Table IV—Antibacterial Activity of Ethyl *N*-Aryl-*S*-(triphenylstannyl)isothiocarbamates and Ethyl *N*-Phenyl-*S*-tritylisothiocarbamate

Compound	*Bacillus subtillis*[a]			*Escherichia coli*			*Micrococcus agilis*			*Staphylococcus aureus*		
	1[b]	10	100	1	10	100	1	10	100	1	10	100
IIIa	−	+	+	−	−	+	2+	2+	2+	2+	2+	2+
IIIb	−	+	2+	−	−	+	2+	2+	2+	2+	2+	2+
IIIc	−	+	2+	−	−	+	2+	2+	2+	2+	2+	2+
IIId	−	+	2+	−	−	+	2+	2+	2+	2+	2+	2+
IIIe	−	+	2+	−	−	+	2+	2+	2+	2+	2+	2+
IV	−	−	−	−	−	−	−	+	2+	−	−	+

[a] Bacteria were obtained from the culture collection of the Department of Biological Sciences, St. John's University. [b] Indicates concentration of compounds employed in micrograms per milliliter; − indicates no inhibition of growth, + indicates partial inhibition of growth, and 2+ indicates complete inhibition of growth.

and filtered to give 2.98 g (65%) of triethylammonium iodide, mp 173–175° [lit. (12) mp 181°].

The benzene was evaporated from the filtrate below 35°, and the mixture was stirred with *n*-heptane and filtered to give 10.88 g (95%) of IIIc, mp 96–106°. Recrystallization from *n*-pentane gave 8.11 g (71%) of IIIc, mp 95–98°. Further recrystallization from *n*-pentane did not change the melting point.

The other compounds in Table I were prepared in a similar manner.

Ethyl *N*-Phenyl-*S*-tritylisothiocarbamate (IV)—A mixture of trityl chloride (5.58 g, 0.02 mole), ethyl *N*-phenylthiocarbamate (13) (3.63 g, 0.02 mole), triethylamine (4.05 g, 0.04 mole), and acetonitrile (200 ml) was stirred at 25° for 47 hr. The solvent was evaporated, the residue was stirred with benzene (200 ml), and the mixture was filtered to give 2.69 g (98%) of triethylammonium chloride, mp 255° [lit. (12) mp 253–254°].

The benzene was evaporated from the filtrate, the residue was stirred with *n*-heptane (100 ml), and the mixture was cooled and filtered to give 7.82 g (92%) of IV, mp 126–135°. Recrystallization from *n*-pentane gave 4.82 g (57%) of IV, mp 133–137°; IR: 1626 s (C=N) cm^{-1}.

Anal.—Calc. for $C_{28}H_{25}NOS$: C, 79.40; H, 5.95; N, 3.31; S, 7.57. Found: C, 79.47; H, 6.05; N, 3.48; S, 7.44.

Biological Methods—The compounds were individually dissolved in tetrahydrofuran except for IIId, which was solubilized in benzene. The preparation of sterile solutions of the compounds, the fungi employed, the antimicrobial testing procedures, and the determination of growth inhibition were reported previously (14).

The compounds also were investigated for antibacterial activity according to the procedure reported earlier (14).

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Antifungal Properties of Halofumarate Esters

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Abstract □ Alkyl esters (C_1–C_4) of the four halofumaric acids were tested for antifungal activity against *Candida albicans, Aspergillus niger, Mucor mucedo,* and *Trichophyton mentagrophytes* at pH 5.6 and 7.0 in the absence and presence of 10% beef serum in Sabouraud dextrose agar. The most toxic compound to each organism was: *C. albicans,* ethyl iodofumarate (0.054 mmole/liter); *A. niger,* methyl bromofumarate (0.090 mmole/liter); *M. mucedo,* methyl fluorofumarate (0.037 mmole/liter); and *T. mentagrophytes,* ethyl iodofumarate (0.020 mmole/liter). The order of overall activity of the six most toxic compounds was: ethyl iodofumarate > ethyl chlorofumarate > methyl iodofumarate = methyl bromofumarate > methyl chlorofumarate > ethyl bromofumarate.

