The Preparation and Fungistatic Properties of Thiocarbohydrazones[1]

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The reaction of thiocarbohydrazide with carbonyl-containing compounds has been reported by several authors investigating heterocyclic ring formation[2-5] and by those suggesting[6,7] that thiocarbohydrazide be used as an analytical reagent. Owing to the presence of the thiocarbonyl group, however, thiocarbohydrazones could be useful as fungicides.[8-10] The work reported here was undertaken in order to investigate this possibility.

The condensation of an aldehyde or ketone with thiocarbohydrazide proceeds readily but there is the question whether a mono (I) or a di derivative (II) is obtained. Stephen and Wilson[3] recognized that the second hydrazine group reacts with ketones much less readily than the first; to obtain II they used excess ketone as the reaction medium. The lack of agreement in melting points reported by different authors for the same thiocarbohydrazones indicates that a simple 2:1 ratio of aldehyde or ketone to thiocarbohydrazide does not normally produce II in good yield.

In the present work, a 1:1 *M* ratio of aldehyde to thiocarbohydrazide was used to produce monothiocarbohydrazones (Table I). In preparing dithiocarbohydrazones, however (Table II), the ratio of carbonyl compound to thiocarbohydrazide used was never less than 4:1, and frequently the former was used as the reaction medium. Evidence that the reaction products reported here have the structures I and II is provided by the elemental analysis[11] and ir absorption data obtained. Where the melting points listed in the tables do not agree with the values reported previously,[2,6,7] it seems probable that a mixture of I and II was obtained in the earlier work.

TABLE I

MONOTHIOCARBOHYDRAZONES OF SOME ALDEHYDES

$RCH{=}NNHCSNHNH_2$ (I)

No.	Aldehyde	Yield,[a] %	Mp, °C	Formula[b]
1	Heptanal	89	165	$C_8H_{18}N_4S$
2	Octanal	71	161	$C_9H_{20}N_4S$
3	Nonanal	93	163	$C_{10}H_{22}N_4S$
4	9-Undecenal	91	161	$C_{12}H_{24}N_4S$

[a] Recrystallized from EtOH. [b] Analyzed for C, H, N, S.[11]

TABLE II

SOME DITHIOCARBOHYDRAZONES

$RR_1C{=}NNHCSNHN{=}CRR_1$ (II)

No.	Aldehyde or ketone	Yield, %	Mp, °C	Formula[c]
5	Heptanal	35[a]	119	$C_{15}H_{30}N_4S$
6	Octanal	41[a]	120	$C_{17}H_{34}N_4S$
7	Nonanal	43[a]	116	$C_{19}H_{38}N_4S$
8	Decanal	92[a]	113	$C_{21}H_{42}N_4S$
9	Undecanal	95[a]	107	$C_{23}H_{46}N_4S$
10	9-Undecenal	89[a]	99	$C_{23}H_{42}N_4S$
11	Dodecanal	95[a]	106	$C_{25}H_{50}N_4S$
12	*m*-Nitrobenzaldehyde	90[b]	245	$C_{15}H_{12}N_6O_4S$
13	2,4-Dichlorobenzaldehyde	61[c]	250	$C_{15}H_{16}N_4SCl_4$
14	2-Octanone	75[a]	60	$C_{17}H_{34}N_4S$
15	2-Heptadecanone	98[d]	82	$C_{35}H_{70}N_4S$

[a] Recrystallized from hexane. [b] Recrystallized from DMF–H_2O. [c] Recrystallized from dioxane–H_2O. [d] Recrystallized from EtOH. [e] Analyzed for C, H, N, and S (also Cl for 13).[11]

All of the thiocarbohydrazones listed in Tables I and II prevent the growth of *Chaetomium globosum* at a concentration of 10 ppm in the tube dilution test.[9] The fungistatic effectiveness of this whole class of compounds in this test is equivalent to that of the best of the commercial fungicides.

Compound **14** was applied to a 10-oz cotton duck[12] by spraying the fabric with or dipping it in a solution of the fungicide which also contained 1% or less of a surfactant. Pieces of this fabric, after drying, were subjected to the plate-disk test method.[9] The following fungistatic effectiveness was observed with disks having a 3 wt % add-on of **14**: with *Aspergillus niger*, no growth on disks; with *C. globosum*, no growth on disks and >3 mm zone of inhibition extending out from the perimeter of the fabric disk. Untreated disks or those treated only with wetting agent showed no zone of inhibition with *C. globosum* and were overgrown by *A. niger* after 24 hr.

Cotton duck on which 1.5% of **14** and ~0.1% wetting agent (on the fabric weight) had been applied showed no loss of breaking strength in the soil burial test.[13] Untreated control fabric lost 70% of the original breaking strength during the same 2-week burial period. The effectiveness of those compounds, listed in the tables, as a class, in preventing the microbial degradation of cellulose is impressive.

Experimental Section

The thiocarbohydrazide and aldehydes and ketones used in this work were the purest grade obtainable from commercial sources. Melting points were measured with a Fisher–Johns apparatus and are corrected. Ir spectra of the thiocarbohydrazones were measured on a Model 21 Perkin-Elmer spectrophotometer (KBr). Elemental analyses were carried out at the microanalytical laboratory of Drs. Weiler and Strauss in Oxford, England.

Preparation for Monothiocarbohydrazones (Table I).—To a solution of thiocarbohydrazide (0.01 mol) in H_2O (30 ml) heated on a steam bath was added a solution of aldehyde (0.01 mol) in EtOH. After 15-min heating, the solid which precipitated out during slow cooling was collected by filtration, washed with cold

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(11) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.
(12) Traditional designation in fabric business.
(13) Canadian Government Specifications Board, Canadian Standard Textile Test Methods (4-GP-2), Method 28.3.

