The Biodegradation of Esters of α-Sulfo Fatty Acids[1]

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ABSTRACT AND SUMMARY

Methyl and aminoalkyl esters of α-sulfonated fatty acids in the C_9-C_{18} range were subjected to aerobic biodegradation in a controlled nutrient medium in which sewage microorganisms were used as the inoculum and the test compound was the sole source of carbon and energy. The extent of biodegradation increased with increasing alkyl chain length of the α-sulfo fatty acid and decreased with increasing substitution on the amino nitrogen and possibly with the increasing molecular weight of the substituent on the amino nitrogen atom. No connection was found between the degree of biodegradability and antimicrobial activity of the test compounds. Methyl α-sulfopalmitate was the compound of this study most inhibitory against *Staphylococcus aureus.*

INTRODUCTION

Previous studies on phosphate-free soap-based detergents showed that anionic or amphoteric lime soap dispersants were most effective in soap-based detergent formulations (1,2). The anionic compounds were usually tallow-derived surfactants which proved to be highly biodegradable under aerobic and microaerophilic conditions (3-5). The amphoteric sulfobetaines of the structure $R\overset{+}{N}(CH_3)_2CH_2CH_2CH_2SO_3^-$ degraded slowly and incompletely, whereas the analogous amido compounds $RCONH(CH_2)_3\overset{+}{N}(CH_3)_2CH_2CH_2CH_2SO_3^-$ degraded rapidly and more completely (6). Presumably, the amide linkage of the latter undergoes enzymatic cleavage (7), and the resulting fatty acid degrades rapidly; whereas the alkylsulfobetaine lacking the amido group could not be cleaved and thus would resist biodegradation.

Recently Smith and co-workers (8) synthesized a series of esters of amino alcohols and α-sulfo fatty acids. These surfactants, in spite of their amphoteric structure, proved disappointing as lime soap dispersants in soap-based detergent formulations. However, since such esters presumably would readily undergo enzymatic hydrolysis and thus biodegrade completely and rapidly, it was of interest to study their biodegradation in comparison with the corresponding methyl esters.

EXPERIMENTAL PROCEDURES

Materials

The sodium salts of the methyl esters of α-sulfo fatty acids were prepared according to the procedure of Stirton et al. (9,10), and the esters of various amino alcohols and α-sulfo lauric acid were those prepared by Smith et al. (8). All of the test compounds used in this study were greater than 97% in purity. Hexachlorophene was obtained through the courtesy of the Givaudan Corporation, Clifton, NJ.

Biodegradation

Biodegradability under aerobic conditions at 25 C was determined by the Esso controlled nutrient procedure (11,12) in which the essential minerals were supplied, and the test compound at 40 ppm was the sole source of carbon and energy. The source of inoculum was activated sludge from a local sewage plant that treats principally domestic sewage. Before each experiment the sludge was acclimated to synthetic sewage (12) for 1 week by following the Soap and Detergent Association procedure (13). The course of biodegradation was followed by measuring the loss of carbon with a Beckman carbonaceous analyzer (3,12). Sensitivity was increased by standardizing the instrument so that a 30 μl sample of 50 ppm sodium oxalate gave a full scale peak height of 100 ppm on the recorder scale.

Bacteriostatic Activity

In screening tests of the surfactants for bacteriostatic activity, 1% stock solutions were prepared by dissolving 100 mg of test compound in 10 ml of 95% ethanol or water. The stock solutions were serially diluted by successively pipetting 2 ml of solution into 18 ml of sterile nutrient agar to obtain 1000, 100, 10, and 1 ppm concentrations of compound. The agar was poured into sterile Petri dishes, allowed to harden, dried at 37 C for 1 hr with covers off, then inoculated with one drop of a 24-hr culture of test microorganism in nutrient broth. The inoculated dishes were then incubated for 48 hr and examined for presence or absence of growth. The test compounds were stable to hydrolysis under the experimental conditions used at essentially neutral pH (8,14).

The following microorganisms were used: *Escherichia coli* ATCC No. 11229, *Staphylococcus aureus* ATCC No. 6538, *Pseudomonas aeruginosa* ATCC No. 8709, *Salmonella typhimurium,* (U.S. HEW, CDC), and *S. enteritidis* (U.S. HEW, CDC). Hexachlorophene was used as the control germicidal standard.

RESULTS AND DISCUSSION

Calculation of the percent biodegradation in these experiments was based on the ratio of carbon content before and after the degradation period. The results are reported in Table I.

Part A of Table I shows the effect of chain length on the extent of biodegradation of the methyl esters of α-sulfo fatty acids. The results, which are the averages of three replicates, were analyzed by analysis of variance and Duncan's Multiple Range Test at the 5% significance level. According to these tests, the degradation data for laurate and pelargonate are significantly different from those for the stearate, palmitate, and myristate. Also, the degradation of myristate is significantly different from that of the stearate but not from that of the palmitate. There is no significant difference between the stearate and palmitate. Thus, the extent of degradation increases with increasing carbon chain length of the α-sulfo esters.

