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p-Alkoxyanilines as Antinitrosamine Agents for Bacon

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As predicted from our work with ethoxyquin in bacon, *p*-alkoxyanilines, both primary and secondary, have been found to be excellent inhibitors of nitrosamine (NA) formation in bacon, with the latter (89–100% inhibition) more efficient than the former (82–93% inhibition). As expected, ortho-, meta-, and phenoxy-substituted anilines also block NA formation but to a lesser extent (37–79% inhibition). The mechanism of inhibition appears to be similar to that of ethoxyquin.

INTRODUCTION

On the basis of the proposed mechanism of action of ethoxyquin, dihydroethoxyquin, and their analogues in inhibiting nitrosamine formation in bacon, described in an earlier paper (Bharucha et al., 1985), it was postulated that aromatic amines, both primary and secondary, possessing an alkoxy substituent in the para position would behave similarly. Several of these anilines were synthesized and tested in bacon. Our findings constitute the subject matter of the present report.

EXPERIMENTAL SECTION

Safety Note: Many nitrosamines have been shown to be highly carcinogenic compounds in test animals, and all experiments should therefore be done in a well-ventilated area. Safety gloves should be worn whenever nitrosamines are being handled.

Preparation of Aromatic Primary and Secondary Amines. The primary amines were purchased from commercial sources when available but otherwise were synthesized in the laboratory. The starting material in the synthesis of most of the compounds was either *o*- or *p*-nitrophenol. The phenol was converted to the alkoxy compound by reaction with alkyl iodide or bromide in the presence of anhydrous potassium carbonate in refluxing acetone. Primary amines were prepared by catalytic (palladium) reduction of the alkoxy nitro compound with hydrogen (Bharucha et al., 1977). The secondary amines were prepared from the primary amines by acylation with acid chlorides followed by reduction of the amide with diborane in tetrahydrofuran (Bharucha et al., 1978). *p*-Methoxy-*N*-*tert*-butylaniline was prepared by the method described by Bondarenko (1967).

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Application of Amine to Bacon and Analysis of Cook-Out Fat. The compounds were added to sequentially sampled commercial pump-cured side bacon in winterized soybean oil as a 4.5% solution, as described earlier (Bharucha et al., 1980).

Bacon was fried under conditions that produce maximum measurable amounts of nitrosamine (12 min), as described earlier (Bharucha et al., 1979).

In all cases the cook-out fat obtained from fried bacon was analyzed for volatile nitrosamines according to our published colorimetric procedure (Cross et al., 1978).

Effect of Primary and Secondary Aromatic Amines on Nitrosamine Level in Fried-Bacon Fat. The primary and secondary aromatic amines were applied to the shingled bacon at a level of 100 ppm, and the bacon was fried immediately.

The primary amine, *p*-(dodecyloxy)aniline was added to sliced bacon in soybean oil at levels of 0, 25, 50, 125, and 250 ppm. Similarly the secondary amine, *p*-methoxy-*N*-dodecylaniline, was added to sliced bacon at levels of 0, 5, 10, 20, 40, and 80 ppm. In another experiment a comparison of the action of the two primary amines *p*-(dodecyloxy)aniline and *p*-(propyloxy)aniline was made at an addition level of 0.181 mmol/k or 50 and 27.4 ppm, respectively. The bacon was fried immediately and the cook-out fat analyzed for nitrosamines.

Nitrosation of *p*-Methoxy-*N*-dodecylaniline. *p*-Methoxy-*N*-dodecylanine (0.5 g, 0.172 mmol) was suspended in a solution of aqueous sulfuric acid (25 mL, 0.8%) The mixture was stirred at room temperature for 10 min and then cooled in an ice bath. The temperature was maintained between 5 and 7 °C as a solution of sodium

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nitrite (1.43 g, 2.06 mmol) and water (5 mL) was added dropwise. The mixture was kept at 5–7 °C for 2 h and then taken up in dichloromethane (ca. 50 mL). The layers were separated, and the aqueous layer was extracted twice with dichloromethane. The combined extract was washed with water (3 × 50 mL), dried over anhydrous sodium sulfate, and evaporated to dryness, yielding 560 mg of orange oil. NMR ($CDCl_3$): δ 0.9 (m, 3 H, terminal CH_3), 1.25 (m, 20 H, CH_2 chain), 3.83 (s, 3 H, OCH_3), 3.95 (m, 2 H, CH_2N), 6.99 and 7.46 (AB pattern, J = 9 Hz, 4 H, aromatic).

