Long-Chain Acetals of Ascorbic and Erythorbic Acids as Antinitrosamine Agents for Bacon

Keki R. Bharucha,* Charles K. Cross, and Leon J. Rubin

Hitherto unknown long-chain acetals of ascorbic and erythorbic acids have been found to be excellent blocking agents for nitrosamine formation in bacon. Unlike ascorbyl palmitate, which tends to lose its activity on storage, the acetals retain their efficacy in bacon even after 35 days at +3 °C.

On the basis of 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· 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, viz., hitherto unknown long-chain acetals of ascorbic and erythorbic acids.

EXPERIMENTAL SECTION

Reagents. *Ascorbyl Palmitate.* Ascorbyl palmitate was obtained from INC Pharmaceuticals Inc.

Ascorbyl Pivalate. Ascorbyl pivalate was synthesized essentially by the method described by Hoffmann-La Roche (1946) for ascorbyl palmitate. Ascorbic acid (16.6 g) was dissolved in concentrated sulfuric acid (200 mL). Pivalic acid (10.2 g) was added, and the solution was allowed to stand at room temperature for 16 h. The solution was poured over cracked ice (750 g) and extracted with diethyl ether (7 × 800 mL). The ether solution was dried over anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator, leaving a slightly pink solid (16.4 g). The solid was triturated with ether (50 mL) filtered, and dried. The resulting white solid (8.2 g) melted at 173–175 °C with prior sintering at 170 °C. The product was crystallized twice from water to give white needles (3.3 g) melting at 174.5–175.5 °C. The molecular weight, determined by titration with 0.1 N sodium hydroxide to 1 equiv, was 257. The theoretical value is 260. Anal. Calcd for $C_{11}H_{16}O_7$: C, 50.76; H, 6.20; O, 43.04. Found: C, 50.84; H, 6.39; O, 43.18.

Ascorbyl Acetonide. Ascorbyl acetonide was prepared by the method of Cutolo and Larizza (1961). Our product had a melting point of 220 °C with decomposition. Anal. Calcd for $C_9H_{12}O_6$: C, 50.00; H, 5.60; O, 44.41. Found: C, 50.03; H, 5.70; O, 44.59.

Hexadecanal. Hexadecanal was prepared from hexadecanol by oxidation with pyridinium chlorochromate (Corey and Suggs, 1975). The alcohol used was analyzed by GC and found to contain the following: C_{15}, 0.6%; C_{16}, 96.7%; C_{18}, 2.7%. Pyridinium chlorochromate (30.6 g) was suspended in dichloromethane (200 mL) at room temperature by using a magnetic stirrer in a 1-L round-bottomed flask. A solution of the alcohol (23.0 g) in dichloromethane (200 mL) was added all at once. The mixture, which quickly turned dark and precipitated a black gum, was sitrred for 1 h. The reaction mixture was poured on a Florisil column (6 in. × $1^1/_2$ in.). The remaining resinlike substance was rinsed from the flask with dichloromethane (5 × 300 mL) into the column. The colorless column eluate was reduced to about 400 mL on a rotary evaporator and washed with 1 N HCl (50 mL) and with water to neutrality. The solution was dried with sodium sulfate and evaporated to dryness. A white crystalline product was obtained (20.3 g, 89%). In a number of runs, the yield ranged from 89 to 98%. The infrared spectrum showed no detectable amount of acid and only a minor amount of alcohol. The aldehyde C=O stretching band was located at 1717 cm^{-1} in chloroform, and the CH stretching band was at 2730 cm^{-1}. This material was used without further purification in the subsequent ketalization step.

Tetradecanal, octadecanal, and Δ^9-octadecenal were prepared in the same way. Dodecanal was obtained from Eastman Kodak.

Ascorbyl Acetals. Two methods were used for the preparation of the acetals, the first being preferred. (*1*) *Method A.* Hexadecanal (45.3 g) was mixed with erythorbic acid (35.9 g) in tetrahydrofuran (200 mL) in a 500-mL round-bottomed flask. A solution of HCl gas in tetrahydrofuran (19.5 mL; 24% w/v) was added. The mixture was stirred mechanically and refluxed in a Soxhlet containing molecular sieve 3A for 2 h. The reaction mixture was allowed to cool to room temperature. The tetrahydrofuran was removed on a rotary evaporator, leaving a slightly sticky residue which was taken up in cold water to form a fine suspension. The crude acetal was filtered on a Buchner funnel. The filter cake was redisperesed in water and filtered, the process being repeated twice. The white, powdery product was dried in a vacuum oven at 50 °C for 16 h. A crude yield of 68 g (91%) was obtained, mp 137–138 °C. The purity by titration with dichlorophenolindophenol in acetone solution was 90 ± 1%. (As a standard for the dichlorophenolindophenol titration, we used analytically pure acetal prepared from hexadecanal and erythorbic acid followed by recrystallizations from ether/hexane.) The crude acetal was washed once by stirring it well in a 1-L beaker with hexane (350 mL). The washed acetal was filtered and dried in a vacuum oven at 50 °C for 3 h to give an off-white solid (58.6 g) of mp 144–145 °C. The purity by titration was 100 ± 1%.

