acid to a distinct blue color, using crystal violet as indicator.

For purposes of comparison, codeine phosphate, chlorpromazine hydrochloride, and amphetamine sulfate were assayed by the methods official in B.P., while papaverine hydrochloride by that official in Ph.Dan. Results of these experiments are recorded in Table I.

Determination of Salts in Tablets

Codeine phosphate, papaverine hydrochloride, chlorpromazine hydrochloride, and amphetamine sulfate in tablets are determined in the same manner as directed in the assay of the salts, the quantity of the powdered tablets being equivalent to the stated amount of the corresponding salt. Results of these determinations are recorded in Table II. Experiments were carried out with standard tablets prepared in this laboratory.

SUMMARY

A simple method for the determination of certain acid salts of organic bases, based on the basic hydrolysis of the salt in the presence of magnesium oxide has been described. The proposed method proved to be a rapid, simple, and accurate one that makes it especially useful for routine work.

Examinations on the possibilities of the application of this procedure to other pharmaceutical preparations will be carried out and reported in a subsequent communication from this laboratory.

Antimicrobial Properties of Aliphatic Thiosemicarbazones

By M. MANOWITZ and G. WALTER

A series of thiosemicarbazones of saturated and α,β-unsaturated aliphatic aldehydes was prepared and tested *in vitro* for activity against various bacteria, yeasts, and molds. Antimicrobial potency was found to depend on chain length and was greatly enhanced by the presence of an unsaturated linkage. Thiosemicarbazones derived from unsaturated aldehydes with chain lengths C-10 to C-12 were the most active against the microorganisms tested.

THE TUBERCULOSTATIC PROPERTIES of thiosemicarbazones have been investigated extensively since the initial publication by Domagk and coworkers (1). A large number of thiosemicarbazones have been prepared and tested for these properties; however, their effect on other microorganisms has received only limited consideration (2–5). Benns, *et al.* (6), determined the antifungal activity of 40 thiosemicarbazones against *Aspergillus niger* and *Chaetomium globosum* and found the most effective compounds were derived from aliphatic aldehydes. Preliminary observations in our laboratories have demonstrated interesting antibacterial properties for certain aliphatic thiosemicarbazones; a further study of the antimicrobial spectrum of a more extensive series of these compounds was indicated. For this purpose, thiosemicarbazones of saturated and unsaturated aliphatic aldehydes were prepared and tested against various bacteria, yeasts, and molds.

EXPERIMENTAL

The thiosemicarbazones of the C-4 through C-12 straight chain saturated aldehydes and of the C-4 through C-13 α,β-unsaturated aldehydes, with the omission of the C-5 unsaturated compound, were included in this investigation. The thiosemicarbazones were prepared by usual methods described in the literature (3).

Antimicrobial Tests.—The antimicrobial properties of the compounds were determined by agar dilution technique employing the following organisms: *Staphylococcus aureus*, ATCC 6538; *S. epidermidis*, ATCC 155; *Bacillus subtilis*, ATCC 9372; *Escherichia coli*, ATCC 11229; *Proteus vulgaris*, ATCC 9920; *Pseudomonas aeruginosa; Bacterium ammoniagenes*, ATCC 6871; *Pityrosporum ovale; Candida albicans*, ATCC 10231; *Trichophyton mentagrophytes*, ATCC 9129; and *Microsporum audouini*, ATCC 11347.

Twofold serial dilutions of the compounds were prepared in alcohol (S.D.30) and 0.2-ml. aliquots of each dilution added to 20-ml. tubes of molten agar. The contents of the tubes were thoroughly mixed and poured into sterile Petri plates. Dextrose tryptone extract agar was employed for the bacteria and Sabouraud's dextrose agar was used for the yeasts and molds.

Bacterial inoculum consisted of 1–100 distilled water dilution of a 24-hour, tryptic soy broth culture grown at 35°. Yeast inoculum was prepared by washing a 3-day-old slant of the organism with 10 ml. of distilled water and diluting the suspended cells 1–100 with distilled water. Mold inoculum consisted of a conidial suspension from the surface growth of a 7-day slant of the organism in 20 ml. of distilled water. Plates were inoculated by placing 1 drop (0.007 ml.) of the inocula on the surface of the hardened agar media with the aid of an Accu-Drop dispenser.[1] Inoculated plates were incubated at 35° for bacterial tests and at 30° for yeasts and molds. Examination of the plates for the presence of growth was made after incubation periods of 48 hours for the bacteria, 4 days for the yeasts, and 14 days for the molds.

RESULTS

Results of the microbiological tests are summarized in Table I. None of the compounds was active against the Gram-negative bacteria (*E. coli, Ps. aeruginosa, Pr. vulgaris*) and therefore these organisms were not included in the table. The data

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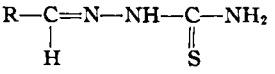
[1] Scientific Products, Flushing, Long Island, N. Y.

