

Soluble Brominated Salicylanilides

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Synopsis—The low aqueous solubility of the brominated salicylanilides (1) limits their usefulness in cosmetic or pharmaceutical preparations. Nonionic surface active agents and compounds chemically related to 1 did not increase solubility. Preparation of the salts of 1 gave compounds with good microbiological activity and aqueous solubility but with limited light stability. Aqueous solutions of formulations utilizing the salts of 1 showed good stability when they were protected from U.V. light.

INTRODUCTION

Antimicrobial agents are used in many cosmetic and pharmaceutical products to protect and preserve them from deterioration due to growth of bacteria, fungi, or yeasts. Many of the higher molecular weight agents such as hexachlorophene, dichlorophene, bithionol, the parabens and substituted salicylanilides have extremely low water solubilities. Because of their low water solubility their use is somewhat limited. Thus, with the great interest today in the salicylanilides it was thought that the preparation of a water soluble compound or mixture would be of value in product formulations.

Recently, considerable interest has centered about the halonitrosalicylanilides (1) and halogenated salicylanilides, especially various chlorinated and brominated derivatives (2, 3). The halogenation of the salicylanilide molecule enhances the activity of the parent compound (4).

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In 1959 Stecker (5) reported that a synergistic mixture existed between 5,4'-dibromosalicylanilide and 3,5,4'-tribromosalicylanilide (Diaphene^{®*}) when combined in the proper proportions. By a modified fresh calfskin technique he demonstrated the substantivity and antibacterial properties of the mixture (6).

At about the same time Mattson (7) reported another similar synergistic mixture consisting of 3,5-dibromosalicylanilide (approximately 15%), 3,5,4'-tribromosalicylanilide (approximately 85%), and traces of a monobromosalicylanilide derivative. This mixture is referred to as ET-394†.

The fact that Stecker (6) noted that the brominated salicylanilides were not inactivated by the presence of various emulsifiers and surface active agents suggested the possibility of physical solubilization of ET-394. All attempts to prepare a 0.1% aqueous solution of ET-394 by the addition of various nonionic or anionic surface active agents were unsuccessful. Even the addition of 50% ethanol failed to dissolve ET-394. Attempts to add ET-394 as a solution in polyethylene glycol 600 monolaurate to various combinations of ethanol, surface active agents and water were also unsuccessful.

Occasionally a chemical similar in structure to the material under investigation is capable of increasing the compound's solubility. Thus, the use of salicylanilide as a solubilizing agent was attempted, but without success.

Recently Russell (8) reported the solubilization of Diaphene to the extent of 0.8% in a mixture of 4% nonionic surface active agent and either 40% detergent or potassium coconut oil soap. Bacteriological testing of these solutions showed activity equal to that of a similar solution containing hexachlorophene.

Thus it appeared that the use of complexation or simple physical means would not work to enhance the solubility of ET-394 in water or in 50% ethanol at low surfactant level. On the other hand, it seemed likely that the brominated salicylanilides as their sodium, potassium or ammonium salts might exhibit improved water solubility.

EXPERIMENTAL

Preparation of Salts

Sodium ET-394 was the first salt to be prepared, and its preparation was accomplished in the following manner: Fifteen milliliters of a 10%

* Diaphene is a registered trademark of the Stecker Chemicals, Inc., Ho-Ho-Kus, N. J.

† Manufactured by The Dow Chemical Company, Midland, Mich.

solution of sodium hydroxide in absolute ethanol was triturated for 30 minutes with 10 g. of ET-394 in a mortar at room temperature. The insoluble product produced was collected on a filter, dried, and recrystallized from hot 25% ethanol. The melting point was $>300^{\circ}\text{C}$, and the salt (sodium ET-394) did not fluoresce under U.V. light. In a similar manner the sodium and potassium salt of the two major components of ET-394 were prepared. These are listed in Table I.

The ammonium salts could not be prepared by the above method. Thus, ammonium ET-394 was prepared by shaking for one hour at room temperature a mixture of 2 g. of ET-394, 50 ml. of 50% ethanol and 50

TABLE I
Salts of Brominated Salicylanilides

| Salicylanilide | Yield | | | Calcd., % | | Found, % | |
|-----------------|---------------|----|-------------------------|-----------|------|----------|------|
| | Salt | % | M.p. $^{\circ}\text{C}$ | C | H | C | H |
| ET-394 | Na | 73 | >300 | ... | .. | ... | .. |
| ET-394 | K | 90 | >300 | ... | .. | ... | .. |
| ET-394 | NH_4 | 55 | 227-9 | ... | .. | ... | .. |
| 3,5,4'-tribromo | Na | 65 | >300 | 33.08 | 1.49 | 32.46 | 1.80 |
| 3,5,4'-tribromo | K | 63 | >300 | 31.99 | 1.45 | 31.63 | 2.02 |
| 3,5,4'-tribromo | NH_4 | 71 | 232-4 | 33.43 | 2.37 | 34.10 | 2.39 |
| 3,5-dibromo | Na | 74 | >300 | 39.95 | 2.04 | 39.55 | 2.20 |
| 3,5-dibromo | K | 65 | >300 | 38.05 | 1.95 | 37.78 | 2.15 |
| 3,5-dibromo | NH_4 | 74 | 140-2 | 40.21 | 3.09 | 39.66 | 3.01 |