Keyphrases □ Halofumarate alkyl esters, various—antifungal activity evaluated □ Antifungal activity—various halofumarate alkyl esters evaluated □ Structure–activity relationships—various halofumarate alkyl esters evaluated for antifungal activity

Interest in developing agents for activity against infections due to opportunistic fungi in debilitated and immunosuppressed patients led to a search for potentially useful classes of compounds (1–3). The fungi that are the most frequent invaders include species of *Candida, Aspergillus, Mucor,* and *Cryptococcus* (4).

DISCUSSION

A previous study of the fungitoxicity of 2-bromo-3-fluorosuccinate esters and related compounds indicated that a systematic examination of the halofumarate esters would be worthwhile (5). Fluorofumaric (6), chlorofumaric (7), bromofumaric (8), and iodofumaric (9) acids were esterified by heating under reflux with methanol, ethanol, 1-propanol,

Table I—Halofumaric Acid Esters (ROOCCX=CHCOOR)

Compound	R	X	Yield, %	Boiling Point (mm) or Melting Point[a] Observed	Literature Value	$n_D^{25°}$	ν neat, cm^{-1} C=O	C=C	Formula		Analysis, % Calc.	Found
Ia	C_2H_5	F	52	62° (0.3)	—[b]	1.4309	1740	1675	$C_8H_{11}FO_4$	C	50.52	50.23
										H	5.83	5.95
										F	9.99	10.21
Ib	n-C_3H_7	F	63	79° (0.2)	—	1.4349	1740	1685	$C_{10}H_{15}FO_4$	C	55.04	54.76
										H	6.93	6.91
										F	8.71	8.65
Ic	n-C_4H_9	F	67	94.5–95.0° (0.1)	—	1.4379	1742	1690	$C_{12}H_{19}FO_4$	C	58.52	58.54
										H	7.78	7.46
										F	7.71	7.71
Id	CH_3	Cl	78	60° (0.2)	108° (15.0)[c]	1.4711	1742	1638	—	C	—	—
										H		
										Cl		
Ie	C_2H_5	Cl	75	110–112° (4.0)	119° (12.0)[c]	1.4600	1742	1638	—	C	—	—
										H		
										Cl		
If	n-C_3H_7	Cl	80	100° (0.3)	131–137° (7.0)[c]	1.4624	1740	1642	$C_{10}H_{15}ClO_4$	C	51.18	51.06
										H	6.44	6.40
										Cl	15.11	15.27
Ig	n-C_4H_9	Cl	59	130–131° (0.08)	166–210° (9.0)[d]	1.4625	1748	1640	$C_{12}H_{19}ClO_4$	C	54.86	54.97
										H	7.29	7.32
										Cl	13.44	13.40
Ih	CH_3	Br	45	30°	27.5–28.5°[c]	—	1750	1638	—	C	—	—
										H		
										Br		
Ii	C_2H_5	Br	72	91–92° (0.07)	145–155° (11.0)[e]	1.4801	1744	1632	—	C	—	—
										H		
										Br		
Ij	n-C_3H_7	Br	73	104° (0.07)	—	1.4770	1742	1632	$C_{10}H_{15}BrO_4$	C	43.03	43.32
										H	5.42	5.29
										Br	28.63	28.46
Ik	n-C_4H_9	Br	84	122–123° (0.1)	170–172° (8.0)[e]	1.4735	1750	1630	—	C	—	—
										H		
										Br		
Il	CH_3	I	85	51–52°	52–52.5°[f]	—	1736	1618	—	C	—	—
										H		
										I		
Im	C_2H_5	I	56	111–112° (1.0)	—	1.5139	1730	1610	$C_8H_{11}IO_4$	C	32.23	32.36
										H	3.72	3.70
										I	42.57	42.69
In	n-C_3H_7	I	80	118–119° (0.2)	—	1.5048	1730	1616	$C_{10}H_{15}IO_4$	C	36.83	37.06
										H	4.62	4.61
										I	38.91	39.02
Io	n-C_4H_9	I	76	140° (0.4)	—	1.4994	1740	1620	$C_{12}H_{19}IO_4$	C	40.69	41.04
										H	5.41	5.41
										I	35.83	35.53

