Table III—Analyses of Fruits of *S. khasianum* and *S. mammosum*[a]

Fruit	Color	Shape	Moisture Content[b], %	Solasodine Content, %
S. khasianum	Green	Round	76.8	0.82 ± 0.04
S. khasianum	Yellow	Round	80.4	1.12 ± 0.06
S. mammosum	Yellow-green	Pear	79.1	1.05 ± 0.04
S. mammosum	Yellow-green	Round	81.9	0.99 ± 0.02
S. mammosum	Yellow-green	Pear with nipples	80.2	0.91 ± 0.03

[a] In all cases, the extraction method used was the one described in this report, and the end-point was determined potentiometrically (n = 3). [b] Determined by drying to constant weight at 105°

error of four titrations was 34.8%. Titrating the same sample with potentiometric end-point detection gave an average deviation of 6.4% in four measurements.

The recovery of added crystalline solasodine to an exhaustively extracted *S. mammosum* sample was quantitative within the experimental error of 2%, demonstrating the reliability of the extraction procedure outlined in this report.

Table III summarizes the results of solasodine determinations performed according to this procedure. Freshly harvested fruits of *S. khasianum* at different ripening stages and fruits of *S. mammosum* of different shapes were analyzed. Experimental errors were at the level previously experienced in the method development assays.

These analyses of *S. mammosum* and *S. khasianum* samples yielded more realistic values than those reported in the literature. Perez-Medina *et al.* (22) found 3.5–4% glycoalkaloids in *S. mammosum*. Maiti and Mathew (23) found 5% solasodine in *S. khasianum*. These investigators used the gravimetric method, and their results showed large variations and unusually high values when compared to solasodine levels found in other *Solanum* species. The present analyses showed alkaloid contents of 0.82–1.12% for these plants (Table III).

The easy operation of the titrating apparatus and the recorded end-point determination method should permit technicians to analyze single samples with sufficient accuracy for field evaluations.

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Antimicrobial Action of Compound 48/80 against Protozoa, Bacteria, and Fungi

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Abstract □ Compound 48/80 inhibited the growth of protozoa, bacteria, and fungi but had no effect on the multiplication of viruses. All susceptible organisms were inhibited by 10 μg/ml of crude compound 48/80, and some were inhibited by as little as 0.1 μg/ml. Against *Tetrahymena pyriformis*, this drug was seven times more potent than quinine. Separation of compound 48/80 into different fractions indicated that some antimicrobial activity could be separated from the histamine-liberating activity. It was found that compound 48/80 is not surface active at 500 μg/ml.

Keyphrases □ Compound 48/80—antimicrobial activity evaluated in protozoa, bacteria, fungi, and viruses □ Antimicrobial activity—compound 48/80 evaluated in protozoa, bacteria, fungi, and viruses □ Phenethylamine polymers—compound 48/80, antimicrobial activity evaluated in protozoa, bacteria, fungi, and viruses □ Polymers of phenethylamine—compound 48/80, antimicrobial activity evaluated in protozoa, bacteria, fungi, and viruses

In 1949, a family of polymers that lowered blood pressure was synthesized from *p*-methoxyphenethylmethylamine and formaldehyde (1). It was proposed that the products were various polymers of phenethylamine and that the most potent oligomer in the mixture was possibly the trimer. A widely distributed batch of this product was

identified by its code name "BW 48-80," which subsequently led to the adoption of the name "compound 48/80." Later, the dimeric and trimeric oligomers were synthesized by an alternative route and found to be inactive (2, 3). In 1972, dialysis and gel filtration were used to obtain evidence that the molecular size of the hypotensive constituents ranged from the tetramer to the octamer (4).