(*2*) *Method B.* Octadecanal (6.0 g) was stirred with ascorbic acid (13.8 g) and *p*-toluenesulfonic acid (1.2 g) in *N*,*N*-dimethylacetamide (60 mL) at 60 °C for 20 h. The reaction mixture was poured into water (100 mL) and extracted with diethyl ether (4 × 80 mL). The combined ether extract was washed with water (5 × 80 mL), dried over sodium sulfate, and evaporated to dryness on a rotary evaporator to give a white solid (8.1 g, 85%), mp 106–115 °C with sintering at 103 °C. The solid was crystallized from ether/hexane (160:250) in the freezer for 16 h to yield

Canada Packers Inc., Toronto, Ontario, Canada M6N 1K4.

0021-8561/80/1428-1274$01.00/0

Table I. Elemental Analyses of the Acetals

ascorbic acid acetal of	mp, °C[a]	formula	M_r	calcd			found		
				% C	% H	% O	% C	% H	% O
dodecanal (C_{12})	122-124.5 (120 S)	$C_{18}H_{30}O_6 \cdot {}^1/_3H_2O$	348.4	62.07	8.81	29.12	62.21	8.88	29.10
tetradecanal (C_{14})	127-130 (125 S)	$C_{20}H_{34}O_6$	370.5	64.84	9.25	25.91	64.55	9.54	25.85
hexadecanal (C_{16})	126-129.5 (125 S)	$C_{22}H_{38}O_6 \cdot 0.2H_2O$	402.1	65.65	9.63	24.72	65.58	9.84	24.20
octadecanal (C_{18})	127-129 (125 S)	$C_{24}H_{42}O_6 \cdot 0.2H_2O$	430.1	67.00	9.94	23.06	66.94	10.01	
Δ^9-octadecenal ($C_{18:1}$)	97-99	$C_{24}H_{40}O_6 \cdot 0.3H_2O$	430.0	66.93	9.52	23.41	66.71	9.88	23.50
erythorbic acid acetal of hexadecanal (C_{16})	139-140	$C_{22}H_{38}O_6$	398.5	66.30	9.61	24.09	66.54	9.88	23.82

[a] S = sinters.

a solid (5.5 g, 68%) melting from 117 to 121 °C with sintering at 103 °C.

This method was also used for the preparation of the acetals of ascorbic acid with dodecanal, tetradecanal, hexadecanal, and Δ^9-octadecenal as well as the acetal of erythorbic acid with hexadecanal. The products were crystallized from ether/hexane several times to give pure samples for the elemental analyses given in Table I. Most of the acetals apparently contained water of crystallization.

Application of Additives to Bacon. The bacon used in this study was commercial pump-cured side bacon (150 ppm of sodium nitrite). Ascorbyl pivalate and ascorbyl acetonide were applied to bacon slices in water solution because of their poor oil solubility. The acetonide was also applied as a slurry in soybean oil. Ascorbyl palmitate and the acetals were applied to the bacon slices as a slurry in antioxidant-free soybean oil, usually 4 mL/lb. 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 and Bharucha, 1979) for *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPyr).

Effect of Storage Time on the Antinitrosamine Activity of Ascorbyl Palmitate. This work was done by using our earlier method of analysis for nitrosamines. Two hundred grams of cook-out fat was required per analysis, and therefore each sample consisted of 2 lb of bacon. Four-pound lots of sliced bacon were sequentially sampled to give two equivalent samples, 1-1 to 1-16 in Table II. Sequential sampling involved distributing consecutive slices from a package over the desired number of sample packages. The samples were treated with soybean oil (controls) or ascorbyl palmitate in soybean oil (tests) to give a level of 500 ppm of ascorbyl palmitate in the bacon. Four pairs of samples were fried, and the cook-out fat was analyzed immediately. Four other pairs were vacuum sealed in pouches and stored at 3 °C for 21 days before being cooked and analyzed. In samples 2-1 to 2-16 and 3-1 to 3-16 the bacon was sampled in lots of 8 lb so that the day 21 samples would be equivalent to the day 0 samples. These samples were treated with 1000 ppm of ascorbyl palmitate.

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 by using equimolar quantities (0.293 mmol/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 mmol/kg on the bacon. The bacon was fried and the cook-out fat analyzed for volatile nitrosamines.

Influence of Sodium Erythorbate on the Antinitrosamine Activity of the C_{16} Acetal of Ascorbic Acid. In this set of experiments, done in triplicate using paired bellies, the input level of nitrite was 150 ppm. The sodium erythorbate was added via the pickle to give either 0 or 550 ppm, designated −A or +A, respectively, in Table III. The bellies were processed normally, sliced, and vacuum packaged. The C_{16} acetal was applied once again in soybean oil at the 250-ppm level on bacon slices designated test −A and test +A in Table III. The bacon was fried as usual under our standardized conditions and the cook-out fat analyzed for nitrosamines.

Effect of Storage Time on the Antinitrosamine Activity of the Acetals of Hexadecanal with Ascorbic and Erythorbic Acids at 1000 ppm. Two-pound lots of bacon were sequentially sampled into four 0.5 lb packs (two controls and two 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 g. The experiment was repeated for a total of 4 times in the case of the erythorbyl acetal and 3 times for the ascorbyl acetal. The samples were chosen to be either unusually fat or unusually lean.

