by the results of duplicate bioassays conducted by Laboratory 2 using the 2-aminopyridine salt of estrone sulfate as the standard; on the powder sample, values of 26.2 and 27.1 mg./Gm. were obtained, while the results on the tablet sample were 1.25 and 1.30 mg./tablet of sodium estrone sulfate.

The variability of the assay on the basis of the data available is about ±10% on a single sample weighing (95% probability limits). This is unsatisfactorily large and further refinements of the assay are now under study with the hope that they will lead to an appreciable increase in the reliability of the assay procedure. It is expected that the details of a reliable method for the rapid determination of the conjugated estrogen content of pharmaceutical preparations, based upon the collaborative work outlined in this report, will be published in the near future.

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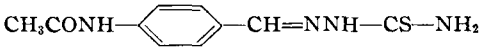
Amithiozone (Tibione) Analogs from Aralkyl Ketones*

By W. LEWIS NOBLES† and J. H. BURCKHALTER‡, §

The antitubercular thiosemicarbazones listed in the literature have been restricted almost exclusively to those derived from aldehydes. In the present report, a group of 18 thiosemicarbazones prepared from aralkyl ketones has been described. The carboxyl or amino group has been introduced into three of the products in an attempt to change their properties or activity. *p*-Chloroacetophenone thiosemicarbazone was found to have four times the *in vitro* effectiveness of 4,4'-diaminodiphenyl sulfone.

THREE years ago, the authors became interested in the synthesis of certain ketonically derived analogs of *p*-acetamidobenzaldehyde thiosemicarbazone (1), the potent antitubercular drug known as amithiozone or Tibione (I), be-

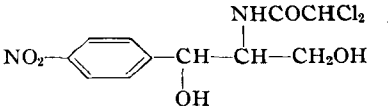


(I)

cause the literature failed to report many derivatives of such origin. Although one laboratory decided that thiosemicarbazones of aldehydes are more active than thiosemicarbazones of ketones, the group failed to list a single ketone derivative (2). Another group described the preparation of more than 100 thiosemicarbazones of aldehydes but only 7 such derivatives of ketones (3). However, a recent report indicates that the thiosemicarbazone of the ketone 4-acetylpyridine appears to have the same order of activity in mice as does amithiozone. (4).

DISCUSSION

All the compounds reported in Table I are ketone thiosemicarbazones. Several (compounds 1, 2, 3, 4, 5, and 6) are obvious variations of amithiozone (I). Others (5, 7, 8, 9, 10, 11, 12, and 13) are related to the antibiotic Chloromycetin (II), while still retaining a similarity to amithiozone. Three



(II)

of the compounds (8, 9, and 14) represent attempts to introduce solubilizing functional groups which might be expected to influence the distribution of the molecule in the host and bacterium.[1] Finally, recalling the intrinsic antibacterial effect of α,β-unsaturated carbonyl compounds (5), it was decided

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† Fellow of the American Foundation for Pharmaceutical Education. Present address: Assistant Professor of Pharmaceutical Chemistry, University of Mississippi, University, Miss.
‡ Professor of Pharmaceutical Chemistry, University of Kansas.
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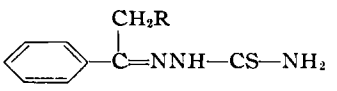
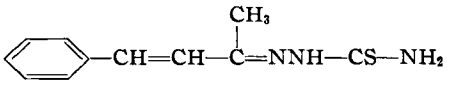
[1] After these studies had been initiated, Behnish, Mietzsch, and Schmidt (2) prepared the semicarbazones of certain aldehydes which were substituted with solubilizing groups. There were no comments on the efficacy of this type.

TABLE I.—THIOSEMICARBAZONES



No.[a]	R	Phenyl Substituents	Procedure	Yield, %	M. P.,[b] °C.	Formula	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
1	H	4-Chloro[c]	A	96	201	$C_9H_{10}ClN_3S$	47.46	47.14	4.42	4.57
2	CH_3	4-Chloro[c]	A	85	176	$C_{10}H_{12}ClN_3S$	49.67	50.01	5.00	5.16
3	H	4-Methoxy[c]	A	94	150	$C_{10}H_{13}N_3OS.H_2O$	49.61	49.59	6.25	6.14
4	H	4-Phenyl[c]	A	90	264	$C_{15}H_{15}N_3S$	66.88	66.80	5.61	5.77
5	H	4-Nitro[d]	A	90	244	$C_9H_{10}N_4O_2S$	45.36	45.87	4.23	4.03
6	H	Replace phenyl with 2-thienyl[e]	A	89	136[r]	$C_7H_9N_3S_2$	42.21	42.20	4.55	4.63
7	α-Dichloroacetamido	4-Nitro[f]	A	88	196	$C_{11}H_{11}Cl_2N_5O_5S$	36.27	36.36	3.06	3.08
8	Amino	4-Nitro[d]	B	80	222	$C_9H_{11}N_5O_2S.HCl$[o]	.....	.....	....	..
9	Dimethylaminomethyl	4-Nitro[g]	B	86	153	$C_{12}H_{17}N_5O_2S.HCl$[p]	.....	.....	....	..
10	Acetoxy	4-Nitro[h]	A	89	212	$C_{11}H_{13}N_5O_4S$	44.60	44.80	4.09	4.20
11	Acetamido	4-Nitro[d]	A	84	240	$C_{11}H_{13}N_5O_3S$	44.73	45.24	4.44	4.77
12	H	3,5-Dinitro[i]	A	90	258	$C_9H_9N_5O_4S$	38.17	38.17	3.21	3.10
13	H	4-(p-Nitrophenyl)[j]	A	77	265	$C_{15}H_{14}N_4O_2S$[q]	57.31	57.77	4.48	4.45
14	α-Carboxymethyl	None[k]	C	82	171	$C_{11}H_{13}N_3O_3S$	52.57	52.47	5.21	5.54