One study (5) reported that compound 48/80 caused the release of histamine and that this was the mechanism by which it lowered blood pressure. Since then, compound 48/80 has often been used as a tool to study the mechanism of mast cell degranulation and the pharmacological effects of the potent agents released from these cells. It is difficult to separate the direct effects of compound 48/80 from its indirect effects caused by the release of these agents, but several reports described actions for compound 48/80 that may not be related to mast cell degranulation: inhibition of the action of acetylcholine (6), central nervous system effects (7), ganglionic blockade (8), competitive inhibition of agonists that produce smooth muscle contraction (9), inhibition of skeletal muscle contraction (10), release of creatine phosphokinase from muscle (11), inhibition of viral-induced giant cell formation (12), and stimulation of tumor growth (13, 14). However, apparently no one has reported an antimicrobial action for compound 48/80.

The effects of compound 48/80 on the growth of protozoa, bacteria, fungi, and viruses were measured, and it was found that compound 48/80 is a moderately potent antimicrobial agent against all of these groups except the viruses. The relation between the antimicrobial activity and the histamine-liberating activity of this drug was also studied. A preliminary account of this work has been presented (15).

EXPERIMENTAL

Protozoa—*Tetrahymena pyriformis* GL (ATCC 30006) was grown in flasks containing Hogg's medium (1% proteose peptone, 0.1% K_2HPO_4, 0.1% dextrose, and 0.1% sodium acetate) on a rotary shaker at 27°. Growth was measured at intervals by counting cells that were freshly killed by dilution with 2.5% formaldehyde in 0.5% saline. In addition to compound 48/80 and its monomer, histamine diphosphate[1], diphenhydramine hydrochloride[2], and quinine hydrochloride[3] were tested for their effects on the growth of *Tetrahymena*.

Two strains of *Plasmodium falciparum* were employed: chloroquine resistant (FVO, Vietnam-Oak Knoll) and chloroquine sensitive (FUP, Uganda-Palo Alto). *P. falciparum*-infected blood was taken from Aotus monkeys and diluted with normal blood to obtain approximately 2% parasitemia. These diluted cultures were then incubated *in vitro* using a rocker dilution technique with a modified Harvard medium (16). The infections were synchronous, and experiments were begun with a majority of asexual parasites in the "ring" stage. At intervals, 100 or more parasites from each blood sample were examined microscopically and classified according to their maturation to trophozoite, schizont, or segmenter stages.

Fungi—Cultures of *Saccharomyces cerevisiae* (ATCC 7754) were grown at 30° in shake flasks containing 2% AC broth[4] on a rotary shaker. At intervals, aliquots of the cultures were diluted with water, and the transmittance of the yeast suspension was read at 755 nm. *Aspergillus niger* (ATCC 1004) was grown by the same procedure; however, growth was measured by weighing the dried mycelium, which was harvested by filtration through preweighed filter paper disks.

Bacteria—*Klebsiella pneumoniae* (ATCC 13883) and *Staphylococcus albus* (ATCC 3004) were grown in SMA broth, *Pediococcus cerevisiae* (ATCC 8081) and *Streptococcus faecalis* (ATCC 8042) were grown in Citrovorum factor assay medium[4] supplemented with 0.1 μg of calcium leucovorin/ml, and *Lactobacillus casei* (ATCC 7469) was grown in folic acid medium[4] supplemented with 0.1 μg of folic acid/ml. Cultures were incubated at 37°, and growth was followed by measuring the transmittance of the culture at 660 nm.

Viruses—Encephalomyocarditis (ATCC VR-129), vesicular stomatitis (ATCC VR-158), and vaccinia (ATCC VR-156) viruses were grown in KB cells at 37° in medium 199 supplemented with 2% calf serum (17). The concentration of the viruses in each inoculum was 100 times that necessary to infect 50% of the cells (100 $TCID_{50}$). The inoculum and compound 48/80 were added to the medium at the same time. Viral growth was followed microscopically by noting the degree of cytopathic effect caused by virus multiplication.