[a] Analytical sample. [b] For ethyl fluoroethylenedicarboxylate, Ref. 10 reported bp 74° (1.5 mm) and $n_D^{20°}$ 1.4330. [c] Reference 8. [d] Reference 11. [e] Reference 12. [f] Reference 9.

and 1-butanol, respectively, using thionyl chloride as the catalyst. Of the 16 esters prepared, the following were reported previously: methyl fluorofumarate (5), ethyl fluoroethylenedicarboxylate (10), methyl chlorofumarate (8), ethyl chlorofumarate (8), *n*-propyl chlorofumarate (11), *n*-butyl chlorofumarate (11), methyl bromofumarate (8), ethyl bromofumarate (12), *n*-butyl bromofumarate (12), and methyl iodofumarate (9). Although *n*-propyl and *n*-butyl chlorofumarates appear in the literature (11), they were not adequately characterized, and the isomeric form of ethyl fluoroethylenedicarboxylate was not established.

The data characterizing the halofumarate esters are contained in Table I. The purity of the esters was verified by GLC.

The compounds were tested against *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 1004), *Mucor mucedo* (ATCC 7941), and *Trichophyton mentagrophytes* (ATCC 9129) in Sabouraud dextrose agar[1] at pH 5.6 and 7.0 in the absence and presence of 10% beef serum[2] according to published methods (1, 13) (Table II). Because these compounds are potential medicinal agents, the highest level tested was 100

[1] Difco.
[2] Miles Laboratories.

Table II—Antifungal Activity of Diesters of Halofumaric Acids at pH 5.6 and 7.0 in Sabouraud Dextrose Agar in the Presence and Absence of Beef Serum[a]

Compound	Level of Inhibition[b]															
	C. albicans				*A. niger*				*M. mucedo*				*T. mentagrophytes*			
	pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0		pH 5.6		pH 7.0	
	–[c]	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+
Methyl fluorofumarate[d]	0.025	0.037	0.12	0.24	0.12	0.31	0.36	—[e]	<0.0062[f]	0.012	0.025	0.037	<0.0062	0.025	0.049	0.25
Ia	0.021	0.074	0.053	0.21	0.084	0.32	0.26	—	<0.0053	0.016	0.016	0.26	<0.0052	0.063	0.011	0.084
Ib	0.018	0.23	0.064	—	0.14	—	0.23	—	0.0091	0.14	0.023	0.28	<0.0049	0.23	<0.0049	—
Ic	0.012	0.24	0.065	—	0.24	—	—	—	0.028	—	0.041	—	<0.0041	0.20	0.012	—
Id	0.090	0.10	0.10	0.10	0.034	0.022	0.10	0.11	0.017	0.039	0.034	0.050	<0.0056	0.017	0.011	0.067
Ie	0.058	0.058	0.058	0.068	0.0096	0.015	0.058	0.097	0.0096	0.048	0.029	0.048	<0.0048	0.015	0.0096	0.058
If	0.026	0.077	0.068	0.21	0.051	0.051	0.21	—	0.017	0.17	0.051	0.34	0.013	0.013	0.017	0.17
Ig	0.053	0.19	0.11	0.19	—	—	—	—	0.011	0.15	0.023	—	0.011	0.11	0.015	0.19
Ih	0.054	0.063	0.081	0.090	0.063	0.072	0.081	0.090	0.013	0.018	0.022	0.063	<0.0045	0.013	0.013	0.040
Ii	0.020	0.056	0.028	0.12	0.020	0.048	0.080	0.16	0.016	0.016	0.032	0.12	<0.0040	<0.0040	<0.0040	0.024
Ij	0.011	0.018	0.032	0.14	0.057	0.036	0.072	—	0.014	0.029	0.043	0.18	<0.0036	0.050	0.011	0.072
Ik	0.029	0.16	0.033	0.23	—	—	—	—	0.026	0.13	0.039	—	0.0065	0.098	0.013	0.098
Il	0.026	0.026	0.052	0.067	0.059	0.059	0.11	0.11	<0.0037	0.0074	<0.0037	0.074	<0.0037	0.0074	0.0074	0.022
Im	0.017	0.020	0.047	0.054	0.054	0.040	0.10	0.10	0.030	0.040	0.040	0.060	<0.0034	0.010	0.0068	0.020
In	0.0092	0.028	0.021	0.12	0.092	0.092	0.15	—	<0.0031	0.028	0.0061	0.18	<0.0031	0.018	0.0091	0.061
Io	—	—	—	—	—	—	—	—	—	—	—	—	0.0056	0.11	0.017	0.11