50% H_2O–EtOH, dried, and recrystallized; characteristic ir absorptions, compounds **1-4**: 3160–3200 (NH); 1635 (C=N and NH_2); 1530 (CNH); 1125 (NCN); 810–830 (C=S) cm^{-1}.

Preparation for Dithiocarbohydrazones (Table II).—A mixture of thiocarbohydrazide (0.01 mol) and aldehyde or ketone (<0.04 mol) was heated from 0.5 to 1 hr at 100°, diluted with hexane, then cooled. The precipitate that formed was collected by filtration, washed with cold hexane, dried, and recrystallized; characteristic ir absorptions, compounds **5-15**: 3120–3200 (NH); 1600–1645 (C=N); 1535–1560 (CNH); 1120–1145 (NCN); 810–850 (C=S) cm^{-1}.

The fungistatic properties of the mono- and dithiocarbohydrazones were evaluated by the tube dilution method,[9] using the tests organisms *C. globosum* (strain USDA 1042.4) and *A. niger* (strain USDA 215-5373.16). Concentrations of the compounds being tested of 10, 100, and 1000 ppm were tried. The criterion of effectiveness was taken to be the absence of fungal growth after a 2-week (*C. globosum*) or a 48-hr (*A. niger*) incubation period.

A typical thiocarbohydrazone, di-2-octanone thiocarbohydrazone, was applied to a 10-oz bleached cotton duck[10] from dioxane or EtOH solution containing a wetting agent. Either dioctyl sodium sulfosuccinate (Alrowet D-65, Geigy) or a linear aliphatic ethoxylate (Igepal A, General Aniline & Film Corp.) were used at a level of 1–10 wt % of the fungicide. Suitable pieces of the treated fabric as well as control pieces were subjected to the plate-disk method[9] and the soil burial method.[10] Breaking strengths of fabric evaluated by the latter method were measured at 21.1° and 65% RH with the Instron tensile tester (type TT-C) using 152.4 × 25.4 mm ravelled strips, with the warp in the long direction. The number of warp yarns in the test samples was always the same, and the average of ten breaking strength values was used in each case.

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Antiprotozoal 4-Aryloxy-2-aminoquinolines and Related Compounds

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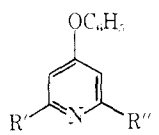
Recently we reported the inhibition of growth of *Tetrahymena pyriformis* and *Crithidia fasciculata* by ethers of 2,6-diamino-4-pyridone.[1] These compounds were shown to interfere with biopterin and/or with enzymes involved in fat metabolism. Since they have an N–C–N–C–N atomic sequence similar to the one in biopterin and other biologically active pteridines we wished to test whether or not this structural feature was necessary for inhibition of the organisms. We therefore synthesized 4-phenoxy-2-aminopyridine for comparison with 4-phenoxy-2,6-diaminopyridine which was the most active ether of the earlier series.

Since quinoline derivatives approximate pteridines in size more than do pyridine derivatives we also prepared and tested 4-phenoxy-2-aminoquinoline. A report of *in vitro* amebicidal activity of 4-propoxy-2-aminoquinoline and its homologs[2] also suggested testing of that compound. Although 4-phenoxy-2-aminoquinoline was a poor inhibitor of *C. fasciculata* it was found to inhibit *T. pyriformis* at considerably lower concentration than 4-phenoxy-2,6-diaminopyridine. In view of these results we prepared several analogs of that compound with substituents in the benzene ring in the hope of finding highly specific inhibitors which could be used as tools in the study of the intermediary metabolism of these organisms, since they (especially *T. pyriformis*) have been widely used in screening programs for antineoplastic compounds.[3]

Curtius degradation of the corresponding esters gave the desired amines. The prerequisite esters were obtained by reaction of ethyl 4-chloroquinaldate with appropriate phenoxides (or thiophenol[4]) and by phenylation of the Na salts of ethyl 4-pyridone-2-carboxylate and ethyl 4-quinolone-2-carboxylate with diphenyliodonium chloride.[5]

The growth of both *T. pyriformis* and *C. fasciculata* is inhibited by 4-phenoxy-2-aminopyridine (culture methods for biological tests were essentially the same as the ones reported previously[1]), but only at concentrations an order of magnitude greater than that of the diamine (Table I). The inhibition of *Crithidia* is not reversed by biopterin as is that caused by the diamine.

TABLE I
INHIBITORY EFFECTS OF 4-PHENOXYAMINOPYRIDINES AND 4-PHENOXYPYRIDINECARBOXYLIC ACID HYDRAZIDES



R'	R''	μmol/ml for half-max inhib. *T. pyriformis*	*C. fasciculata*
NH_2	H	0.085 (3)[a]	0.245 (3)
$CONHNH_2$	H	0.053 (2)	0.393 (1)
$CONHNH_2$	$CONHNH_2$	0.5 (2)	0.123 (5)
NH_2	NH_2	0.0084[b]	0.028[b]

[a] Number in parentheses is the number of experiments. [b] See ref 1.

The intermediate 4-phenoxypicolinic acid hydrazide was also tested and found to be active against *T. pyriformis* but not against *C. fasciculata*. In contrast, 4-phenoxydipicolinic acid dihydrazide[1] inhibited significantly only the latter organism (Table I). The substituted quinaldic acid hydrazides like the monohydrazide of the pyridine series inhibited *T. pyriformis* but were only slightly active against *C. fasciculata*.

The substituted aminoquinolines were significantly more active against *T. pyriformis* but not against *C. fasciculata* (Table II). The inhibitory effects of none of the quinoline derivatives was susceptible to reversal by biopterin or folic acid. A few preliminary tests with crude materials have shown that liver fraction 1, (Nutritional Biochemicals Corp.) contains a sub-

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