The increasing activity of the saturated acids with increasing chain length suggests that a similar chain lengthening mechanism may operate in the synthesis of saturated fatty acids. The radioactivity of each saturated acid was approx double that of the previous acid. This might be interpreted as a series, starting with palmitic acid as the precursor. However, if this is true, the biosynthetic system must be under a control distinct from that for the monoene acids; oleic, eicosenoic and erucic, since no changes in the relative amt of the saturated acids were recorded in fatty acid composition of the high and zero erucic acid oils (Table I). If the chain lengthening mechanism for the saturated acids was under the same control as for the monoethenoid acids, then no stearic, arachidic or behenic acids would be expected in seed from the zero erucic acid plants. Thus a chain lengthening system for the saturated acids is not precluded but, if it exists, evidently it is not identical to that proposed for the monoethenoid acids.

It is interesting to note that, under the conditions of the present study, the specific activities for oleic, linoleic and linolenic acids (Table II) are approx equal and are each lower than stearic acid.

The results of this study show the presence of at least two separate biosynthetic pathways in rapeseed fatty acid biosynthesis. The chain lengthening system under direct genetic control operates to add an acetate molecule to the carboxylic portion of oleic acid to form eicosenoic acid, and the addition of a second acetate molecule to form erucic acid. This genetic control is indicated by the fatty acid composition of the oil from the five genotypes studied, where the substitution of a (−) gene for a (+) gene reduced the amt of erucic acid by 9–10% under the experimental conditions used (Table I). A similar chain-lengthening system has been shown for the formation of archidonic acid in animal tissue where linoleic acid is the precursor, although genetic control has not been demonstrated in this animal system (7). It is of interest to speculate on the type of genetic control operating in the plant system. It is possible that the genes control the enzymes which regulate the production of substrates or precursors necessary for the synthesis of eicosenoic and erucic acids or it may be that the genes control the quantity of enzyme produced for the chain elongation system. These possibilities are under further investigation and will be the subject of future publications.

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Two New Stable Polybrominated Salicylanilides for Antibacterial Use in Soap and Detergent Products

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Abstract

The versatility of two brominated salicylanilides as antiseptic agents and germicides in soap and detergent products for use on skin, hair, hard surfaces and in fabrics is discussed.

Both preparations show stability at elevated temp and pressure and compatibility with many surfactant vehicles. They are free of primary irritation and sensitization, including sunlight-induced irritation.

A mixture of di- and tribromosalicylanilide is currently being used in toilet bars, shampoos, laundry products, disinfectant sprays and multi-purpose disinfectant products. The substantivity to fabrics is further characterized by stability to heat and hydrolysis by alkalinity.

Essentially pure 3,4′,5 tribromosalicylanilide is resistant to discoloration, making it particularly suitable for use in white and pastel colored toilet bars. It has excellent substantivity to skin and provides long-lasting action against odor-causing and pathogenic bacteria.

Introduction

ANTIBACTERIAL AGENTS have been finding increased application in household products in recent years. They are being used in toilet soaps, detergent products and hard surface cleansers, as well as personal products such as ointments, creams, lotions, underarm deodorants and shampoos. These germicides have proved effective in sprays and multi-purpose disinfectant cleansers specially formulated to combat the alarming rise of infections of Staphylococcus aureus and other pathogens in hospitals.

Desirable performance specifications of germicides are:

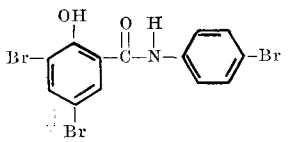
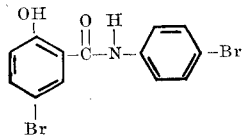
1. Activity at low concn
2. Compatibility with surface active systems and cosmetic vehicles
3. Heat, odor and color stability
4. Sustained residual activity
5. Safety in handling and use; absence of primary irritation, sensitization and photosensitization
6. Substantivity to both animate and inanimate surfaces
7. Economical

Germicides used in toilet soaps include hexachlorophene, bithionol, trichlorocarbanilide and formerly tetrachlorosalicylanilide. Each of these has some shortcoming (1,2) in that not all of the above outlined specifications are satisfied.

The halogenated salicylanilides have been studied quite extensively because of their exceptionally high germicidal activity, as discussed by LeMaire et al. (3). The conformance of certain derivatives of salicylic acid to the above specifications suggests that they are the antimicrobial agents of choice for soap and detergent products. Of the halogenated salicylanilides in-

TABLE I

Physical Properties of PBS and TBS

Structural formulas		
		3,4′,5 tribromosalicylanilide
		4′,5 dibromosalicylanilide

	PBS	TBS
Molecular weight	395–410	442–450
Melting point	190–200°C	220–226°C
Color	White-buff	White
Form	Free-flowing powder	Free-flowing powder
Odor	None	None
Taste	None	None

vestigated by LeMaire et al., the brominated derivatives were found to possess greater activity and mildness superior to chlorinated derivatives. For example, 5 chloro, 2′,4′ dibromosalicylanilide was found (4) to be more active as a skin germicide than 2′,4′,5 trichlorosalicylanilide.

