LONG-CHAIN ACETALS OF ASCORBIC AND ERYTHORBIC ACID AS ANTINITROSAMINE AGENTS FOR BACON

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INTRODUCTION

Based on the mechanism of formation of N-nitrosopyrrolidine (NPyr) in bacon, proposed in an earlier publication (Bharucha, et al.,1979) from this laboratory, it was postulated that a good nitrosamine-blocking agent should inter alia satisfy the following requirements:

1. Serve as a good NO$^{\cdot}$ radical trap;
2. Be fat-soluble;
3. Be non-steam-volatile;
4. Be stable up to the maximum frying temperature of about 174°C.

A number of compounds, both old and new, which have the above attributes were synthesized and, as expected, were found to inhibit the nitrosamine formation in bacon. The present communication describes our work with one such class of compounds, the hitherto unknown long-chain acetals of ascorbic and erythorbic acid.

EXPERIMENTAL

Ascorbyl Palmitate: Ascrobyl palmitate was obtained from ICN Pharmaceuticals Inc.

Fatty Aldehydes:

Tetradecanal, hexadecanal, octadecanal, and Δ9-octadecenal were prepared from the corresponding alcohols by oxidation with pyridinium chlorochromate (Corey & Suggs, 1975). Dodecenal was obtained from Eastman Kodak.

Ascorbyl Acetals:

Two methods were used for the preparation of the acetals. Details will be given elsewhere.

The products were crystallized from ether/hexane several times to give pure samples which gave satisfactory elemental analyses. Most of the acetals apparently contained water of crystallization. The melting points are given in Table I.

Application of Additives to Bacon

The bacon used in this study was commercial pump-cured side bacon (150 ppm sodium nitrite). Ascorbyl palmitate and the acetals were applied to the bacon slices as a slurry in antioxidant-free soybean oil, usually 4 mL per pound. The slurry containing the appropriate amount of additive was poured over the shingled bacon slices and spread with a spatula. The bacon was either fried immediately or vacuum sealed in packages for storage at 3°C.

Frying Conditions : The bacon was fried as previously described. (Bharucha et al., 1979).

Analysis of Volatile Nitrosamines

For the most part, the analytical methods described in an earlier publication (Cross et al., 1978) were used to measure the volatile nitrosamines in bacon cook-out fat. Fried bacon rashers were analyzed by the revised densitometric procedure (Cross & Bharucha, 1979) for N-Nitrosodimethylamine (NDMA) and N-Nitrosopyrrolidine (NPyr).

The Effect of Storage Time on the Antinitrosamine Activity of Ascorbyl Palmitate

Ascorbyl palmitate was used at 2 levels - 500 and 1000 ppm. The bacon, both test and control, was fried immediately or after a storage period of 21 days. The results will not be reported in detail here, but will be commented on later.

The Influence Of The Length of the Acetal Side Chain on Antinitrosamine Activity

The acetals prepared from ascorbic acid and C_{12}, C_{14}, C_{16}, and C_{18} aldehydes were tested for their antinitrosamine activity using equimolar quantities (0.293 mmoles/Kg) corresponding to the C_{12} acetal at 100 ppm. The sliced bacon was sampled sequentially to give two groups of five equivalent samples. One sample of each group was treated with soybean oil while the other was treated with slurries of the individual acetals in soybean oil to give a level of 0.293 mmoles/Kg on the bacon. The bacon was fried and the cook-out fat analyzed for volatile nitrosamines.

The Effect of Storage Time on the Antinitrosamine Activity of the Acetals of Hexadecanal with Ascorbic & Erythorbic Acids at 1000 ppm

Two pound lots of bacon were sequentially sampled into four ½ pound packs (2 controls and 2 tests). The controls were treated with soybean oil (2 mL) while the tests were treated with the acetals of ascorbic or erythorbic acid (227 mg) as a slurry in soybean oil (2 mL). One sample each of the control and test was fried immediately and both the rasher and cook-out fat were analyzed for volatile nitrosamines. The other two samples were vacuum sealed and stored at 3°C for 35 days before being cooked and analyzed. The analytical procedure employed was the revised thin-layer densitometric procedure (Cross & Bharucha, 1979) which requires a sample size of only 20 grams. The experiment was repeated for a total of four times in the case of the erythorbyl acetal and three times for the ascrobyl acetal. The samples were chosen to be either unusually fat or unusually lean. The results for only the ascorbyl acetal are presented here.