Effect of the C_{16} Acetal of Ascorbic Acid on Nitrosamine Formation in Bacon under Household Frying Conditions. One-pound packages of bacon were sampled sequentially into two packages each containing 0.5 lb of bacon. A total of 8 lb was divided in this manner, resulting in 16 packages consisting of 8 pairs of equivalent samples. One sample of each pair (the control) was treated with soybean oil while the other (the test) was treated with the acetal of ascorbic acid and hexadecanal in soybean oil to give a level of 1000 ppm of acetal on the bacon as described in the previous experiment. The samples were fried by a home economist in our test kitchen under so-called "home frying" conditions. The pan was set to 340 °F, and the bacon was fried on both sides until it was

Table II. Effect of Storage Time on the Antinitrosamine Activity of Ascorbyl Palmitate (AP)[a]

sample	additive, ppm	storage, days	nitrosamine, ppb TLD[b] NDMA	NPyr	total	% redn
1-1 control	0	0	7	35	42	
1-2 test	AP, 500	0	3	13	16	62
1-3 control	0	0	3	56	59	
1-4 test	AP, 500	0	2	13	15	75
1-5 control	0	0	6	47	3	
1-6 test	AP, 500	0	2	12	14	74
1-7 control	0	0	nd	61	61	
1-8 test	AP, 500	0	1	14	15	75
1-9 control	0	21	15	93	108	
1-10 test	AP, 500	21	6	37	43	60
1-11 control	0	21	6	46	52	
1-12 test	AP, 500	21	5	16	21	60
1-13 control	0	21	27	72	99	
1-14 test	AP, 500	21	18	60	78	21
1-15 control	0	21	18	90	108	
1-16 test	AP, 500	21	7	33	40	63
2-1 control	0	0	26	53	79	
2-2 control	0	21	25	64	89	
2-3 test	AP, 1000	0	2	4	6	92
2-4 test	AP, 1000	21	10	18	28	69
2-5 control	0	0	12	34	46	
2-6 control	0	21	43	53	96	
2-7 test	AP, 1000	0	1	3	4	91
2-8 test	AP, 1000	21	12	8	20	79
2-9 control	0	0	25	47	72	
2-10 control	0	21	27	48	75	
2-11 test	AP, 1000	0	2	4	6	92
2-12 test	AP, 1000	21	11	12	23	69
2-13 control	0	0	14	46	60	
2-14 control	0	21	33	56	89	
2-15 test	AP, 1000	0	4	4	8	87
2-16 test	AP, 1000	21	12	14	26	71
3-1 control	0	0	50	48	98	
3-2 control	0	21	35	37	72	
3-3 test	AP, 1000	0	11	6	17	83
3-4 test	AP, 1000	21	8	7	15	79
3-5 control	0	0	29	71	100	
3-6 control	0	21	24	49	73	
3-7 test	AP, 1000	0	10	11	21	79
3-8 test	AP, 1000	21	3	8	11	85
3-9 control	0	0	36	71	107	
3-10 control	0	21	31	46	78	
3-11 test	AP, 1000	0	10	8	18	83
3-12 test	AP, 1000	21	4	7	11	86
3-13 control	0	0	16	64	80	
3-14 control	0	21	14	62	66	
3-15 test	AP, 1000	0	4	7	11	86
3-16 test	AP, 1000	21	2	8	10	85

[a] nd = not detected at about 0.1 ppb. [b] TLD = thin-layer densitometric method (Cross et al., 1978).

judged visually to be medium done. The bacon fried under these conditions was still flexible. In four samples only the fried rasher was analyzed for volatile nitrosamines. In another four samples both the fried rasher and cook-out fat were analyzed.

Effect of Adding the Acetal to the Frying Pan before the Bacon on the Nitrosamine Level Found in Fried Bacon. In a series of four experiments the acetal was added to the cold frying pan as a slurry in soybean oil or as the dry powder. The sliced bacon, sampled sequentially to give equivalent samples, was placed over the acetal in the pan so that a level of 1000 ppm of acetal was present. Controls in these experiments were untreated, treated with soybean oil alone, or treated with a slurry of acetal in soybean oil as in our normal laboratory procedure. The samples were fried, and both the rashers and cook-out fat were analyzed for nitrosamines.

Table III. Influence of Sodium Erythorbate (550 ppm) on the Antinitrosamine Activity of the Ascorbyl C_{16} Acetal (250 ppm) in Cook-Out Fat

sample	residual nitrite, μg/g	nitrosamine, μg/kg NDMA	NPyr	total	% redn
control −A	87	16	46	62	
test −A		4	7	11	82
control +A	8	10	42	52	
test +A		5	6	11	79
control −A	103	6	38	44	
test −A		0.4	4	4	91
control +A	6	4	34	38	
test +A		1	3	4	89
control −A	70	15	37	52	
test −A		4	5	9	83
control +A	2	15	21	36	
test +A		3	0.4	3	91

Comparison of the Effect of Smoking and Not Smoking on the Nitrosamine Levels in Fried Bacon. Three paired bellies were cured. One belly of each pair was smoked normally while the other was put through the same temperature cycle without smoke. The bellies were cooled, sliced, and packaged. Corresponding sections of the pair were fried and analyzed for nitrosamines as usual with or without acetal treatment.