Antibacterial activity, both *in vitro* and *in vivo*, of the general class of polybrominated salicylanilides has been studied and described by Vinson and coworkers (5).

Discussion

Investigations have shown that certain polybrominated salicylanilides possess the desirable properties of germicides to the maximum extent. These new products are:

1. A 1:1 mixture of 4′,5 dibromosalicylanilide and 3,4′,5 tribromosalicylanilide, hereafter referred to as PBS (trade name TEMASEPT by Fine Organics, Inc.)
2. An essentially pure 3,4′,5 tribromosalicylanilide (containing a small percentage of 3,5 dibromosalicylanilide), hereafter referred to as TBS (trade name TEMASEPT II by Fine Organics, Inc.)

The physical properties and solubility characteristics of PBS and TBS are summarized in Tables I and II, respectively.

Activity at Low Concentrations. Bacteriostatic effect against various organisms has been determined, and activity at low concn has been confirmed for both PBS and TBS.

Laboratory studies were conducted to determine the bacteriostatic effect of various mixtures of 4′,5 dibromosalicylanilide (DBS) and TBS. Solutions were prepared containing 10% pure DBS, 10% pure TBS and various mixtures thereof (maintaining the total germicide concn at 10%). Solubilization of the germicides is readily effected in an alkaline-alcoholic solution employing isopropyl alcohol. These stock solutions were used at different concn in the final rinse water of a standard laundry wash cycle for washing bed linens. The minimum effective concn to produce a zone of inhibition by the treated linen disks (15 mm) on a nutrient agar plate seeded with S. aureus was determined. The results are presented in Table III.

By analyzing data in Table III, we find that pure DBS is more active than pure TBS. However, combinations of DBS and TBS (such as 40% DBS–60% TBS, or a 1:1 mixture, or 60% DBS–40% TBS) are distinctly more active than either germicide alone. It should be noted that these activities are based on the fabric substantivity test.

TABLE II

Solubility of PBS and TBS

Water	IS	Chloroform	SS
Acetone	VS	Cyclohexane	SS
Methyl alcohol	S	*Dimethylformamide*	VS
Ethyl alcohol	S	Pyridine	VS
Isopropyl alcohol	S	Benzene	S
Ethyl ether	S	Ethylene glycol dimethyl ether (Cellosolve)	VS
Petroleum ether	IS	Methylene glycol monoethyl ether (Carbitol)	VS
Dioxane	VS		
1,2 dimethoxyethane	VS		
2,2 dimethoxypropane	IS		

Key: VS—very soluble >4%
S—soluble 0.5 –4.0%
SS—sparingly soluble 0.01–0.5%
IS—insoluble <0.01%

This synergistic action of the two germicides was also noted on human skin in a study employing the Serial Basin Wash Test. The germicidal mixtures used were incorporated at a 1% level into a standard soap bar, and a modified Price Serial Basin Wash Test was conducted on panels of ten subjects each. The test procedure followed was as described by Cade (6). The average reduction in hand counts for each germicide mixture after two weeks of regular use of the experimental bar is given in Table IV.

Data in Table IV show that in degerming the skin, pure TBS is superior to pure DBS, which is the reverse found in the test on fabrics and hard surfaces. Again, however, mixtures of the two germicides in the range of a 1:1 ratio exhibit distinctly superior germicidal activity over pure DBS and appear to be slightly more active than pure TBS.

Historically, PBS was developed first as the commercial antimicrobial agent of choice for general use because of the synergistic effect shown by the results of the above discussed experiments.

In this connection, it is interesting to note that contrary to the claims made by Stecker (7) that 4′,5 DBS is devoid of antibacterial activity, it was found that 4′,5 DBS is indeed active, and in the 1:1 mixture range with TBS, it provides the synergistic action against bacteria on animate and inanimate surfaces shown above. Antibacterial activity of 4′,5 DBS has also been observed by LeMaire et al. (3).

Recommended concn of PBS and TBS for use in various products are presented in Table V.

Compatibility. The synergistic action noted for the binary mixtures of DBS and TBS in alcoholic solution is also observed when this mixture is present in detergent systems comprised of soap, synthetic detergents or mixtures thereof. The synergistic effect of the binary mixtures described herein is also observed in the treatment of laundry to render it bacteriostatic.

Stability. The essentially pure TBS is particularly applicable for use in white or pastel colored soap because of its resistance to discoloration caused by UV

TABLE III

Bacteriostatic Effect of Mixtures of DBS and TBS

Percent of each germicide in binary system		Minimum effective concentration (in ppm) of germicide *mixture to give a zone of* inhibition against S. aureus
4′,5 DBS	3,4′,5 TBS	
0	100	20
5	95	19
25	75	15
40	60	8
50	50	5
60	40	6
75	25	7
95	5	9

TABLE IV

Serial Basin Wash Data of Soaps Containing 1% of Germicidal Mixtures

Percentage of each germicide in binary system		Percent reduction in hand counts (average) serial basin wash test
4',5 DBS	3,4',5 TBS	
0	100	89
25	75	89
40	60	91
50	50	97
60	40	91
75	25	84
100	0	68

light. In an investigation to determine the qualitative differences in discoloration, soap bars were exposed to a 20 w G.E. black light (UV) for 16 hr at a distance of 8 in. A commercial white floating soap, pastel colored with D & C colors, was used as a control. The test bars, in addition to color, contained germicides at a 1% concn. The observations are presented in Table VI.