ml. of 28% ammonium hydroxide in a pressure bottle containing glass beads. After cooling of the mixture, the pressure bottle was opened. The mixture was warmed to remove the excess ammonia, filtered and cooled to precipitate the product. After collection on a filter and recrystallization from 25% ethanol, a 55% yield of ammonium ET-394 was obtained which fluoresced under U.V. light and melted at $227-9^{\circ}\text{C}$. The ammonium salts of the components of ET-394 were similarly prepared.

Infrared Absorption Data

As proof of salt formation, the infrared spectra of the various new compounds were compared with those of the parent molecules.

3,5,4'-Tribromosalicylanilide gave bands at 3525 for stretching OH, 1645, 1590, and 1540 for the amide group and 1175 cm^{-1} for the deformation of the OH group. The higher amide bands and the presence of the stretching OH bands indicated that the parent molecule exists in the β form (9) [H-bonding between (NH) and (OH) with the carbonyl group

free]. Sodium, potassium and ammonium 3,5,4'-tribromosalicylanilide showed no phenolic stretching band but a sharp band at 1225 (Na), 1210 (K), and 1220 cm.^{-1} (NH_4) which correlated with the (OH) deformation vibration. Ammonium 3,5,4'-tribromosalicylanilide showed a (NH) stretching vibration at 3410 cm.^{-1} . In all cases shifts of the amide I bands of the salts were slight, but the amide II band shifted to a slightly higher frequency due to the absence of H-bonding with (NH).

In the case of the salts of 3,5-dibromosalicylanilide and the parent molecule, the infrared spectra indicated that the absence of the 4'-bromo

TABLE II
Solubility of Salts of Salicylanilides in Various Solvents^a

| Compounds | | Gm./100 ml. at 25° | |
|---------------|-------------------------------|--------------------|--------------------|
| | | Water | 50% Ethanol |
| | ET-394 | 0.00086 | 0.050 |
| Na | ET-394 | 0.270 | 3.055 |
| K | ET-394 | 0.645 | 10.737 |
| NH_4 | ET-394 | 0.106 | 3.866 |
| | 3,5,4'-Tribromosalicylanilide | 0.0042 | ^b |
| Na | 3,5,4'-Tribromosalicylanilide | 0.347 | |
| K | 3,5,4'-Tribromosalicylanilide | 0.972 | |
| NH_4 | 3,5,4'-Tribromosalicylanilide | 0.145 | 3.448 |
| | 3,5-Dibromosalicylanilide | 0.036 | ^b |
| Na | 3,5-Dibromosalicylanilide | 1.298 | |
| K | 3,5-Dibromosalicylanilide | 3.645 | |
| NH_4 | 3,5-Dibromosalicylanilide | 0.555 | 4.731 |

^a The solubilities were determined gravimetrically.

^b Solubilities of the various salicylanilides could not be determined at this time because of lack of material for study.

group had very little effect on the (OH) or amide bands. Thus, spectral changes due to salt formation closely approximated those described for the tribromo compound.

Solubility Determinations

Distilled water and 50% ethanol were chosen as solvents for the determination of the solubility of the various salts as well as of the base compounds.

In all cases accurately weighed samples of the compounds were shaken for at least 24 hours in the given solvent at room temperature. The insoluble material from each sample was collected on a filter, the filter dried and weighed, and the solubilities calculated. Table II gives the solubilities of the brominated salicylanilides and their salts.

Antibacterial Studies

Antibacterial studies of the ET-394 salts (0.5% in 50% ethanol in amber bottles) were conducted over a period of six months to determine whether or not a loss of activity occurred. As shown in Table III, the salts retained their potency during this time.

The tests were conducted with six different organisms. Nutrient agar plates were smeared with the organisms, and discs were placed on the smears. The plates were incubated for 72 hours at 37°C. Ethanol (50%) was used as a standard.

Stability Studies

Aqueous solutions of the salts of the brominated salicylanilides turned brown within two weeks after exposure to room light. Solutions of the salts in 50% ethanol decomposed much more slowly and to a lesser extent. Solutions in amber bottles did not show a color change after one year. It appeared that light was necessary for the degradation. Salt solutions containing a trace of iron as an oxidizing catalyst did not change color in amber bottles.

Several antioxidants were used in an attempt to prevent the degradation. To 0.1% solutions of sodium and potassium ET-394 were added 0.1% of sodium bisulfite, sodium nitrite, sodium sulfite, ascorbic acid, and sodium metabisulfite, respectively. In every solution an immediate precipitate appeared. Similarly glycerin and 2-methyl-2,4-pentanediol in concentrations of from 0.05–5.0% yielded yellow solutions with heavy precipitation.