RESULTS AND DISCUSSION

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 functions by destroying nitrite and in the process they themselves undergo oxidation to dehydroascorbate or dehydroerythorbate. However, neither of these compounds is very effective; even when added at the 2000-ppm level, the reduction in nitrosamine formation is only about 70%. These findings are by no means surprising since these substances being water soluble do not meet the second requirement of lipophilicity mentioned above in the beginning of the paper. Fat-soluble derivatives of ascorbic or erythorbic acid were clearly indicated, and toward this end attention was initially directed to commercially available ascorbyl palmitate (AP).

A large number of experiments involving the use of AP incorporated on bacon slices in soybean oil showed, as expected, that it is far more effective than sodium ascorbate or erythorbate in reducing nitrosamine formation in bacon. The results, depicted in Table II, are in agreement with Sen's published values (Sen et al., 1976). However, the activity of AP was inconsistent and sometimes tended to decrease with storage time. Thus, at the 500-ppm level, there was an average decrease from about 70% reduction at zero time to about 50% reduction after 21 days. At the 1000-ppm level, in the first of the experimental series (2-1 to 2-16), there was a decrease from about 90% at zero time to 70% after 21 days, while in the second series (3-1 to 3-16) no such decrease was observed. The erratic behavior of AP could be due to oxidation to the corresponding dehydro compound or to the chemical or enzymatic hydrolysis of the ester moiety to ascorbic acid and palmitic acid. Unreported results in which the sodium salt of AP was found to be a poor antinitrosamine agent in bacon suggested that chemical hydrolysis of ascorbyl palmitate may be involved.

Table IV. Effect of Ascorbyl Pivalate on Nitrosamines in Bacon

sample	ascorbyl pivalate, ppm	nitrosamine, μmol × 10^{-2}/kg TLD[a] NDMA	NPyr	total	EP[b]	% redn on EP
control	0	9	14	23	24	
test	1000	7	6	13	9	63
control	0				36	
test	1000				24	33
control	0				44	
test	1000				26	41

[a] TLD = thin-layer densitometric method (Cross et al., 1978). [b] EP = colorimetric method (Cross et al., 1978) gives total nitrosamine and therefore is expressed in μmol × 10^{-2}/kg.

Table V. Effect of Ascorbyl Acetonide on Nitrosamines in Bacon

sample	carrier	ascorbyl acetonide, ppm	nitrosamine, μmol × 10^{-2}/kg (EP)	% redn
control	water	0	18	
test	water	1000	11	39
control	soybean oil	0	32	
test	soybean oil	1000	16	50

Attempts were therefore made to circumvent the problem by introducing into the ascorbic and erythorbic acids oil-solubilizing groups that would not be susceptible to ready hydrolysis.

Initially, the sterically hindered ester of pivalic acid with ascorbic acid, which should be very stable to hydrolysis, came readily to mind. Ascorbyl pivalate was therefore synthesized and tested. Unfortunately, it was not very effective, as shown by the data presented in Table IV, presumably because it was not sufficiently soluble in fat.

Attention was then turned to the ketals and acetals of ascorbic acid, which would be expected to be alkali stable. The known acetonide of ascorbic acid was therefore synthesized, but it too did not prove to be very effective (see Table V), once again presumably due to its poor lipophilicity.

The discouraging results with the pivalate and acetonide prompted the synthesis and testing of hitherto unknown long-chain acetals of ascorbic and erythorbic acids, which certainly should be lipophilic. The initial choice was the acetal from commercially available dodecanal. As expected, this ascorbyl acetal (Figure 1, n = 10) was oil soluble and showed excellent inhibition (>90%) of nitrosamine in the bacon cook-out fat even at the 500-ppm level (Table VI). The sodium salt when applied to bacon slices in water was equally effective at the 500-ppm level. At the 250-ppm level the C_{12} acetal was slightly less efficacious (78% inhibition), and even at 100 ppm it still showed significant activity. Clearly then, the C_{12} acetal, as expected, was more efficacious than ascorbyl palmitate. Its use in bacon, however, has one drawback; it imparts an objectionable soapy aftertaste.

This flavor problem was not quite unexpected. On the basis of experience with fats containing lauric (C_{12}) and myristic C_{14}) glycerides, where similar flavor problems were noted, it was reasoned that longer chain acetals would probably circumvent the problem. This indeed proved to be the case. The ascorbyl C_{14} (I; n = 12), C_{16} (I; n = 14, C_{18} (I; n = 16), and $C_{18:1}$ acetals were therefore synthesized from their corresponding aldehydes and subjected to testing. In organoleptic evaluation, the bacon treated with ascorbyl C_{14} acetal had a slight but noticeable flavor, while bacon treated with the C_{16}, C_{18}, and $C_{18:1}$ ascorbyl acetals was indistinguishable from the commercial product. When applied to bacon in soybean oil at the 1000-ppm level, all showed excellent inhibition of nitrosamine formation (~96%) in the cook-out fat, as shown by the results in Table VII.