RESULTS AND DISCUSSION

All of our results will not be reported here. A full version of this paper will be published in the Journal of Agricultural and Food Chemistry. Thus the acetals of both ascorbic and erythorbic acid behave similarly and

the choice as to which to use must be based on considerations other than efficacy. Only the results with ascorbyl acetal will be given here, although those obtained with erythorbyl acetal will be indicated. There are other omissions which do not seriously affect the main argument.

It has been well established (Herring, 1973) that the incorporation of sodium ascorbate or erythorbate in curing pickle will lower nitrosamine levels in cooked bacon, albeit slightly. The ascorbate or erythorbate reduction in nitrosamine levels when streaked in soybean oil on bacon. Its sodium salt was also very effective (> 95% inhibition) when applied as an aqueous solution at the 1000 ppm level to the bacon slices just prior to frying (see Table V).

In the above work stringent frying conditions were used to maximize nitrosamine formation. When frying was done by a home economist under what can be termed "home frying" conditions the concentration of nitrosamines in both the rasher and cook-out fat of the control samples were, as expected, much lower. The C_{16} ascorbyl acetal nevertheless brought about a substantial reduction in nitrosamine content of the test samples, in most instances to levels less than 1 ppb. These results are not reported here.

The mode of application of the acetal is also without effect on its antinitrosamine activity. Thus the C_{16} ascorbyl acetal, when sprinkled as a solid or added as a solution in soybean oil in the frying pan in which the bacon slices were subsequently fried, gave nearly the same amount of reduction in nitrosamine content as when applied directly to the bacon slices in soybean oil at the same level (1000 ppm). The results are not reported here. The results are also very interesting from the mechanistic standpoint in that they are indicative that the nitrosamines are not produced directly in the rashers but are introduced into it from the nitrosamines in the rendered fat. In other words most, if not all, of the nitrosamines produced during frying of bacon seem to be formed in the rendered fat. An alternative explanation, that the reduction in the nitrosamine content of the rasher is brought about by the acetal dissolved in the cook-out fat which subsequently equilibrates with the fat in the rasher, appears less likely. In either event, the results indicate that the nitrosamines, be they formed in the rasher or cook-out fat, are produced essentially, if not exclusively, in the fat phase and the action of the blocking agents is also mediated in the same phase (Bharucha et al., 1979).

The effect of storage on the antinitrosamine effect of the C_{16} ascorbyl acetal in bacon is demonstrated by the data summarized in Table VI. The results show that the acetal applied to bacon at the 1000 ppm level retains its activity for at least 35 days at +3°C, in contrast to the erratic behaviour of the ascorbyl palmitate referred to above in the text. Two types of bacon were used in these studies, one decidely fat and the other extra lean. Generally speaking, more nitrosamines were produced with fat than lean bacon, as would be expected. The acetals were equally effective in both types of bacon bringing about > 90% reduction of nitrosamines in the majority of cases, the effect being more pronounced with nitrosopyrrolidine (NPyr) than dimethylnitrosamine (NDMA). The residual nitrosamine content in the rasher and cook-out fat averaged 0-3 ppb and 1-4 ppb respectively. Considering that frying conditions producing maximum amounts of nitrosamines were used, the results clearly show that the C_{16} acetal of ascorbic acid (and of erythorbic acid, for which the results are not given here) is an excellent blocking agent of nitrosamines in bacon.

The persistent survival of minute amounts of nitrosamines in acetal-treated bacons suggested, presumably, that an alternative, albeit minor, pathway exists for nitrosamine formation which is not subject to blockage by the acetals. It is for this reason that, when the nitrosamine levels in control bacon are low, the percent reduction becomes an unreliable indicator of the effectiveness of the antinitrosamine agent.