TBS showed stability to UV light, for the test bars containing TBS did not show any discoloration greater than that shown by the control bars which contained no germicide. Considerable discoloration was observed with the hexachlorophene bar; the PBS bar showed moderate discoloration. The discoloration was compared to the unirradiated bars. It should be noted that even the control bars colored white, amber and green displayed a slight discoloration, whereas that shown by the blue control bar was moderate.

Extensive heat stability studies were conducted on PBS and TBS. No evidence of breakdown into aniline compounds was found. The principal components of PBS and TBS should be regarded as brominated monophenylamides of salicyclic acid. Because salicylic acid is a strong acid, its derivatives can be expected to be relatively stable. Indeed, the amide of salicylic acid does not break down at the elevated temp occurring in the autoclave or at the pH of soaps and detergents. The test developed for the analysis requires heating an alcoholic-aqueous solution of PBS at a pH of 10 in an autoclave at 20 psi for 20 min. Any aniline or halogenated aniline that may have been formed is then solubilized by adding a sufficient quantity of 1 *N* HCl to the mixture to make it acidic. After 15 min of agitation, the material is filtered and diazotized with sodium nitrite. A pink color would be developed with sodium β naphthol if aniline or halogenated aniline were present in a concn as low as 0.4 ppm. The color of the solution is compared to a standard containing 0.01 mg p-bromoaniline/ml (solubilized with equimolar HCl). No measurable color was obtained with either PBS or TBS thus treated, signifying the absence of detectable quantities of aniline or halogenated aniline. The test developed for this analysis is similar to that developed independently by M. S. Schechter (8) of the U.S. Dept. of Agriculture, who has confirmed the findings presented here.

TABLE V

Recommended Concentrations in Various Products

Product	Concn	Germicide	Use
	%		
Soap	0.5 –1	PBS or TBS	Skin degerming and deodorant action
Household detergents	0.25–0.5	PBS or TBS	*Sanitizing*
Shampoo	1.0 –3.0	PBS or TBS	Skin degerming and anti-dandruff action
Creams	0.1 –0.25	TBS	Skin degerming
Underam deodorants	0.1 –0.25	TBS	Skin degerming and deodorant action
Aerosol sanitizers	0.5 –1	PBS or TBS	Disinfecting/ sanitizing
Liquid disinfectant cleaners	3.0 –6	PBS alone or with phenolics	Disinfecting
Laundry final rinse treatment	3.0 –5	PBS or TBS	Sanitizing

TABLE VI

Degree of Discoloration of Soap Bars 16 Hr UV Exposure

	White	Amber	Green	Blue
Control (no germicide)	Slight	Slight	Slight	Moderate
Control + 1% hexachlorophene	Marked	Marked	Marked	Marked
Control + 1% Trichlorocarbanilide	Slight	Slight	Slight	Slight
Control + 1% PBS	Moderate	Moderate	Moderate	Moderate
Control + 1% TBS	Slight	Slight	Slight	Slight

Residual Activity. An important application of PBS is in hospital sanitizers and disinfectant cleaners, alone or in combination with other germicides. It provides quick kill and long-lasting residual action against Staphylococcus aureus and other hospital pathogens. It has been observed that pathogens do not develop resistance to this germicide. Vinson, Dineen and Schneider (9) have described products such as aerosols, disinfectant cleaners, and laundry antiseptic preparations containing PBS.

Safety. Because of published indications (1) that tetrachlorosalicylanilide is a photosensitizer, there has been a natural tendency to proceed with caution in the use of other halogenated salicylanilides in products which would contact human skin. Extensive studies of the dermatological effect of both PBS and TBS have been conducted. In one investigation (2) which was concerned with measuring the effect of several halogenated salicylanilides, TBS included, the authors concluded that:

". . . on subjects photosensitized to T_4CS (tetrachlorosalicylanilide), it was found that:

1. TBS was the only halogenated salicylanilide tested that did not demonstrate cross-photosensitivity.
2. In general, the skin reacts more to the chlorinated salicylanilides under test than to the brominated preparations.
3. Tetrahalogenated salicylanilides are stronger cross-photosensitizers than the trihalogenated derivatives."

A summary (10) of research conducted to date on the dermatological effect of TBS and PBS concluded that neither of these products is a primary irritant, sensitizer, photosensitizer or cross-sensitizer at recommended use concn.

Substantivity. Both PBS and TBS are substantive to skin and fabrics. This conclusion follows after considering the procedure employed to determine bacteriostatic effect, described above, which included a water rinse prior to testing for bacteriostatic action.

Economy. Because of the high germicidal power of these two antimicrobial agents, low concn can be employed effectively. The economy inherent in a low concn requirement is obvious.

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