Part B of Table I shows the effect of increased substitution on the amino nitrogen, as well as possibly that of increased molecular weight of substitutents on the amino nitrogen atom. The number of replicates for all of the experiments of Parts B and C was too low to allow application of Duncan's Multiple Range Test. However, since the experiments of Parts A, B, and C of Table I were run in an identical manner, it is reasonable to assume that their results are comparable. The extent of biodegradation of all amino esters was less than that of the α-sulfolauric acid. While there was very little difference between the ethanolamine and methyl esters of α-sulfolauric acid, there was a notable decrease in the extent of biodegradation with in-

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TABLE I

Biodegradation and Antibacterial Properties of Methyl and Aminoalkyl Esters of α-Sulfo Fatty Acids

			Inhibitory concentration, ppm				
A. Methyl esters	Percent biodegradation[a]	Time, days	S. *aureus*	*E. coli*	*Ps. aeruginosa*	*S. typhimurium*	*S. enteritidis*
$C_7H_{15}CH(SO_3Na)COOCH_3$	54^C	27	>1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3Na)COOCH_3$	54^C	27	1000	>1000	>1000	>1000	>1000
$C_{12}H_{25}CH(SO_3Na)COOCH_3$	74^B	27	>100	>1000	>1000	>1000	>1000
$C_{14}H_{29}CH(SO_3Na)COOCH_3$	78^{BA}	27	100	>1000	>1000	>1000	>1000
$C_{16}H_{33}CH(SO_3Na)COOCH_3$	83^A	27	1000	>1000	>1000	>1000	>1000
B. α-Sulfolauric acid and esters							
$C_{10}H_{21}CH(SO_3H)COOH$	52	22	1000	1000	1000	1000	1000
$C_{10}H_{21}CH(SO_3Na)COOCH_3$	51	22	1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}H_3$	49	22	>1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}H_2CH_3$	44	22	>1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}H(CH_3)_2$	38	22	1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}(CH_3)_3$	40	22	1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}H_2CH_2CH_2OH$	38	22	1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)CO\text{-}OCH_2CH_2OCH_2CH_2\overset{+}{N}H_3$	31	22	1000	>1000	>1000	>1000	>1000
$C_{10}H_{21}CH(SO_3^-)COOH + HOCH_2CH_2OCH_2CH_2\overset{+}{N}H_2$	48	22	—	—	—	—	—
C. α-Sulfopalmitates							
$C_{14}H_{29}CH(SO_3Na)COOCH_3$	78	22	100	>1000	>1000	>1000	>1000
$C_{14}H_{29}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}H_3$	49	22	1000	>1000	>1000	>1000	>1000
$C_{14}H_{29}CH(SO_3^-)CO\text{-}OCH_2CH_2\overset{+}{N}(CH_3)_3$	47	22	>1000	>1000	>1000	>1000	>1000
$C_{14}H_{29}CH(SO_3^-)CO\text{-}OCH_2CH_2OCH_2CH_2\overset{+}{N}H_3$	42	22	>1000	>1000	>1000	>1000	>1000
D. Control germicidal standard							
Hexachlorophene	—	—	1	10	10	100	1

[a] Means with the same letter are not significantly different, whereas means with no letters in common are significantly different.

creased substitution on the amino nitrogen atom of the ethanolamine esters. The quaternary and tertiary esters were nearly equal with respect to the percent biodegradation. The diglycolamine ester was the least biodegradable of the α-sulfolaurates. It was suspected initially that the poor biodegradability of the diglycolamine ester might be due to its hydrolysis to the α-sulfo acid and diglycolamine, which conceivably could inhibit degradation. However, a determination of the biodegradation of the mixture of equimolar amounts of α-sulfolauric acid and diglycolamine showed this not to be the case since there was virtually no difference between the extent of biodegradation of α-sulfolauric acid, its methyl ester, and the mixture of acid and diglycolamine. Thus, the poor biodegradability of the diglycolamine ester was apparently an inherent characteristic of the ester.

Part C of the table shows analogous biodegradation data for amino esters of α-sulfopalmitic acid. Since the extent of biodegradation of methyl α-sulfopalmitate was so much greater than that of the sulfolaurate, the decrease in biodegradability of its amino esters was even more striking. Again, the diglycolamine ester was the least biodegradable of the series.

The fact that some of these esters resist biodegradation suggested that they might possess antibacterial properties if there is an analogy between the α-sulfo fatty acids and the unsubstituted fatty acids. Kabara et al. (14) have shown that lauric acid was the most inhibitory of straight chain fatty acids, whereas the C_6, C_8, C_{18}, and C_{20} acids showed no inhibition at the maximum concentration tested (1.0 mg/ml = 1000 ppm). Also, in earlier work, Wyss and coworkers (15) showed that both pelargonic and lauric acids inhibited *S. aureus* at about the same molar concentration that Kabara found for lauric acid (2.49 μm = ca. 500 ppm).

Table I shows the inhibitory concentrations of α-sulfo fatty acids and esters against one gram positive and four gram negative microorganisms. Unlike the unsubstituted fatty acids and esters, which had maximum inhibition at C_{12}, the α-sulfo derivatives had maximum inhibition (100 ppm) against *S. aureus* at C_{16}. Of all the compounds in this study, only sulfolauric acid was effective at 1000 ppm against the five microorganisms used. The amino esters possessed no greater antibacterial properties than did the methyl esters. It is thus concluded that poor biodegradability of some of the compounds of this study is unrelated to antimicrobial activity.

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