Table VI. Ascorbic Acid Dodecanal (C_{12}) Acetal in Soybean Oil Applied to Bacon Slices

	nitrosamine in cook-out fat, μmol × 10^{-2}/kg	% redn
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 VII. Antinitrosamine Effect of Ascorbyl C_{12}, C_{14}, C_{16}, C_{18}, and $C_{18:1}$ Acetals at the 1000-ppm Level in Bacon Cook-Out Fat

sample	nitrosamine, μmol × 10^{-2}/kg colorimetric EP	TLD NDMA	NPyr	total	% redn
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	
$C_{18:1}$ acetal	<3 (1.3)	1	1.4	1.4	98

Table VIII. 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

sample	additive, ppm	nitrosamine, μmol × 10^{-2}/kg TLD NDMA	NPyr	total	% redn
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
2A	control	18	47	65	
2B	C_{12} acetal, 100	13	23	36	45
2C	C_{14} acetal, 109	5	20	25	62
2D	C_{16} acetal, 118	18	19	37	43
2E	C_{18} acetal, 127	10	20	30	54

To establish the relative efficacies of the various acetals, we carried out an experiment in which these acetals were all tested at the same time on an equivalent molar basis (0.293 mmol/kg of bacon, equivalent to 100 ppm of C_{12} acetal). The results depicted in Table VIII show that the activity is constant throughout the series.

The C_{16} acetal of ascorbic acid was therefore selected for in-depth study. At the 500-ppm level, it showed 94% 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

Table IX. Antinitrosamine Effect of C_{16} Ascorbyl Acetal (500 ppm) and Its Sodium Salt (1000 ppm)

additive, ppm	nitrosamine, μmol × 10^{-2}/kg	% redn
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 X. Influence of C_{16} Ascorbyl Acetal on Nitrosamine Formation in Bacon Fried under Household Conditions[a]

	nitrosamine, μg/kg					
	NDMA		NPyr		total	
sample	rasher	cook-out fat	rasher	cook-out fat	rasher	cook-out fat
1C	1.4		5.4		6.8	
1T	0.5		nd		0.5	
2C	1.8		3.7		5.5	
2T	1.3		0.3		1.6	
3C	1.0		1.6		2.6	
3T	0.4		nd		0.4	
4C	1.1		2.1		3.2	
4T	0.4		nd		0.4	
5C	0.3	5.0	0.6	7.6	0.9	12.6
5T	nd	nd	nd	nd	nd	nd
6C	0.4	1.4	nd	2.6	0.4	4.0
6T	0.2	3.0	nd	0.2	0.2	3.2
7C	0.4	4.4	0.2	3.5	0.6	7.9
7T	nd	0.7	nd	nd	nd	0.7
8C	0.4	8.0	0.1	5.3	0.5	13.3
8T	0.5	0.4	nd	nd	0.5	0.4

[a] nd = not detectable at about 0.1 μg/kg; C = control sample; T = test sample.

1000-ppm level to the bacon slices just prior to frying (see Table IX).

In another set of experiments done in triplicate, the ascorbyl C_{16} acetal was applied in soybean oil at the 250-ppm level to bacon slices prepared with and without 550 ppm of sodium erythorbate in normal pickle (150 ppm of nitrite). The results in Table III show that even at this low level (250 ppm) there is 80–90% reduction of nitrosamines in the cook-out fat. When one compares the controls with and without erythorbate designated as control +A and control −A, respectively, one sees practically no difference in nitrosamine content (52 vs. 62 μg/kg and 38 vs. 44 μg/kg) in the first two series and only a slight difference in the third one (36 vs. 52 μg/kg), suggesting, as mentioned earlier in the text, that sodium salts of ascorbic or erythorbic acids are poor antinitrosamine agents for bacon. The data in Table III also show that the presence of erythorbate in the bacon does not materially influence the activity of the acetal; nearly the same reduction in nitrosamines was obtained in bacon samples prepared with and without the erythorbate.

Frying conditions also have little effect on the antinitrosamine activity of the C_{16} ascorbyl acetal as shown by results in Table X where the frying of bacon was done by a home economist under what can be termed home frying conditions. In the above work more stringent frying conditions were used to maximize nitrosamine formation. Although the concentrations of nitrosamines in both the rasher and cook-out fat of the control samples are, as expected, very low, the C_{16} acetal nevertheless brings about a substantial reduction in nitrosamine content of the test samples, in most instances to levels less than 1 ppb.

