SO_4, then was heated to boiling. Crystalline 5 began to separate from the hot solution. After cooling, the product was collected and recrystallized from ethanol.

Compounds 6 and 8 were prepared by the same method, from 2 and 3, respectively. The acid ethanolic solution was treated with excess 10% Na_2CO_3 to precipitate the products; these were then crystallized from aqueous ethanol.

Compound 4 could best be dehydrated to 10 by the following procedure: a solution of 4 (1.1 Gm., 3.0 mmoles) in glacial acetic acid (2.0 ml.) was refluxed for 1 hr., then was poured into cold 10% Na_2CO_3 (50 ml.). The solid was collected and recrystallized from aqueous ethanol.

The hydrochlorides 7, 9, and 11 were prepared from the corresponding bases in the conventional fashion, and were purified by crystallization from absolute ethanol.

Structure Proof of 1-Benzyl-3,4-diphenyl-4-imidazolin-2-thione (Compound 5)—Treatment of 2-benzylaminoacetophenone hydrochloride (8) (VIII) with phenyl isothiocyanate, according to the procedure described by McCombie and Scarborough (*cf.* 6) for the preparation of 1,3,4-triphenyl-4-imidazolin-2-thione, gave a 15% yield of 5; IR $\lambda_{max.}$ (nujol) 6.70, 6.91, 7.15, 7.71, 7.95, 8.30, 9.35, 10.40, 13.40, 13.59, 14.24 μ.

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Keyphrases

Imidazolthiones—synthesis
3,4-Diphenyl-4-oxazolin-2-thione—derivatives
Pharmacological screening
IR spectrophotometry—structure

New Compounds: *N*-Hydroxy- and *N*-Acetoxy-Metabolites of *N,N'*-(2,7-Fluorenylene)bisacetamide

By NELLIE W. PITZER and FRANCIS E. RAY*

The partial catalytic reduction of 2,7-dinitrofluorene (I) in dimethylformamide, ethyl acetate, and acetic anhydride has yielded: *N*-hydroxy-*N*-(2-fluorenyl-7-nitro)acetamide (VII); *N*-acetoxy-*N*-(2-fluorenyl-7-nitro)acetamide (II); *N'*-hydroxy-*N,N'*-(2,7-fluorenylene)bisacetamide (XI); *N,N'*-dihydroxy-*N,N'*-(2,7-fluorenylene)bisacetamide (VIII); and *N,N'*-diacetoxy-*N,N'*-(2,7-fluorenylene)bisacetamide (IV).

THE ISOLATION and subsequent synthesis of *N*-hydroxy-*N*-(2-fluorenyl)acetamide as a proximate carcinogenic metabolite of *N*-(2-fluorenyl)-acetamide (1) led to the undertaking of the synthesis of a similar *N*-hydroxy compound as a possible metabolite for *N,N'*-(2,7-fluorenylene)bisacetamide (VI) which is known to produce carcinoma of the glandular stomach in rats (2).

It has been reported recently that *N*-acetoxy-*N*-(2-fluorenyl)acetamide is an ultimate carcinogen of *N*-(2-fluorenyl)acetamide (3). Therefore the acetoxy esters synthesized have taken on increased biological interest.

The procedure for the catalytic partial reduction and the acetylation of 2-nitrofluorene in ethyl acetate and the subsequent hydrolysis in ammonium hydroxide (4) applied to 2,7-dinitrofluorene (I) gave a small yield of *N*-hydroxy-*N*-(2-fluorenyl-7-nitro)acetamide (VII).

After hydrolysis the ethyl acetate solution contained a mixture of reduction products. This prompted the use of *N*-(2-fluorenyl-7-nitro)acetamide (III) for catalytic reduction, as the only *N*-hy-

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Received August 28, 1967, from Pharmaceutical Chemistry Research Laboratory, University of Florida, Gainesville, FL 32601

Accepted for publication November 11, 1967.

This work was supported by Public Health Research grants No. 5 RO1 CA 08186 and No. 2 RO1 CA 07737 from the National Cancer Institute.

The authors wish to thank Dr. Danuta Malejka for her assistance in preparing this manuscript.

* Deceased November 25, 1966.