apparent increase in carcass iron (iron absorption) was observed to occur in the limited (1 hour) time period prior to sacrificing and determination in any of the iron treated groups.

From these data on the control groups, an average percentage of approximately 12% of the total iron present was found in the gastrointestinal tract, leaving approximately 88% in the remainder of the carcass. The average iron content per mouse was 1.427 mg.; the average iron content per gram mouse was 53 mcg.

From the determination of the iron content in the feces from two groups of mice, the average iron content of the feces per mouse in the control group was 27 mcg. and in the iron treated group 13 mcg. This would appear to indicate that estimates of fecal iron could be ignored in selected studies.

SUMMARY AND CONCLUSIONS

As modified in this laboratory, the Fisher method was observed to be relatively accurate within concentration limits and comparatively easy to accomplish. The digestion of the animal was the most tedious part of the technique.

An average estimate of the total amount of iron present per mouse was 1.457 ± 0.152 mg. This represented an average of 52 ± 6 mcg. per Gm. body weight of mouse with approximately 11.5% of the total iron per mouse present in the gastrointestinal tract (168 ± 54 mcg.) and 88.5% in the remainder of the carcass (1.31 ± 0.14 mg.).

In the animals treated orally with 1, 2, and 4 mg. of iron 1 hour prior to sacrificing and determination, the average percentages of iron present in the gastrointestinal tracts were 21, 25, and 28, with 79, 75, and 72% in the remainder of the carcasses, respectively. Low inherent carcass iron content must be considered even when comparisons are made on a microgram iron content per gram weight basis as no apparent increase in carcass iron (iron absorption) was observed to occur in the limited (1 hour) time period prior to sacrificing and determining in any of the iron treated groups.

Employing this method, the quantity of iron apparently unaccounted for in the animals treated orally with iron was in direct linear relationship to the amount of iron administered or added and due to the inherent properties of the colorimetric method itself; therefore, the quantities of iron determined are relative and indicative of the true quantities of iron present.

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3-Bromopyruvic Acid, a Highly Selective Antimicrobial Agent Against Certain Yeasts

By WALTER A. ZYGMUNT and TELLIS A. MARTIN

3-Bromopyruvic acid has been shown to be a highly effective growth inhibitor of certain yeasts. Wide differences were noted in the inhibitory activity of the compound among different species and genera of yeast.

THE USE of halogens as antimicrobial agents is well documented, and certain halogenated metabolites possess potent antimetabolite activity. Among such compounds are fluoroacetic acid (1), halogenated phenylalanines (2), 5-halogenated pyrimidines (3), and many others.

Busch *et al.* (4) have reported that β-halogenated pyruvic acids have pharmacological activity as cholinesterase inhibitors. However, there appears to be only limited information in the literature on the activity of such compounds as possible antimicrobial agents. 3-Bromopyruvic acid was selected as a representative compound. This report summarizes our findings on its inhibitory activity against a variety of microorganisms.

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EXPERIMENTAL

Since significant growth inhibition was noted only with certain yeasts and yeast-like fungi, description of the testing techniques employed will be restricted to this group of microorganisms. Yeast nitrogen base medium (Difco), supplemented with 1.5% dextrose and an initial medium pH of 4.5, were used. Solutions of 3-bromopyruvic acid and—where applicable—of reversal agents were adjusted to pH 4.5, sterilized by Seitz filtration, and portions added to previously autoclaved flasks containing sterile distilled water and basal medium. Only freshly prepared solutions of test compound and reversal agents were used. The final volume was 10 ml. per 50-ml. conical flask. In the preparation of inocula, all cells were carefully washed and standardized to a constant absorbance reading. All flasks were agitated on a rotary shaker during incubation for 20 hours at 28°. Growth was measured turbidimetrically as absorbance using a Coleman Junior spectrophotometer at a wavelength of 620 mμ.

All yeast cultures tested, except for *Candida albicans* (ATCC 10231), were kindly supplied by Dr. L. J. Wickerham, Northern Utilization Research

TABLE I.—INHIBITION IN PER CENT OF YEAST AND YEAST-LIKE FUNGAL GROWTH BY 3-BROMOPYRUVIC ACID

Culture	3-Bromopyruvic Acid, mcg./ml. 1.0	2.5	5.0	10	20	40
C. lipolytica	80	98	100	100	100	100
C. brumpti	63	94	97	100	98	97
C. zeylanoides	44	64	91	100	100	100
C. flareri	0	11	47	88	100	100
C. guilliermondii	12	21	33	72	91	95
C. utilis	0	20	24	42	61	81
C. albicans (477)	6	36	44	62	82	89
C. albicans (10231)	3	12	18	45	68	94
C. monosa	0	5	21	62	97	100
C. krusei	0	0	0	27	94	100
C. pulcherrima	0	0	0	0	0	2
C. reukaufii	0	0	0	0	0	0
C. tropicalis	0	0	0	0	0	0
H. anomala	17	46	85	97	98	98
G. candidum	0	0	0	7	23	78
S. cerevisiae	0	0	0	0	7	23

candidum is moderately sensitive, and *S. cerevisiae* is only slightly susceptible. Potassium bromide at concentrations up to 100 mcg./ml. shows no growth inhibition.