CONCLUSIONS

1. Ascorbyl palmitate (AP), as predicted, is far more effective (70 - 90% inhibition at 500 - 1000 ppm level) than sodium ascorbate or erythorbate in reducing nitrosamine formation in bacon. However, its activity tends to decrease with storage time.
2. The long-chain acetals (C_{12}, C_{14}, C_{16}, C_{18}, and $C_{18}^{=}$) of ascorbic acid bring about 93 - 98% reduction of nitrosamines in the cook-out fat when streaked on bacon slices at the 1000 ppm level. All of these acetals are more or less equipotent. The C_{12} ascorbyl acetal, and to a much less extent the C_{14} homologue, leave a soapy after-taste. This is however not true of the higher members of the series; in organoleptic testing, the bacons treated with ascorbyl C_{16}, C_{18}, and $C_{18}^{=}$ acetals were indistinguishable from the commerical samples. For in-depth study therefore the C_{16} acetals of ascorbic and erythorbic acids were chosen.
3. Under "household" frying conditions, the C_{16} ascorbyl-acetal-treated bacon gives vanishingly small amounts (> 1 ppb) of nitrosamines.
4. The mode of application of the acetal is not critical. Thus the ascorbyl C_{16} acetal, when sprinkled as a solid or added as a solution in soybean oil to the frying pan in which the bacon slices are subsequently fried, gave the same excellent (> 90%) reductions in nitrosamine content as when applied directly to the bacon slices in soybean oil at the same level (1000 ppm).
5. Unlike AP, the C_{16} acetals of both ascorbic and erythorbic acids retain their activity (> 90% inhibition of nitrosamines) for at least 35 days at +3°C when applied to bacon at the 1000 ppm level; the reduction is more pronounced with NPyr than with NDMA. The residual nitrosamine contents in the rashers and cook-out fats were 0-3 and 1-4 ppb respectively despite the fact that frying conditions producing maximum amounts of nitrosamines were used.

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FIGURE 1 : ASCORBIC ACID ACETAL

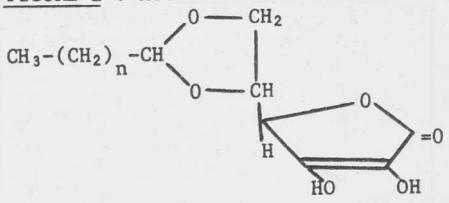

TABLE 1 - Melting Points of the Acetals

Ascorbic Acid Acetal of	m.p. °C
Dodecanal (C_{12})	S* 120 122 - 124.5
Tetradecanal (C_{14})	S 125 127 - 130
Hexadecanal (C_{16})	S 125 126 - 129.5
Octadecanal (C_{18})	S 125 127 - 129
9-Octadecenal ($C_{18}^{=}$)	97 - 99
Erythorbic Acid Acetal of Hexadecanal (C_{16})	139 - 140

S* - sinters at ....

TABLE II - Ascorbic Acid Dodecanal (C_{12}) Acetal in Soybean Oil Applied to Bacon Slices

	Nitrosamines µmole x 10^{-2}/Kg in cook-out fat	% Reduction
CONTROL	67	
TEST (1000 ppm)	3	97
CONTROL	47	
TEST (500 ppm)	4	92
CONTROL	39	
TEST (500 ppm as Na salt)	5	87
CONTROL	58	
TEST (250 ppm)	13	78
CONTROL	58	
TEST (100 ppm)	25	57

TABLE III - The Antinitrosamine Effect of Ascorbyl C_{12}, C_{14}, C_{16}, C_{18} and $C_{18}^{=}$ Acetals at the 1000 ppm Level in Bacon Cook-Out Fat