15	...	None[l]	A	95	147	$C_{11}H_{13}N_3S$	.....	.....	...	..
16	...	4-Nitro[i]	A	92	250	$C_{11}H_{12}N_4O_2S$	49.98	50.06	4.57	4.67
17	...	2-Chloro[m]	A	86	185	$C_{11}H_{12}ClN_3S$	52.06	52.50	4.77	4.71
18	...	2,3-Dimethoxy[n]	A	91	188	$C_{13}H_{17}N_3O_2S$	55.89	56.00	6.13	6.16

[a] Compounds 1, 3, 4, 6, 14, and 17 are white; 2, 5, 7, 8, 9, 11, 12, 13, 15, 16, and 18 are various shades of yellow; and 10 is orange.
[b] Melting points are uncorrected.
[c] Intermediate ketones from Eastman Kodak Co.
[d] Ketone obtained through the courtesy of Parke, Davis and Co.
[e] 2-Acetothienone obtained through the courtesy of Socony-Vacuum.
[f] α-Dichloroacetamido-p-nitroacetophenone prepared according to L. M. Long and H. D. Troutman, *J. Am. Chem. Soc.*, **73**, 481(1951).
[g] β-Dimethylamino-p-nitropropiophenone hydrochloride, m. p. 186–189°, prepared according to J. H. Burckhalter and S. H. Johnson, Jr., unpublished report.
[h] p-Nitrophenacyl acetate prepared according to C. Engler and C. Zielke, *Ber.*, **22**, 203(1889).
[i] For ketone, see experimental part.
[j] Ketone prepared according to W. S. M. Grieve and D. H. Hey, *J. Chem. Soc.*, **1933**, p. 968.
[k] β-Benzoylpropionic acid prepared according to "Organic Syntheses," Coll. Vol. II, p. 81.
[l] Thiosemicarbazone previously reported by S. Gheorghui, *Bull. soc. chim.*, [5] **1**, 97(1934).
[m] Supplied by Dr. S. H. Johnson; see ref. 5.
[n] Supplied by Mr. J. R. Campbell; see ref. 5.
[o] Calculated for Cl: 12.24. Found: 12.46.
[p] Calculated for Cl: 10.68. Found: 10.82.
[q] Purified only by successive treatments with hot solvents such as benzene, water, and alcohol.
[r] Ref. 6 gives 148–149°.

to prepare compounds 15, 16, 17, and 18 which might also be classed as vinylogous relatives (5) of compounds 1–6.

All the thiosemicarbazones were prepared by treatment of the ketone with thiosemicarbazide in alcohol-water solution in the presence of a few drops of mineral acid. Usually, fifteen to twenty minutes of heating at steam-bath temperature was sufficient time to gain complete reaction and satisfactory yields. This is in contrast to the period of six to eight hours reported by others (6). In several cases the product precipitated spontaneously when solutions containing the reagents were mixed. In other cases it was necessary, after heating for the required time, to cool the mixture to induce crystallization. With p-chloropropiophenone, the product appeared as a yellow oil after the period of heating, and vigorous agitation induced crystallization.

The intermediate ketones were, in general, prepared by the well-established methods of the literature. Because of the difficulty of obtaining and keeping p-nitrobenzaldehyde, as a result of the oxidizing effect of alkali and air, the best procedure for obtaining p-nitrobenzalacetone was found to be through the nitration of benzalacetone at −20° (7). Another ketone, 3,5-dinitroacetophenone, was prepared by modifying the general method for ketones developed by Walker and Hauser (8).

Biological Results.—The thiosemicarbazones of Table I were submitted to Dr. G. P. Youmans of Northwestern University for *in vitro* screening. Thus far, results of testing of 10 of the 18 compounds have been received: Nos. 1, 3, 4, 5, 6, 11, 12, 14, 15, and 17. p-Chloroacetophenone thiosemicarbazone (No. 1) is four times as active as 4,4′-diaminodiphenyl sulfone, the reference drug. β-Benzoylpropionic acid (No. 14) was devoid of activity at 10 mg. %. The remaining 8 compounds were reported to be active, though less so than compound 1.

While it is realized that a large percentage of compounds which show *in vitro* activity fail to possess *in vivo* effectiveness (9), several of the products of Table I have been submitted for *in vivo* testing.