Gel Filtration—Compound 48/80 was fractionated in a 111 × 1.5-cm column of Sephadex G-25 medium[5]. A solution of 0.03 *N* acetic acid adjusted to pH 3.0 with hydrochloric acid was passed through the column at a flow rate of 12 ml/hr. Ten-milligram samples dissolved in 1 ml of acetic acid solution were placed on the column. The effluent was continuously monitored at 280 nm, and 8.5-ml fractions were collected.

Blood Pressure—Wistar male rats (300–600 g) were anesthetized with pentobarbital sodium[6] (50 mg/kg) and monitored on a polygraph[7]. Arterial pressure was measured with a liquid-pressure transducer[8] connected by a cannula to the right femoral artery. Heparin sodium[9] (10 mg/kg) and the fractions to be assayed were given through a cannula inserted into the right femoral vein. In each animal, the blood pressure was noted just before the infusion and again 5 min later. A dose–response curve was prepared for compound 48/80. The column fractions were analyzed in quadruplicate, and their potencies were expressed in terms of equivalent micrograms of compound 48/80 by reference to the dose–response curve.

Surface Activity—Test solutions were passed through a drop counter at a rate of approximately 15 drops/min. The volume obtained from 4000 drops provided a measure of drop size and, therefore, of surface tension. Benzalkonium chloride (0.01%) and other surfactants produced a volume that was 40–60% of the volume obtained with pure water.

RESULTS

The effects of compound 48/80 on the growth of microorganisms are presented in Table I. The growth of each protozoan, fungal, and bacterial species tested was inhibited, but there was no effect on the viruses used. The most sensitive species was *Staph. albus*, which did not grow at all in medium containing 2.5 μg of compound 48/80/ml. *L. casei* was also very sensitive, with 0.1 μg/ml causing a 37% inhibition of growth. The least sensitive of the affected organisms were *K. pneumoniae* and *A. niger*. In every case where the main reactant (*p*-methoxyphenethylmethylamine) for the synthesis of compound 48/80 was tested, it had no effect or was far less effective than an equal amount of compound 48/80.

Also shown in Table I are the effects of quinine, histamine, and diphenhydramine on the growth of *T. pyriformis*. Quinine and the antihistamine inhibited growth, whereas histamine had no significant effect. Quinine was about one-seventh as active as compound 48/80. Low levels of compound 48/80 had a static effect on *T. pyriformis* rather than a killing effect. After 24 hr of growth inhibition, dilution of the medium allowed growth to resume.

In separate experiments, compound 48/80 was about as active as pyrimethamine in inhibiting the maturation of *P. falciparum*, but it was only 10% as active as chloroquine. Compound 48/80 was more active against the chloroquine-sensitive strain of *Plasmodium* than it was against the chloroquine-resistant strain.

The activities of the fractions of compound 48/80 prepared by chromatography on Sephadex G-25 are shown in Fig. 1. The fractions that were the most potent histamine liberators also contained most of the antimicrobial activity. However, some fractions possessed antimicrobial activity but no hypotensive activity. When these fractions were tested against *P. falciparum*, all were active, including peaks 1, 2, and 3. Each fraction was more active against the chloroquine-sensitive strain of this protozoan than against the chloroquine-resistant strain.

In a preliminary experiment, peak 5 from the Sephadex G-25 column effluent was further purified by rechromatography on G-25 and then was

[1] City Chemical Corp.
[2] Tiffany Chemical Co.
[3] Merck and Co.
[4] Difco.
[5] Pharmacia.
[6] Abbott.
[7] Grass model 7.
[8] Statham P23Dc.
[9] Nutritional Biochemicals.

