at $\tau$ = 2.91 (8H), assigned to the aromatic protons; a singlet at $\tau$ = 6.60 (3H), assigned to the methyl protons on the nitrogen of the acridane nucleus (10-methyl); a singlet at $\tau$ = 6.95 (3H), assigned to one of the methyl group protons of the amide; a singlet at $\tau$ = 7.65 (3H) assigned to the protons of the other methyl group of the amide; and a singlet at $\tau$ = 8.40 (3H) assigned to the 9-methyl protons. The UV spectrum showed $\lambda_{max.}^{MeOH}$ at 288 mμ ($1.7 \times 10^4$). The IR spectrum showed $\lambda_{max.}^{CHCl_3}$ at 3.32(s), 3.41(m), 6.08(v.s.), 6.15(v.s.), 6.3(s), 6.8(v.s.), 6.9(s), 6.95(s), 7.2(s), 7.41(v.s.), 7.85(m), 7.9(s), 8.5(m), 8.7(m), 8.8(s), 9.2(s), 9.6(m), 11.3(m), 11.55(m), 13.8(m)broad μ. Three more recrystallizations from diluted ethanol afforded the analytical sample, white needles, m.p. 183–185°.

*Anal.*—Calcd. for $C_{18}H_{20}N_2O$: C, 77.11: H, 7.19; N, 9.99. Found: C, 77.19; H, 7.09; N, 9.86.

**$LiAlH_4$ Reduction of N,N-Dimethyl-9-carboxamido-9,10-dimethylacridane (IV) to 9,10-Dimethylacridane (Ib)**—In a two-neck, 100-ml. flash equipped with a condenser funnel and a nitrogen outlet 0.5 g. (13.0 mmoles) of lithium aluminum hydride in 25 ml. of dry ether was refluxed while stirring under nitrogen for 45 min. At this time, a suspension of 0.3 g. (1.07 mmoles) of *N,N*-dimethyl-9-carboxamido-9,10-dimethylacridane in 10 ml. of ether was introduced. These substances were heated, under reflux while stirring and passing nitrogen, for 12 hr. The excess lithium aluminum hydride was decomposed by adding ethyl acetate while cooling in an ice bath. This was followed by 20 ml. of 40% aqueous sodium potassium tartrate. The mixture was filtered and the ethyl acetate layer separated. The aqueous layer was extracted successively with ethyl acetate and chloroform. The combined organic extracts were distilled at 45° under reduced pressure. The white crystalline residue, m.p. 125–134°, was recrystallized twice from methanol to yield 0.213 g. (95.3% yield), of 9,10-dimethylacridane (*Ib*) as white needles, m.p. 130–134°. Three more recrystallizations from methanol afforded the analytical sample as white needles, m.p. 136–137°, which did not depress the melting point of an authentic sample of 9,10-dimethylacridane [synthesized according to Semon's procedure (3) and recrystallized from methanol], and had identical IR and UV spectra.

*Anal.*—Calcd. for $C_{15}H_{15}N$: C, 86.08; H, 7.22; N, 6.69. Found: C, 86.08; H, 7.19; N, 6.67.

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# Insoluble Erythromycin Salts


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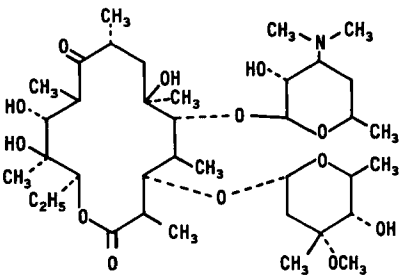
**Abstract** □ Fifteen salts of erythromycin were prepared and their relative water solubilities and bitterness levels measured. The water solubilities of the salts were found to be related to the size of the alkyl group attached to the acid. The level of bitterness, however, was related not only to the size of the alkyl group but also to the stability of the salt. The stability of the salt was a function of the strength of the acid used to prepare the salt and could be measured by the downfield shift of the *N*-methyl protons in the NMR spectrum of the salt. The stearyl sulfate salt was the least bitter of the salts.



**Keyphrases** □ Erythromycin salts, insoluble—synthesis □ Solubility, aqueous—alkyl group effect □ Bitterness, erythromycin salts—alkyl group, stability relationship □ Stability, erythromycin salts—acid strength, salt formation □ NMR spectroscopy—identity


Erythromycin, a member of the macrolide family of antibiotics (1) is highly active against a broad spectrum of Gram-positive bacteria. Its structure is shown (2).