Sodium formaldehyde sulfoxylate and tetrasodium ethylenediamine-tetraacetic acid (38%) in concentrations of 0.001–0.1% in combinations with the sodium or potassium salts of ET-394 (0.1%) dissolved in water or 50% ethanol produced varying shades of brown with sedimentation. Chemical degradation also occurred even when various ultraviolet light absorbers were added to the solutions.

At this point it was realized that the mechanism and kinetics of the degradation of the salts in solution was beyond the scope of this project. Thus, this information will be presented in a separate publication.

Practical Use

Because of its unusually high solubility, potassium ET-394 was selected for incorporation into an aftershave lotion, a preshave lotion and a shampoo.

TABLE III
Antibacterial Activity of Salts of ET-394^a

| Organism | ET-394 ^b | G-11 ^b | Na ET-394 | | | K ET-394 | | | NH ₄ ET-394 | | |
|-----------------------|---------------------|-------------------|-----------|----|----|----------|----|----|------------------------|----|----|
| | 0 | 0 | 0 | 3 | 6 | 0 | 3 | 6 | 0 | 3 | 6 |
| <i>E. coli</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>A. faecalis</i> | 6 ^c | 11 | 3 | 2 | 3 | 3 | 6 | 3 | 2 | 2 | 3 |
| <i>Ps. aeruginosa</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>S. lutea</i> | 21 | 18 | 11 | 14 | 12 | 15 | 20 | 13 | 13 | 14 | 11 |
| <i>Staph. aureus</i> | 8 | 12 | 15 | 16 | 11 | 12 | 17 | 15 | 11 | 12 | 13 |
| <i>Staph. albus</i> | 17 | 13 | 8 | 11 | 14 | 11 | 19 | 16 | 8 | 13 | 13 |

^a A 0.5% solution in 50% ethanol, using agar plates incubated 72 hr. at 37°C.

^b ET-394 (mixture of tri- and di-brominated salicylanilides) (0.025%) in 0.5% cetyl alcohol and a sufficient quantity of 70% isopropanol to make 100 ml. Same solvent for 0.1% G-11 (hexachlorophene).

^c Numbers are millimeters of inhibition.

A typical aftershave formulation containing sorbitol, menthol, boric acid, perfume oil, dye, and 50% denatured alcohol was prepared. A sample of the product was kept as a control. To the remaining solution was added 0.2% potassium ET-394. The solution immediately changed to a lighter color which could not be rematched exactly to the intensity of the control by the addition of more dye. The solution was divided in half; one sample was stored in a clear glass bottle, and the other sample was stored in an amber bottle. These two solutions and the control were subjected to periodic antibacterial testing.

The second formulation, a preshave lotion, contained sorbitol, a nonionic surface active agent, perfume, color, and 50% ethanol. Again three samples were prepared, one as a control and two containing the antibacterial agent.

The third preparation, a triethanolamine lauryl sulfate liquid shampoo, was prepared and divided into three samples, two of which contained potassium ET-394.

After two months of storage the preparations were subjected to antibacterial testing. The same organisms and methods were used as described under "Antibacterial Studies." The products containing potassium ET-394 were extremely effective against gram-positive organisms and displayed little, if any, inhibitory effect on gram-negative organisms. The zones of inhibition closely approximated those given in Table III for potassium ET-394.

The aftershave lotion decomposed after standing in direct sunlight

for several days in a clear bottle. The preshave lotion and the shampoo retained their original appearances even after remaining in direct sunlight for a month. All of the preparations retained their original color when stored in amber bottles.

CONCLUSIONS AND DISCUSSION

Unsuccessful attempts were made to increase the solubility of ET-394 by various physical methods. It was found possible, however, to increase its solubility in aqueous media by formation of its alkali metal salts without any hydrolysis of the amide linkage.

When the potassium, sodium, and ammonium salts are dissolved in distilled water or ethanol (50%) and exposed to sunlight, they developed a brown color with precipitation after short periods of time. Amber bottles, which have been used in the past to prevent light degradation, proved useful in preventing chemical decomposition of the salt solutions. Solutions of potassium, sodium, and ammonium ET-394 (0.5%) in ethanol (50%) were tested for antibacterial activity when fresh and after six months' storage in amber bottles. At the end of the six month period the solutions retained their original antibacterial activities, clearness and colorless appearance. Thus, it seems that solubility is not a primary factor in the mechanism of action of the brominated salicylanilides. I.R. studies of the various salts indicated further that only slight changes in the internal bonding of the compounds took place during salt formation.

Because of its high solubility, good antibacterial action and ease of manufacture, potassium ET-394 (0.2%) was eventually tested in each of three formulations, an aftershave lotion, a preshave lotion, and a shampoo. After a few days a brown color was detectable only in the aftershave lotion in the clear glass bottle, while the remaining preparations appeared unchanged. Antibacterial testing, however, produced no evidence to support loss of activity by degradation since the activity of the brown solution was equal to that of the clear ones.

It is concluded that the salts of brominated salicylanilides have solubility properties which can be utilized in preparations in which other antibacterial agents would be insoluble or possibly inactivated by components of the system.

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