Growth of three different species of bacteria in chemically defined media, *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium phlei*, is not effectively antagonized by 3-bromopyruvic acid. In all cases, the minimal inhibitory concentrations are greater than 100 mcg./ml.

The fungistatic nature of the growth inhibition is suggested by the increased concentrations of 3-bromopyruvic acid required for complete growth inhibition of *C. lipolytica* with increased incubation periods. Where at 24 hours growth is inhibited by 2.5 mcg./ml., these concentrations increase to 5, 10, and greater than 80 mcg./ml. at 48, 72, and 96 hours, respectively. Thus, the fungicidal concentration of the compound is greater than 80 mcg./ml.

Of the seven organic acids tested (Table II), acetate and pyruvate are the most effective reversal agents for 3-bromopyruvic acid growth inhibition in *C. lipolytica*. This growth inhibition is not easily reversed, however, in that a ratio of 2000 to 1 of pyruvic acid to test compound (based on carbon concentrations of both compounds) is needed to cause a 90% reversal of the growth inhibition at 1 mcg./ml. of test compound. Therefore, these data suggest an interference in the metabolism of pyruvic acid by 3-bromopyruvic acid in those yeasts which are highly susceptible to growth inhibition by this compound. Alternately, those species of yeast which are relatively insensitive to the inhibitory activity of this compound may possess such permeability characteristics that the test compound cannot readily permeate the cells.

TABLE II.—PER CENT REVERSAL OF 3-BROMOPYRUVIC ACID GROWTH INHIBITION IN *Candida lipolytica* BY VARIOUS ORGANIC ACIDS[a]

Organic Acids, mcg./ml. as C	Pyruvate I	Pyruvate II	Acetate I	Acetate II	α-Ketoglutarate I	α-Ketoglutarate II	Succinate I	Succinate II	Fumarate I	Fumarate II	L-Malate I	L-Malate II	Citrate I	Citrate II
0.4	8	0	21	0	0	0	0	0	5	0	18	0	0	0
4	44	0	34	0	22	0	1	0	0	0	1	0	12	0
40	50	0	42	0	18	0	0	0	25	0	0	0	0	0
400	92	28	79	13	33	0	19	0	44	0	7	0	14	0
4000	100	86	[b]		52	12	86	3	[b]		75	2	38	4

[a] 3-Bromopyruvic acid was added at concentrations of 1.0 mcg./ml.(I) and 10 mcg./ml.(II). [b] Growth inhibition in the absence of test compound.

Laboratories, U. S. Department of Agriculture, Peoria, Ill. These cultures included *C. albicans* (NRRL Y-477), *C. guilliermondii* (NRRL Y-488), *C. flareri* (NRRL Y-245), *C. krusei* (NRRL Y-301), *C. monosa* (NRRL Y-1079), *C. pulcherrima* (NRRL Y-775), *C. reukaufii* (NRRL Y-1348), *C. utilis* (NRRL Y-900), *C. zeylanoides* (NRRL Y-106), *C. tropicalis* (NRRL Y-1410), *C. brumpti* (NRRL Y-311), *C. lipolytica* (NRRL Y-1094), *Geotrichum candidum* (NRRL Y-552), *Hansenula anomala* (NRRL Y-1737), and *Saccharomyces cerevisiae* (NRRL Y-9763).

3-Bromopyruvic acid was synthesized according to the method of Dickens and Williamson (5). Identity of the compound was established by bromine analysis (Calcd. for $C_3H_3BrO_3$: Br, 47.86. Found: Br, 47.57), infrared spectrum, and condensation with thiourea to form a known derivative (2-imino-4-thiazoline-4-carboxylic acid) (6).

RESULTS AND DISCUSSION

Data in Table I show the potent antimicrobial activity of 3-bromopyruvic acid against certain yeasts and yeast-like fungi. The results also clearly indicate wide differences in sensitivity to the inhibitory activity of the compound within a single yeast genus. Among 13 cultures of *Candida* representing 12 different species, a minimum of 50% growth inhibition occurs at less than 1 mcg./ml. of 3-bromopyruvic acid with two cultures, one culture at 2.5 mcg./ml., four cultures at 10 mcg./ml., and three cultures at 20 mcg./ml. Three cultures show no growth inhibition at 40 mcg./ml. of test compound.

In addition, single species of three other yeast genera also vary widely in susceptibility to the compound. *H. anomala* is highly sensitive to growth inhibition by 3-bromopyruvic acid; whereas *G.*

Previously, Kalnitsky and Barron (1) showed that fluoroacetate, and much less effectively bromoacetate, inhibited oxidation of acetate and pyruvate in *S. cerevisiae*. Interestingly, inhibition of pyruvate oxidation by fluoroacetate was much less marked in several species of bacteria.

In summary, wide differences in the susceptibility to the growth inhibitory activity of 3-bromopyruvic acid exist among different genera of yeast and also between different species of yeast within the same genus. Data suggest that the observed growth inhibition may be due to an interference in pyruvate metabolism.

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