ERYTHROMYCIN

Although the antibiotic has been shown to be effective therapeutically, it has an inherently bitter taste which must be masked for oral administration. For adults, the taste problem is overcome by administration of the antibiotic in coated tablets or in capsules. However, in preparations intended for pediatric use, the preferred formulations are chewable tablets and suspensions. The bitterness in these pediatric dosage forms can be minimized by making an insoluble ester derivative of the hydroxyl group on the basic sugar such as the ethyl succinate (3), the ethyl carbonate (4), or the propionate ester (5), or by making an insoluble salt of the amine. Salts of erythromycin can be formed by reaction of the tertiary amine group with acids. This paper describes the authors' studies on the preparation of various salts of erythromycin and their properties.

## RESULTS

Two factors were considered in choosing the acids for the preparation of the salts. These were the strength of the acid and the size of the alkyl group attached to the acidic function. Four types of acids with different acid strengths were chosen: carboxylic acids

**Table I**—Erythromycin Salts: Physical and Biological Properties

| Erythromycin Salt | Compd. No. | Microbiological Assay, mcg./mg.[a] Theory | Found | Water Solubility at 25°C., mcg./ml. | NMR of Protonated Dimethylamine, c.p.s. Absorbance | Shift from Erythromycin 137 c.p.s. | Relative Bitterness Level[b] |
|---|---|---|---|---|---|---|---|
| Formate | I | 940 | 911 | >20,000 | 157 | 20 | 5 |
| Laurate | II | 786 | 722 | 571 | 151 | 14 | 4 |
| Stearate | III | 720 | 665 | 182 | 147 | 10 | 3 |
| Phosphate | IV | 880 | 728 | >20,000 | 154 | 17 | 5 |
| Ethyl phosphate | V | 853 | 772 | >20,000 | 165 | 28 | 5 |
| Monostearyl phosphate | VI | 676 | 564 | 1,096 | 165 | 28 | 3 |
| Distearyl phosphate | VII | 548 | 468 | 205 | 165 | 28 | 3 |
| Sulfamate | VIII | 881 | 886 | >20,000 | 178 | 41 | 5 |
| Cyclohexylsulfamate | IX | 803 | 590 | >20,000 | 174 | 37 | 5 |
| Octylsulfamate | X | 777 | 745 | 4,100 | 177 | 40 | 4 |
| Lauryl sulfamate | XI | 734 | 600 | 624 | 176 | 39 | 3 |
| Stearyl sulfamate | XII | 676 | 590 | 316 | 176 | 39 | 2 |
| 2-Ethylhexyl sulfate | XIII | 944 | 772 | 1,800 | 179 | 42 | 4 |
| Lauryl sulfate | XIV | 734 | 759 | 320 | 179 | 42 | 3 |
| Stearyl sulfate | XV | 676 | 670 | 108 | 179 | 42 | 1 |

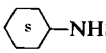
[a] Plate microbiological assay using *B. subtilis* organism, based on erythromycin as 1,000 mcg./mg. [b] 1, tasteless; 2, very slightly bitter; 3, slightly bitter; 4, moderately bitter; 5, very bitter.

phosphoric acids, sulfamic acids, and sulfuric acids. Salts of erythromycin were prepared with various alkyl derivatives of these acids. These salts are listed in Table I together with their biological activities, water solubilities, bitterness levels, and NMR absorptions of their protonated dimethylamino groups.

## DISCUSSION

The results tabulated in Table I indicate that the solubility of an erythromycin salt is an inverse function of the length of the alkyl group of the acid. It is known that increasing the length of the