EXPERIMENTAL[2]

3,5-Dinitroacetophenone.—The following procedure is similar to the general method of Walker and Hauser (8) but differs in several important details. Into a 300-ml. flask equipped with a mechanical stirrer, dropping funnel, and thermometer, 3.9 Gm. (0.11 mole) of magnesium turnings, 0.5 ml. of dry carbon tetrachloride, and 2.5 ml. of absolute alcohol were placed. As soon as the magnesium began to react with the alcohol, 25 ml. of dry chlorobenzene was added rapidly, and the reaction was allowed to proceed to completion.

A solution of 17.6 Gm. (0.11 mole) of diethyl malonate, 12.5 ml. of chlorobenzene, and 10 ml. of absolute alcohol was added to the cooled and stirred mixture at a rate to keep the temperature at about 65°. When the reaction had proceeded to the extent that removal of the cooling bath did not result in a rise in temperature, the mixture was heated slowly to 85° and kept there until the amount of unreacted magnesium became constant (usually about ninety minutes).

The clear, dark solution was cooled to 25°, and a solution of 23.1 Gm. (0.1 mole) of 3,5-dinitrobenzoyl chloride (Eastman) in 63 ml. of xylene was added with stirring and cooling so that the temperature did not exceed 35°. When about half of the acid chloride had been added, a brown gelatinous mass precipitated, and stirring (which was maintained for thirty minutes) became difficult. The flask was then cooled with ice, and a solution of 7 ml. of sulfuric acid in 50 ml. of water was slowly added. The mixture was transferred to a separatory funnel and the chlorobenzene layer was separated and concentrated *in vacuo*. The residue was heated at reflux temperature with a solution of 30 ml. of glacial acetic acid, 4 ml. of concentrated sulfuric acid, and 20 ml. of water for six hours, after which decarboxylation was complete. The mixture was added slowly with stirring to 400 Gm. of cracked ice. The solid product was filtered, washed with water, and then melted under 100 ml. of water and stirred while 12 Gm. of sodium bicarbonate was added. The product was again filtered and treated with 4 Gm. of sodium bicarbonate. After recrystallization from alcohol, 14.8 Gm. (67% yield) of off-white 3,5-dinitroacetophenone was obtained; m. p. 82–83°, reported (10) 82–84°.

***p*-Nitrobenzalacetone.**—To 400 Gm. of concentrated sulfuric acid cooled to 0°, 170 Gm. (1.17 moles) of benzalacetone was slowly added with stirring. By use of an acetone-dry ice bath, the temperature was lowered to −20°, and a nitrating solution containing 90 Gm. (1.18 moles) of concentrated nitric acid (sp. gr. 1.42) in 200 Gm. of concentrated sulfuric acid was added as rapidly as possible without allowing the temperature to rise above −15°. Large amounts of dry ice in the bath allowed rapid addition. Stirring was continued for three hours whereupon the yellow solid was collected on a fritted glass funnel, washed with water, and air dried. After recrystallization from alcohol, 155 Gm. (69% yield) of light yellow product was obtained; m. p. 109–110°, reported (7) 110°.

The *ortho* isomer was not isolated, since no trace of it was visible in the original filtrate of the *para* isomer. However, it has been isolated when the nitration proceeded at higher temperature (5).

ARALKYL KETONE THIOSEMICARBAZONES

Procedure A.—The ketone (0.05 mole) was dissolved in the minimum amount of alcohol. Concurrently, 4.5 Gm. (0.05 mole) of thiosemicarbazide was dissolved in the necessary volume of warm water. To a mixture of the two solutions, first 10 drops of concentrated hydrochloric acid and then 2 Gm. of sodium acetate trihydrate were added. The resulting mixture was heated on the steam bath for fifteen to twenty minutes during which time a heavy precipitate generally formed. The flask was cooled in an ice bath, and its contents were collected, washed with warm water, and dried in the air. Usually, thiosemicarbazide was more water soluble than the product; hence, trituration of the crude product with boiling water served as an excellent method of purification. An analytical sample was obtained by recrystallization from alcohol or diluted alcohol.

Procedure B.—The amino ketone hydrochloride (0.05 mole) was dissolved by warming in diluted alcohol to which a few drops of concentrated hydrochloric acid had been added. At the same time, 4.5 Gm. of thiosemicarbazide was dissolved in a minimum amount of warm water. A mixture of the two solutions was heated for almost twenty minutes. After cooling the solution overnight in an ice chest, the yellow crystals which had formed were collected on a funnel. An analytical sample was obtained by repeated recrystallizations from acidified diluted alcohol.

Procedure C.—A mixture of 8.9 Gm. (0.05 mole) of β-benzoylpropionic acid and 4.1 Gm. of sodium acetate trihydrate was dissolved in 125 ml. of alcohol. This solution was mixed with another containing 4.5 Gm. (0.05 mole) of thiosemicarbazide, 0.5 ml. of concentrated hydrochloric acid, and 100 ml. of warm alcohol. The mixture was heated at reflux temperature for about forty-eight hours. After cooling the solution overnight in an ice chest, the white solid product was collected and air dried. It was recrystallized from alcohol.

[2] Microanalyses by Mr. C. W. Beazley, Skokie, Ill.

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