[a] *C. albicans* and *M. mucedo* were incubated at 37° for 20 hr, and *T. mentagrophytes* and *A. niger* were incubated at 28° for 5 days. [b] Minimal concentrations of compound in millimoles per liter causing 100% inhibition of test organisms by recalculation of test data obtained in micrograms per milliliter. Activity to 10 μg/ml was obtained in increments of 1 μg/ml; activity from 10 to 20 μg/ml was obtained in increments of 2 μg/ml; and activity from 20 to 100 μg/ml, the highest level tested, was obtained in increments of 10 μg/ml. All tests were carried out in duplicate in "I" plate petri dishes. [c] – and + = absence and presence of 10% beef serum, respectively. [d] Data taken from Ref. 5. [e] — = not inhibitory below 100 μg/ml. [f] < = inhibitory at 1 μg/ml, the lowest level tested.

μg/ml; the results of greatest interest were those obtained at pH 7.0 in the presence of 10% beef serum.

Adjustment of the test medium from pH 5.6 to 7.0 and addition of 10% beef serum resulted in the reduction of antifungal activity in most cases. The methyl and ethyl esters of the halofumaric acids were more active than the *n*-propyl and *n*-butyl homologs. The most toxic compound to each organism was: *C. albicans*, ethyl iodofumarate (0.054 mmole/liter); *A. niger*, methyl bromofumarate (0.090 mmole/liter); *M. mucedo*, methyl fluorofumarate (0.037 mmole/liter); and *T. mentagrophytes*, ethyl iodofumarate (0.020 mmole/liter).

The first six most toxic compounds against each organism were ranked in order of activity. After averaging the ranks, the following order of overall antifungal activity was obtained: ethyl iodofumarate > ethyl chlorofumarate > methyl iodofumarate = methyl bromofumarate > methyl chlorofumarate > ethyl bromofumarate.

Methyl and ethyl chlorofumarates, methyl, ethyl, and *n*-butyl bromofumarates, and methyl iodofumarate were reported to possess antifungal properties (12–14). The mechanism of action was attributed to interference with the Krebs cycle (12). All four halofumaric acids are fumarase substrates (15).

EXPERIMENTAL[3]

To prepare *n*-propyl fluorofumarate, fluorofumaric acid (6) (1.5 g, 0.011 mole), 1-propanol (15 ml), and thionyl chloride (0.5 ml) were heated under reflux overnight. Sufficient water was added to cause the ester to come out of solution. The mixture was extracted (chloroform), the extract was washed (water) and dried (sodium sulfate), and the solvent was removed in a rotary still. Then the residue was fractionated under reduced pressure.

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[3] Melting points were taken in a Thomas-Hoover melting-point apparatus and are uncorrected. GLC was performed on a Varian Aerograph model 1200 gas chromatograph with a flame-ionization detector to which was attached a Varian Aerograph model 20 recorder. The purity of the acids was established by GLC on a column of 3% Dexsil 400 on Anachrom A (90–100 mesh) purchased from Analabs, New Haven, Conn. All samples tested microbiologically were at least 95% pure. IR spectra were obtained with a Perkin-Elmer model 221 spectrophotometer, and refractive indexes were taken with an Abbe-3L B&L refractometer.