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 (Table XI) as when applied directly to the bacon slices in soybean oil at the same level (1000 ppm). The results in Table XI 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 them 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 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} acetals of both erythorbic and ascorbic acids in bacon is demonstrated by the data summarized in Tables XII and XIII, respectively. The results show that the acetals applied to bacon at the 1000-ppm level retain their activity

Table XI. Influence of Addition of the Acetal to the Frying Pan before the Bacon on the Nitrosamine Content of Fried Bacon Rasher and Cook-Out Fat[a]

		nitrosamine, pbb							
		NDMA		NPyr		total		% redn	
sample	treatment	rasher	fat	rasher	fat	rasher	fat	rasher	fat
1-C1	S/B	3.3	7.2	11.8	34.8	15.1	42.0		
1-C2	acetal in S/B added to rasher	1.0	0.3	0.9	1.1	1.9	1.4	87	97
1-T	acetal in S/B added to pan	0.3	0.5	0.5	0.8	0.8	1.3	95	97
2-C1	as 1-C1	1.2	11.1	6.7	44.3	7.9	55.4		
2-C2	as 1-C2	0.5	0.9	0.2	1.5	0.7	2.4	91	96
2-T	as 1-T	2.2	0.3	0.5	0.4	2.7	0.7	65	99
3-C	none	2.7	10.0	10.2	36.8	12.9	46.8		
3-T	acetal powder added to pan	0.5	2.0	0.6	4.4	1.1	6.4	92	86
4-C1	as 1-C1	3.6	3.9	19.4	28.0	23.0	31.9		
4-C2	as 1-C2	1.1	0.8	3.7	1.5	4.8	2.3	80	93
4-T	as 3-T	0.9	0.6	2.7	1.3	3.6	1.9	84	95

[a] Acetal added to give 1000 ppm on the bacon. S/B = soybean oil; C, C1, and C2 = control samples; T = test sample.

Table XII. Effect of Storage on the Antinitrosamine Activity of C_{16} Erythorbyl Acetal at 1000 ppm in Bacon[a]

		rasher								cook-out fat							
		nitrosamine, μg/kg								nitrosamine, μg/kg							
		NDMA		NPyr		total		% redn		NDMA		NPyr		total		% redn	
sample	% cook-out fat	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35
fat bacon (C)	50	4.5	3.4	11	14	15.5	17.4			10.2	5.8	44.6	50.1	54.8	55.9		
fat bacon (T)		2.3	1.8	0.8	0.3	3.1	2.1	80	88	3.5	0.7	2.0	1.4	5.5	2.1	90	96
lean bacon (C)	31	3.0	1.4	5.1	2.2	8.1	3.6			9.6	3.5	20.4	18.1	30.0	21.6		
lean bacon (T)		2.6	2.2	0.5	nd	3.1	2.2	62	39	1.8	1.6	0.7	0.4	2.5	2.0	92	91
fat bacon (C)	50	3.1	1.6	9.2	6.3	12.3	7.9			8.0	5.2	40.3	29.1	4.3	34.3		
fat bacon (T)		2.8	1.3	1.5	0.6	4.3	1.9	65	76	1.8	0.8	1.9	1.6	3.7	2.4	92	93
lean bacon (C)	31	2.4	1.3	10.8	3.8	13.2	5.1			3.3	2.2	47.3	23.1	50.6	25.3		
lean bacon (T)		1.4	1.0	0.7	nd	2.1	1.0	84	80	1.6	1.9	1.4	0.9	3.0	2.8	94	89
fat bacon (C)	53	10.9	2.6	13.5	5.	24.4	8.5			11.1	8.9	33.7	28.9	44.8	37.8		
fat bacon (T)		2.5	0.2	0.6	nd	3.1	0.2	87	98	1.0	0.7	nd	0.6	1.0	1.3	98	97
lean bacon (C)	19	4.0	0.4	8.0	2.3	12.0	2.7			4.5	1.3	24.0	12.1	28.5	13.4		
lean bacon (T)		2.2	0.2	nd	nd	2.2	0.2	82	93	1.9	0.5	1.0	0.4	2.9	0.9	90	93
fat bacon (C)	47	4.5	1.1	11.1	6.0	15.6	7.1			11.3	4.7	53.7	21.0	65.0	31.7		
fat bacon (T)		1.9	0.5	nd	0.3	1.9	0.8	88	89	0.8	0.1	0.9	0.9	1.7	1.0	97	97
lean bacon (C)	20	1.2	0.9	4.4	2.0	5.6	2.9			3.4	1.0	27.4	11.4	30.8	12.4		
lean bacon (T)		1.5	0.1	nd	nd	1.5	0.1	73	97	1.5	0.4	1.3	1.0	2.8	2.3	91	81

[a] nd = not detectable at about 0.1 ppb; (C) = control sample; (T) = test sample.

Table XIII. Effect of Storage on the Antinitrosamine Activity of C_{16} Ascorbyl Acetal at 1000 ppm in Bacon[a]

		rasher								cook-out fat							
		nitrosamine, μg/kg								nitrosamine, μg/kg							
		NDMA		NPyr		total		% redn		NDMA		NPyr		total		% redn	
sample	% cook-out fat	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35	day 0	day 35
fat bacon (C)	47	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)	23	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)		nd	nd	nd	nd	nd	nd	>98	>97	1.5	0.7	1.3	1.4	2.8	2.1	87	90
fat bacon (C)	44	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)	21	0.3	nd	6.9	3.5	7.2	3.5			2.0	0.4	32.6	19.9	34.6	20.3		
lean bacon (T)		nd	nd	nd	nd	nd	nd	>99	>97	1.2	0.6	0.9	0.5	2.1	1.1	94	95
fat bacon (C)	47	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)	23	0.2	nd	6.0	3.1	6.2	3.1			3.9	2.9	32.1	32.5	36.0	35.4		
lean bacon (T)		nd	nd	nd	0.2	nd	0.2	>98	94	0.9	1.4	0.9	2.5	1.8	3.9	95	89

[a] nd = not detectable at about 0.1 ppb; (C) = control sample; (T) = test sample.