**Table II**—Erythromycin Salts: Methods of Preparation

| Acid Used to Prepare Salt | | M.p. °C. | Empirical Formula | Elemental Analysis Theory | Found | Preparation Solvent | Crystallization Solvent |
|---|---|---|---|---|---|---|---|
| HCOOH | I | 164–165 | $C_{38}H_{69}NO_{15}$ | C, 58.51<br>H, 8.92 | C, 58.21<br>H, 8.72 | ether | 2-butanone |
| $C_{11}H_{23}COOH$ | II | 105–110 | $C_{49}H_{91}NO_{15}$ | C, 62.99<br>H, 9.82 | C, 63.02<br>H, 9.85 | acetone–water | acetone–water |
| $C_{17}H_{35}COOH$ | III | 103–107 | $C_{55}H_{103}NO_{15}$ | C, 64.86<br>H, 10.19 | C, 64.69<br>H, 10.10 | acetone–water | acetone–water |
| $H_3PO_4$ | IV | 151–154 | $C_{37}H_{71}NO_{17}P$ | C, 53.35<br>H, 8.59<br>P, 3.72 | C, 53.07<br>H, 8.72<br>P, 3.34 | acetone | chloroform |
| $C_2H_5OPO_3H_2$ | V | 130–139 | $C_{39}H_{74}NO_{17}P$ | C, 54.47<br>H, 8.67<br>P, 3.60 | C, 54.57<br>H, 8.80<br>P, 3.41 | acetone | chloroform–hexane |
| $C_{18}H_{37}OPO_3H_2$ | VI | 103–105 | $C_{55}H_{106}NO_{17}P$ | C, 60.92<br>H, 9.85<br>P, 2.85 | C, 60.66<br>C, 10.11<br>P, 2.58 | ether | acetone–hexane |
| $(C_{18}H_{37}O)_2PO_2H$ | VII | 81–83 | $C_{73}H_{142}NO_{17}P$ | C, 65.58<br>H, 10.70<br>P, 2.32 | C, 65.48<br>H, 10.62<br>P, 2.27 | ether | acetone–hexane |
| $H_2NSO_3H$ | VIII | 192–195 | $C_{37}H_{70}N_2O_{16}S$ | C, 53.47<br>H, 8.49<br>S, 3.86 | C, 52.95<br>C, 8.11<br>S, 3.85 | acetone–water | acetonitrile |
|  | IX | 171–181 | $C_{43}H_{80}N_2O_{16}S$ | C, 56.55<br>H, 8.83<br>S, 3.51 | C, 56.47<br>C, 8.61<br>S, 3.48 | acetonitrile | acetonitrile |
| $C_8H_{17}NHSO_3H$ | X | 145–150 | $C_{45}H_{86}N_2O_{16}S$ | C, 57.30<br>H, 9.19<br>S, 3.40 | C, 57.11<br>H, 9.22<br>S, 3.51 | acetone–hexane | acetone–hexane |
| $C_{12}H_{25}NHSO_3H$ | XI | 129–132 | $C_{49}H_{94}N_2O_{16}S$ | C, 58.89<br>H, 9.48<br>S, 3.21 | C, 58.89<br>H, 9.62<br>S, 3.09 | chloroform | 2-butanone |
| $C_{18}H_{37}NHSO_3H$ | XII | 115–117 | $C_{55}N_{106}N_2O_{16}S$ | C, 60.97<br>H, 9.86<br>S, 2.96 | C, 60.43<br>H, 9.76<br>S, 3.11 | chloroform | 2-butanone |
| $C_4H_9CHCH_2OSO_3H$<br>$C_2H_5$ | XIII | 120–140 | $C_{45}H_{85}NO_{17}S$ | C, 57.24<br>H, 9.07<br>S, 3.39 | C, 57.25<br>H, 9.12<br>S, 3.59 | acetone–water | acetone–water |
| $C_{12}H_{25}OSO_3H$ | XIV | 128–132 | $C_{49}H_{93}NO_{17}S$ | C, 58.83<br>H, 9.37<br>S, 3.21 | C, 58.85<br>H, 9.17<br>S, 3.27 | acetone–water | acetone–water |
| $C_{18}H_{37}OSO_3H$ | XV | 125–128 | $C_{55}H_{105}NO_{17}S$ | C, 60.91<br>H, 9.76<br>S, 2.76 | C, 60.61<br>H, 9.78<br>S, 2.76 | acetone–water | acetone–water |

alkyl chain of the acid increases its hydrophobic properties as well as those of the corresponding salt. Magerlein also noted this relationship when he prepared a series of alkyl sulfamic acid salts of the antibiotic, lincomycin (6).

The results in Table I also show that the salts made from acids with similar alkyl groups but with different acid strengths have similar water solubilities, *i.e.*, III, VII, XII, and XV; II, XI, and XIV; X and XIII. The small differences in the solubilities within these groups may be due to a minor influence of acid strength. The increased solubility of the salt of stearyl phosphoric acid (VI) is probably due to the free acid group on the phosphate. Thus one must infer that the strength of the acidic group has little influence on the water solubility of the salts and the solubility is related primarily to the length of the alkyl group of the acid.

From a comparison of the bitterness level of each salt (Table I) one can see that within each series of salts, I–III; IV–VII; VIII–XII; XIII–XV; the bitterness level correlates well to the solubility in water. However, the bitterness of salts with similar alkyl groups but different acid functions, *i.e.*, III, VII, XII, and XV; II, XI, and XIV; X and XIII; shows little or no correlation to water solubility. The taste appears to be a function of the strength of the acid used to make the salt as well as of the water solubility.