Table I—Effect of Compound 48/80 on the Growth of Microorganisms[a]

Group	Organism	Drug	Dose, μg/ml	Growth, % of Control
Protozoa	*T. pyriformis*	Compound 48/80	1	85[b]
		Compound 48/80	5	57
		Compound 48/80	25	19
		Monomer	100	95[c]
		Quinine	5	97[b]
		Quinine	25	81
		Quinine	100	21
		Histamine	10	101
		Histamine	100	106
		Diphenhydramine	1	89
		Diphenhydramine	10	73
		Diphenhydramine	100	17
	P. falciparum	Compound 48/80	2.5	75[d]
		Compound 48/80	25	0
		Monomer	10	100
Fungi	*S. cerevisiae*	Compound 48/80	5	100[e]
		Compound 48/80	10	15
		Compound 48/80	20	1
		Monomer	100	100
	A. niger	Compound 48/80	5	89[f]
		Compound 48/80	25	72
		Compound 48/80	100	1
		Monomer	100	47
Bacteria	*K. pneumoniae*	Compound 48/80	2.5	77[g]
		Compound 48/80	10	64
		Compound 48/80	25	37
		Compound 48/80	100	25
	L. casei	Compound 48/80	0.1	63
		Compound 48/80	0.25	40
		Compound 48/80	2.5	7
		Compound 48/80	25	0
		Monomer	200	93
	Ped. cerevisiae	Compound 48/80	2.5	68
		Compound 48/80	10	1
		Monomer	200	93
	Staph. albus	Compound 48/80	2.5	0
		Compound 48/80	10	0
		Monomer	100	71
	Strep. faecalis	Compound 48/80	2.5	87
		Compound 48/80	25	4
		Monomer	200	99
Viruses	Vesicular stomatitis	Compound 48/80	20	100[h]
	Encephalomyocarditis	Compound 48/80	40	100[i]
	Vaccinia	Compound 48/80	10	100[j]

[a] The assay at each concentration was performed two to five times. Only representative experiments at selected concentrations are shown. Monomer = *p*-methoxyphenethylmethylamine. [b] Cells per cubic millimeter after 24 hr. [c] Cells per cubic millimeter after 8 hr. [d] Number of parasitized red cells in which parasites grew to schizont stage in 35 hr. [e] Optical density of culture at 755 nm after 24 hr. [f] Mycelial dry weight after 24 hr. [g] Optical density of culture at 660 nm after 24 hr. [h] Cytopathic effect of the viruses in KB cells after 48 hr at the maximum nontoxic dose for 48 hr. [i] Cytopathic effect of the viruses in KB cells after 24 hr at the maximum nontoxic dose for 24 hr. [j] Cytopathic effect of the viruses in KB cells after 120 hr at the maximum nontoxic dose for 120 hr.

chromatographed on paper as previously described (4). The four resulting components were eluted from the paper and then analyzed for hypotensive activity and for activity against *L. casei*. One component displayed hypotensive activity only, another showed antibacterial activity only, and the other two components were inactive.

In another preliminary experiment, repurified peak 5 was chromatographed on a Bio Gel P-4[10] column. Four peaks were obtained in the effluent, representing compounds that were evidently separated by adsorption effects. As in the case of the paper chromatography, one peak showed hypotensive activity only, another had antibacterial activity only, and the other two were inactive.

Since compound 48/80 contains ionized nitrogen atoms, its antimicrobial and histamine-liberating activities were compared with those of a typical quaternary ammonium detergent. Benzalkonium chloride[11] was 100 times more potent than compound 48/80 as a hemolytic agent and 10 times more potent as an antimicrobial agent against yeast. However, benzalkonium chloride was one-third as potent as compound 48/80 as a histamine-releasing agent when tested *versus* mast cells *in vitro* and was only 1% as potent as a hypotensive agent. Detergents liberate histamine from mast cells by disrupting the plasma membrane, whereas compound 48/80 liberates histamine by a selective, noncytolytic action (18). The drop size procedure showed that compound 48/80 has no surface activity at 500 μg/ml; therefore, its antimicrobial activity is not attributable to surface activity or detergency.