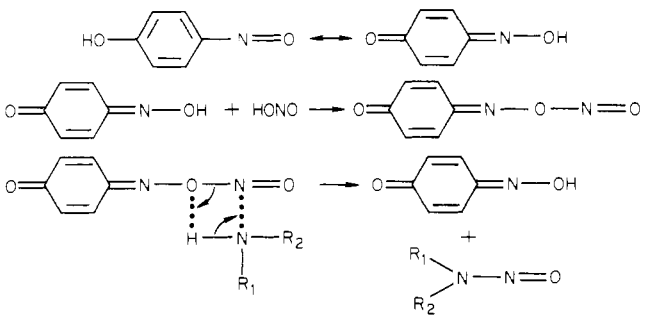
Table XIV. Effect of Smoking and Not Smoking on the Nitrosamine Levels in Fried Bacon[a]

sample	treatment	(wt fat × 100)/(wt fat + rasher)	nitrosamine, ppb						nitrite before frying, ppm
			NDMA		NPyr		total		
			rasher	fat	rasher	fat	rasher	fat	
1A }	S, NA	30	0.7	5.5	3.9	28.4	4.6	33.9	96
1B }	NS, NA	29	1.3	8.0	4.4	32.7	5.7	40.7	104
1C }	S, NA	28	0.9	6.0	4.9	31.0	5.8	37.0	97
1D }	NS, NA	31	nd	8.5	3.2	36.4	3.2	44.9	131
1E }	S, NA	41	0.9	8.5	5.0	25.7	5.9	34.2	84
1F }	NS, NA	38	1.2	6.5	9.0	31.5	10.2	38.0	107
2A }	S, A	39	0.3	1.0	nd	0.4	0.3	1.4	
2B }	NS, A	38	0.6	1.1	0.2	1.2	0.6	2.3	
2C }	S, A	57	0.4	0.7	0.2	0.9	0.6	1.6	
2D }	NS, A	54	1.0	0.9	0.5	0.4	1.5	1.3	
3A }	S, A	46	0.7	1.0	nd	0.9	0.7	1.9	23[b]
3B }	NS, A	45	0.2	1.6	nd	1.2	0.2	2.8	33[b]
3C }	S, A	59	1.5	2.2	1.4	2.9	2.9	5.1	33[b]
3D }	NS, A	59	2.8	1.9	2.7	2.9	5.5	4.8	100[b]
3F }	S, A	66	1.0	1.3	1.7	3.0	2.7	4.3	50[b]
3F }	NS, A	65	1.6	1.3	1.8	2.9	3.4	4.2	89[b]

[a] S = smoked; NS = not smoked; A = treated with 1000 ppm of acetal; NA = not treated with acetal; a brace indicates paired bellies; nd = not detected at about 0.1 ppb. [b] Nitrite analyzed 1 week after frying using refrigerator-stored samples.

for at least 35 days at +3 °C, in contrast to the erratic behavior of the ascorbyl palmitate referred to above. Two types of bacon were used in these studies, one decidedly 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 was more pronounced with nitrosopyrrolidine (NPyr) than with dimethylnitrosamine (NDMA). The residual nitrosamine content in the rasher and cook-out fat averaged 0–3 and 1–4 ppb, respectively, for both the acetals. Considering that optimized frying conditions producing maximum amounts of nitrosamines were used, the results clearly show that the C_{16} acetals of ascorbic and erythorbic acids are excellent blocking agents 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. Since phenols are natural constituents of smoke and since the presence of C-nitrosophenols in bacon has been demonstrated (Knowles et al., 1975), it occurred to us that conceivably the C-nitrosophenols may catalytically transnitrosate amines or amino acids present in the bacon to give nitrosamines according to the scheme [see Walker et al. (1979)]



The acetals would then be ineffective in preventing formation of nitrosamines during the frying process. To test the validity of this hypothesis, we used cured paired bellies; one belly was smoked normally while the other was put through the same temperature cycle but without smoke. Both bellies were sliced and packaged, and the corresponding sections were fried and analyzed either with or without acetal treatment in the normal way. The results are presented in Table XIV. Samples 2A and 2B were from the corresponding bellies 1E and 1F. The third column, a measure of the fat content, shows that even when paired bellies are used, it is essential to use corresponding sections of the pair. The results in Table XIV clearly show very little difference between smoked and nonsmoked bacons with or without the acetal treatment, suggesting that C-nitrosophenols are not implicated in the formation of nitrosamines. In fact, the unsmoked bacon samples generally tended to contain more nitrosamine presumably because of their higher nitrite content at the time of frying.

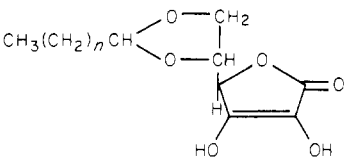


Figure 1. Ascorbic acid acetal.

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 $C_{18:1}$) 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, leaves a soapy aftertaste. 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 $C_{18:1}$ acetals were indistinguishable from the commercial samples. For in-depth study therefore the C_{16} acetals of ascorbic and erythorbic acids were chosen.