TABLE I.—ANTIMICROBIAL ACTIVITY OF THIOSEMICARBAZONES



Compd. No.	R	Minimum Concn., mcg./ml., Completely Inhibiting Growth of Organism[a]							
		S.a.	*S.e.*	*B.s.*	*B.a.*	*P.o.*	*C.a.*	*T.m.*	*M.a.*
I	$CH_3(CH_2)_2$	x[b]	x	x	x	x	x	50	50
II	$CH_3(CH_2)_3$	x	x	x	x	x	x	100	50
III	$CH_3(CH_2)_4$	x	x	x	x	x	x	100	50
IV	$CH_3(CH_2)_5$	x	x	x	x	x	x	50	50
V	$CH_3(CH_2)_6$	x	x	x	x	x	x	50	50
VI	$CH_3(CH_2)_7$	100	50	100	x	x	x	100	100
VII	$CH_3(CH_2)_8$	12.5	12.5	25	x	x	x	100	50
VIII	$CH_3(CH_2)_9$	12.5	12.5	12.5	12.5	x	x	100	50
IX	$CH_3(CH_2)_{10}$	x	x	x	x	x	x	x	x
X	$CH_3CH{=}CH$	x	x	x	x	x	x	x	x
XI	$CH_3(CH_2)_2CH{=}CH$	50	25	50	100	x	x	50	50
XII	$CH_3(CH_2)_3CH{=}CH$	50	50	100	50	100	x	100	50
XIII	$CH_3(CH_2)_4CH{=}CH$	12.5	6.25	12.5	12.5	50	50	25	12.5
XIV	$CH_3(CH_2)_5CH{=}CH$	3.12	3.12	6.25	6.25	25	25	12.5	3.12
XV	$CH_3(CH_2)_6CH{=}CH$	1.56	.78	1.56	1.56	x	100	3.12	1.56
XVI	$CH_3(CH_2)_7CH{=}CH$	.78	.78	.78	1.56	x	x	3.12	3.12
XVII	$CH_3(CH_2)_8CH{=}CH$	.78	.39	.78	.78	x	x	x	100
XVIII	$CH_3(CH_2)_9CH{=}CH$	x	x	50	50	x	x	x	x

[a] *S.a.* = *S. aureus*; *S.e.* = *S. epidermidis*; *B.s.* = *B. subtilis*; *B.a.* = *B. ammoniagenes*; *P.o.* = *P. ovale*; *C.a.* = *C. albicans* *T.m.* = *T. mentagrophytes*; *M.a.* = *M. audouini*. [b] x denotes growth at 100 mcg./ml.

demonstrated that the thiosemicarbazones of the α,β-unsaturated aldehydes were more potent antimicrobial agents than the thiosemicarbazones of the corresponding saturated aldehydes. Activity against Gram-positive bacteria, in the unsaturated group, increased with increasing chain length from C-4 through C-12, then abruptly decreased at C-13. Compounds XV, XVI, and XVII were the most effective against the bacteria, inhibiting growth at 1 mcg./ml. or less. The unsaturated thiosemicarbazones also demonstrated a corresponding chain length–activity effect against the molds but only up through C-11 (Compound XVI). Activity against yeasts for this group was exerted by Compounds XII, XIII, XIV, and XV.

Most of the saturated thiosemicarbazones possessed slight activity against *T. mentagrophytes* and *M. audouini* (50–100 mcg./ml. range); however, all were inactive against the yeasts and Gram-negative bacteria. Among the saturated compounds, only Compounds VI, VII, and VIII inhibited the growth of the Gram-positive bacteria.

Microbiological tests were also conducted on the individual aldehydes from which the thiosemicarbazones were prepared. Results of these tests demonstrated activity against *T. mentagrophytes* and *M. audouini* at the 50–100 mcg./ml. range for most of the unsaturated aldehydes and for undecanal and dodecanal. None of the aldehydes was effective against bacteria or yeasts at the highest concentration tested (100 mcg./ml.), except 2-dodecenal which inhibited the growth of Gram-positive bacteria at 50 mcg./ml.

DISCUSSION AND SUMMARY

This study has revealed that considerable activity is present among the thiosemicarbazones of aliphatic aldehydes. It is evident that the presence of an α,β-unsaturated linkage in the aldehyde from which the thiosemicarbazone is derived markedly enhances the effectiveness of these compounds. There is an apparent structure-activity pattern displayed by this series of compounds that is a function of the chain length of the molecule. Thiosemicarbazones prepared from aldehydes with chain lengths C-10 to C-12 demonstrated the greatest activity against the bacteria and molds tested.

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