	Nitrosamines µmole x 10^{-2}/Kg				
	Colori-	TLD			
Sample	metric EP	NDMA	NPyr	Total	%Red^n
control	35				
C_{12} acetal	<3(2.5)				93
control	48	6	36	42	
C_{14} acetal	<3(1.6)	1	0.6	1.6	97
C_{16} acetal	<3(1.6)	1	0.6	1.6	97
C_{18} acetal	<3(1.6)	1	0.8	1.8	97
control	66	20	53	73	
$C_{18}^{=}$	<3(1.3)	1	1.4	1.4	98

TABLE IV - Comparison of the Antinitrosamine Effect of Ascorbyl C_{12}, C_{14}, C_{16} and C_{18} acetals in Bacon on an Equimolar Basis, Equivalent to 100 ppm of C_{12} Acetal

		Nitrosamines µmole x 10^{-2}/Kg			
Sample	ppm Additive	NDMA	NPyr	TLD Total	% Red^n
1A	control	16	51	67	
1B	C_{12} acetal 100	10	20	30	55
1C	C_{14} acetal 109	10	18	28	58
1D	C_{16} acetal 118	9	17	26	61
1E	C_{18} acetal 127	10	19	29	57

TABLE V - The Antinitrosamine Effect of C_{16} Ascorbyl Acetal (500 ppm) and its Sodium Salt (1000 ppm)

ppm Additive	Nitrosamines µmole x 10^{-2} / Kg	% Reduction
control 0	67	
C_{16} acetal 500	4	94
control 0	35	
C_{16} acetal Na salt 1000	<3 (2)	95
control 0	117	
C_{16} acetal Na salt 1000	5	96
control 0	93	
C_{16} acetal Na salt 1000	5	95

TABLE VI

The Effect of Storage on the Antinitrosamine Activity of C_{16} Ascorbyl Acetal at 1000 ppm in Bacon

	Nitrosamines - µg/Kg															
	Rasher								Cook-out Fat							
	NDMA		NPyr		Total		% Reduction		NDMA		NPyr		Total		%Reduction	
Sample	Day0	Day35	Day0	Day35	Day0	Day35	Day0	Day35	Day0	Day35	Day0	Day35	Day0	Day35	Day0	Day35
Fat Bacon (C)	3.9	4.7	15.6	13.1	19.5	22.8			9.4	6.0	50.4	39.4	59.8	45.4		
Fat Bacon (T)	1.0	0.7	0.9	0.7	1.9	1.4	90	94	0.7	1.6	1.5	1.8	2.2	3.4	96	93
Lean Bacon (C)	0.8	0.2	3.9	3.4	4.7	3.6			5.5	2.8	16.5	18.3	22.0	21.1		
Lean Bacon (T)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>98	>97	1.5	0.7	1.3	1.4	2.8	2.1	87	90
Fat Bacon (C)	2.5	0.9	17.1	10.0	19.6	10.9			5.9	3.9	55.1	27.9	61.0	31.8		
Fat Bacon (T)	0.3	0.2	0.9	1.0	1.2	1.2	94	89	1.4	0.9	2.6	1.7	4.0	2.6	93	92
Lean Bacon(C)	0.3	n.d.	6.9	3.5	7.2	3.5			2.0	0.4	32.6	19.9	34.6	20.3		
Lean Bacon(T)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>99	>97	1.2	0.6	0.9	0.5	2.1	1.1	94	95
Fat Bacon (C)	4.1	3.6	19.8	23.9	23.9	27.5			6.6	9.2	46.0	60.2	52.6	69.4		
Fat Bacon (T)	1.2	0.3	1.4	1.2	2.6	1.5	89	95	0.6	1.0	1.2	2.6	1.8	3.6	97	95
Lean Bacon(C)	0.2	n.d.	6.0	3.1	6.2	3.1			3.9	2.9	32.1	32.5	36.0	35.4		
Lean Bacon(T)	n.d.	n.d.	n.d.	0.2	n.d.	0.2	>98	94	0.9	1.4	0.9	2.5	1.8	3.9	95	89

n.d. - Not detectable at about 0.1 ppb

(C) - Control Sample

(T) - Test Sample