(3) The C_{16} ascorbyl acetal when streaked at the 250-

ppm level in soybean oil on bacon slices, prepared with and without incorporation of sodium erythorbate (550 ppm) in the normal pickle (150 ppm of nitrite), gave 80–90% reduction of nitrosamine formation in the cook-out fat.

(4) Under household frying conditions, the C_{16} ascorbyl acetal treated bacons give vanishingly small amounts (<1 ppb) of nitrosamines.

(5) 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).

(6) Unlike AP, the C_{16} acetals of both ascorbic and erythorbic acids retain their activities (>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 optimized frying conditions producing maximum amounts of nitrosamines were used.

(7) The smoking operation during the processing of bacon has little, if any, effect on the nitrosamine levels of cooked bacon, suggesting that C-nitrosophenols are not implicated in nitrosamine formation, e.g., via transnitrosation.

LITERATURE CITED

Bharucha, K. R., Cross, C. K., Rubin, L. J., *J. Agric. Food Chem.* **27**, 63–69 (1979).
Corey, E. J., Suggs, J. W., *Tetrahedron Lett.*, 2647 (1975).
Cross, C. K., Bharucha, K. R., *J. Agric. Food Chem.* **27**, 1358–1360 (1979).
Cross, C. K., Bharucha, K. R., Telling, G. M., *J. Agric. Food Chem.* **26**, 657–660 (1978).
Cutolo, E., Larizza, A. *Gazz. Chim. Ital.* **91**, 964–972 (1961).
Herring, H. K., *Proc. Meat Ind. Res. Conf.*, 47–60 (1973).
Hoffmann-La Roche, British Patent 579 333, July 31, 1946.
Knowles, M. E., Gilbert, J., McWeeny, D. J., *J. Sci. Food Agric.* **26**, 267–276 (1975).
Sen, N. P., Donaldson, B., Seaman, S., Iyengar, J. R., Miles, W. F., *J. Agric. Food Chem.* **24**, 397–401 (1976).
Walker, E. A., Pignatelli, B., Castegnaro, M., *J. Agric. Food Chem.* **27**, 393–396 (1979).

Received for review February 8, 1980. Accepted May 8, 1980.

Metal Uptake by Crops Grown over Entrenched Sewage Sludge

Lawrence J. Sikora,* Rufus L. Chaney, Nicholas H. Frankos, and Charles M. Murray

Oats (*Avena sativa* L.), wheat (*Triticum aestivum* L. em. Thell), and chard (*Beta vulgaris* var. Cicla) were grown on plots containing entrenched digested and limed raw sewage sludge to determine the metal uptake by the crop and the effect of surface pH adjustment on this uptake. The digested and control plots were split with half receiving surface applications of lime to determine the effects of liming the surface soil on metal uptake. The limed raw sludge plot was not split because its surface soil was already pH 6.7. Metal uptake by the crops reflected metal content of the sludge and the pH of the sludge and subsoil more than the pH of the surface soil. Chard accumulated higher metal levels than either wheat or oats which had similar metal accumulations. Crops grown in limed raw sludge plots had metal levels equal to or less than those of crops grown on control plots with limed surface soil. Although liming the surface soil reduced metal uptake by crops grown on digested sludge plots, metal uptake was still significantly greater than that of crops grown on limed raw sludge or control plots. These results suggested that land containing entrenched limed sludges (raw or digested) may be used to grow agricultural crops if the sludge is relatively low in heavy metals.

The principal method currently used for the disposal of limed raw sewage sludge produced at the Blue Plains Wastewater Treatment plant in Washington, DC is entrenchment. The regional, 309 million gallon per day facility produces about 200 wet tons of digested sludge per day which is land spread on cooperator farms and about 700 wet tons of limed raw sludge, of which one-half is composted and the other half entrenched at selected sites in Montgomery and Prince Georges counties, Maryland. Entrenchment or "trenching", which has been conducted since 1974, is expected eventually to be replaced by sewage sludge composting because of the resource recovery benefits of the sludge compost product (Hornick et al., 1979).

The method for sludge entrenchment involves placing sludge in trenches which are 60 cm wide, 75–135 cm deep, and 60–90 cm apart. The disposal rate for sewage sludge filter cake of about 20% solids is 2900 metric tons/ha, which is significantly higher than that allowed for surface application and one of the major benefits of the method. When sludge is surface applied, it is recommended that the rate equal the nitrogen fertilizer requirements of the crop which for most crops equals an application rate in the range of 5–40 metric tons ha, dry weight basis (CAST, 1976). Because entrenched sludge is buried relatively near the soil surface, agricultural benefits from such sludge were foreseen and demonstrated in a greenhouse experiment (Taylor et al., 1978).

The methods used for sludge entrenchment vary with the amount of sludge to be buried on a daily basis (Sikora and Colacicco, 1979). After a site has been used for sludge entrenchment, the recommended practice is to level the mounds which result when the sludge-filled trenches are

Biological Waste Management and Organic Resources Laboratory, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Beltsville, Maryland 20705 (L.J.S. and R.L.C.), and Maryland Environmental Service, Annapolis, Maryland (N.H.F. and C.M.M.).