DISCUSSION

The data show that compound 48/80 has a broad spectrum of activity in inhibiting the growth of microorganisms. In this respect, it resembles cationic detergents; however, the results indicate that compound 48/80 does not display the hemolytic and surface activities of a detergent. Although compound 48/80 is only moderately potent as an antimicrobial agent, it is not a pure substance; Fig. 1 shows that some components are inactive or are only weakly active. Thus, it is possible that the mixture may contain some very potent compounds.

In addition, since the assays were performed in the presence of growth media, complex formation with medium components may have reduced the concentration of free antimicrobial agent. (Each nitrogen atom of the polymer bears a positive charge.) Since different media were used for the growth of different organisms, the percentage of compound 48/80 complexed (if any) may have varied from one organism to another. Therefore, the relative sensitivities observed may be approximate. However, the salient point is that compound 48/80 inhibited the growth of all bacterial, fungal, and protozoal cultures tested.

Compound 48/80 (at 2–10 μg/ml) blocks the division of fertilized sea urchin (*Tripneustes gratilla*) eggs[12]. In KB cells and in sea urchin eggs, compound 48/80 alters the appearance of the cytoplasm and the shape of the nucleus. Evidently, compound 48/80 inhibits the division of a wide variety of cells, possibly by a similar mechanism.

[10] Bio-Rad.
[11] Zephiran, Calbiochem.
[12] R. Hino, School of Medicine, University of Hawaii personal communication.

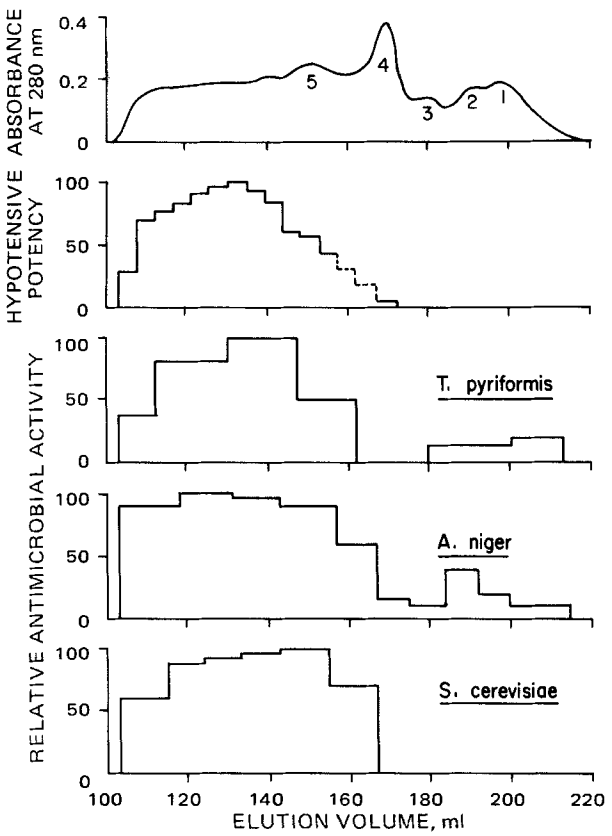

Figure 1—*Histamine-liberating and antimicrobial activities of fractions of compound 48/80. Compound 48/80 was fractionated by chromatography on Sephadex G-25 as previously described (4). The top curve is the tracing produced by a UV monitor. In the other four charts, the most potent fraction was assigned an activity of 100%.*

There are several indications that the histamine-liberating and antimicrobial activities of compound 48/80 may reside in different constituents of this mixture. Figure 1 shows that some antimicrobial activity was separated from the hypotensive fractions by chromatography on Sephadex G-25. A similar partial separation was achieved as measured by activity against *P. falciparum*. When peak 5 (Fig. 1) was separated into four components by chromatography on paper or on Bio Gel P-4, preliminary data indicated that a separation of the two activities had been effected. The fact that peak 5 contained at least four different compounds, all of approximately the same molecular size, suggests that the reaction for the synthesis of compound 48/80 may be more complex than previously assumed.

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