Since the dimethylamino group of erythromycin A is a weakly basic group (pKa 8.8), the stability of the corresponding salt is dependent on the strength of the acid used in the preparation of the salt. Salts made from weak acids such as carboxylic or phosphoric acids are unstable and easily revert back to the free acid and base (4, 7). Several examples of this phenomenon were observed throughout this study, *i.e.*, the formic and acetic acid salts could be converted back to erythromycin base merely by drying the salt under high vacuum. This observation was not seen with the stronger acids such as sulfamic and sulfuric which form stable salts. Thus for a series of salts with a particular alkyl group the bitterness level is dependent not on the water solubility of the salt but on its relative stability.

The relative stabilities of the salts could be determined by measuring in the NMR spectra the chemical shifts of the *N*-methyl hydrogens of the protonated dimethylamine. These shifts together with the shift measured from the nonprotonated form (erythromycin, 137 c.p.s.) are tabulated in Table I. It was previously shown (8–11) that the resonance peak of a *N*-methyl group is shifted downfield on protonation and that the magnitude of this shift is dependent on the strength of the acid used to form the salt. These increasing shifts correlate well to the acid strengths and to the salt stability. Thus the sulfate salts (shifts of 42 c.p.s.) from erythromycin are the most stable followed closely by the sulfamates (39 c.p.s.). Much more unstable are the phosphates (28 c.p.s.) and finally the carboxylic acid salts (10 c.p.s.).

It was concluded that the water solubility of an erythromycin salt is dependent primarily on the size of the alkyl group of the acid. The bitterness level, however, is related not only to the water solubility of the salt and hence to the size of the alkyl group, but also to the stability of the salt and hence to the acid strength. Thus, the most taste-free salt of erythromycin was made from the strongest acid with the largest alkyl group, which in this series is the stearyl sulfuric acid salt (XV).

## EXPERIMENTAL

The NMR spectra were recorded on a spectrophotometer,[1] using deuteriochloroform solutions; values are measured in c.p.s. downfield from tetramethylsilane as an internal standard. Melting points were determined using a Koeffler hot stage.

**Acids**—The acids used to prepare Salts I–IV and VIII–IX were obtained from standard commercial sources. The alkyl phosphoric acids used to prepare Salts V–VII were prepared by hydrogenolysis of the corresponding diphenyl phosphate esters (12, 13). The alkyl sulfamic acids were prepared by the method of Magerlein (6). *n*-Octyl sulfamic acid was a new compound, m.p. 184–186°.

*Anal.*—Calcd. for $C_8H_{19}NO_3S$: C, 45.90; H, 9.15; N, 6.69; S, 15.32. Found: C, 45.94; H, 8.98; N, 6.54; S, 15.07.

The Salts XIII–XV were prepared from the sodium salts of the corresponding acids. The sodium salts for XIII and XIV were obtained from commercial sources. Sodium stearyl sulfate for XV was prepared by the literature method (14).

**Preparations of Erythromycin Salts**—The Salts I–XII were prepared by combination of stoichiometric quantities of the corresponding acid and erythromycin base in an appropriate solvent from which the salt was isolated either by filtration or by evaporation of the solvent. The salt was recrystallized from a second solvent or solvent pair. The Salts XIII–XV were prepared by adjusting an equimolar solution of the sodium salt of the acid and erythromycin in aqueous acetone to pH 6 with 1 *N* HCl (7). Salts were crystallized from aqueous acetone mixtures. The salts are listed in Table II with their empirical formulas, melting points, elemental analyses, and the solvents used for preparation and recrystallization.

**Solubility and Taste Studies**—The solubility of each salt was determined by placing 300 mg. of the salt and 5 ml. of water in a sealed ampul. The ampul was shaken on a shaker[2] for 2 hr. in a 25° water bath. The solution was prefiltered through a cotton pledget before filtering through a 0.45-$\mu$ membrane. (Millipore) The solution was diluted and assayed by a chemical method utilizing arsenomolybdate (15). The results are an average of two runs. The relative taste levels were qualitatively determined by holding a small sample ($\sim$3 mg.) of each salt in the mouth for 30 sec. Each salt was placed into one of five levels of bitterness. The results are tabulated in Table I.

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[1] Varian A-60.

[2] Vibro mixer.