A colorimetric test (Cross et al., 1978) for *N*-nitroso group indicated 110% of the calculated *N*-nitroso content. After 6 h in dichloromethane the *N*-nitroso content had dropped to 100%. Gas chromatography of the reaction mixture showed three peaks reminiscent of crude nitrosated ethoxyquin, but the retention times were very long under the conditions described previously (Bharucha et al., 1985). The oven temperature program was changed so that the oven was initially set at 170 °C with the injection port at 210 °C. It was held at 170 °C for 2 min after injection and then programmed at 4°/min to 230 °C where it was held. Under these conditions the three peaks had retention times of 15.8, 17.2, and 27.1 min. All three peaks were identified through the mass spectral data.

Effect of Heating Crude Nitrosated *p*-Methoxy-*N*-dodecylaniline in Air. Samples of the crude nitrosated *p*-methoxy-*N*-dodecylaniline (approximately 60 mg) were placed in 50 mL round-bottom flasks and immersed in oil baths at 160, 180, 200, and 220 °C for 10 min. The amount of *N*-nitroso group remaining was measured by the colorimetric method. In addition, samples (12–15 mg) were weighed from the heated mixtures into 10-mL volumetric flasks and made up to 10 mL with dichloromethane. Aliquots (100 μL) were removed, methyl stearate (10.0 mg) was added, and the solutions were analyzed by gas chromatography. The identity of each component detected by gas chromatography was known from mass spectral evidence. A response of 1 relative to methyl stearate was assumed.

Chromatography of unheated, crude nitrosated *p*-methoxy-*N*-dodecylaniline on a 250-μm layer of alumina that had been dried at room temperature showed only one pink spot at R_f 0.73 in a developing system of 20% diethyl ether in hexane. The spot was eluted from the plate and subjected to gas chromatography. The compound eluted at a retention time of 27.1 min.

On irradiation of the TLC plate with both long- and short-wave ultraviolet light two more spots became visible: a purple spot at R_f 0.49 and an orange spot at R_f 0.19.

Solutions 0–4 (10 μL) were spotted on a 20 × 20 cm air-dried alumina plate. The compound *p*-methoxy-*N*-dodecylaniline was also applied to one spot. The plate was developed for 10 cm with 20% diethyl ether in hexane.

Solution 0 (2 mL, 2.59 mg) was evaporated to dryness and dissolved in 200 μL of dichloromethane. The total sample was streaked on an alumina plate and developed in 20% diethyl ether in hexane. The plate was covered and a small area irradiated with ultraviolet light to locate the streaks. The streak corresponding to the compound with R_f 0.19 was removed and eluted with dichloromethane. Analysis of a portion of the eluted material by the colorimetric method for *N*-nitroso compounds showed a very strong positive result equivalent to about 425 μg of *N*-nitroso-*C*-nitroso-*p*-methoxy-*N*-dodecylaniline. The remaining solution not used for the colorimetric *N*-nitroso determination was evaporated to dryness under vacuum and transferred to a separatory funnel with water and dichloromethane. The solution was made basic with aqueous potassium hydroxide and extracted with dichloromethane. The organic layer was dried with anhydrous sodium sulfate, evaporated to dryness, and subjected to analysis by gas chromatography and thin-layer chromatography.

Finally, the plate was irradiated with ultraviolet light for 15 min in order to denitrosate the *N*-nitroso-*p*-methoxy-*N*-dodecylaniline. The streak (R_f 0.49) was extracted with dichloromethane, and the components were separated by gas chromatography.

Table I. Effect of Primary Aromatic Amines at 100 ppm on the Nitrosamine Level Found in Fried-Bacon Fat

	sample	mol wt	nitrosamines,[a] μmol × 10^{-2}/kg: test	control	redn, %
1	*p*-methoxyaniline	123	6	81	93
2	*p*-ethoxyaniline	137	4	81	95
3	*p*-(propyloxy)aniline	151	5	97	95
4	*p*-*n*-butoxyaniline	165	7	97	93
5	*p*-(hexyloxy)aniline	193	4	43	91
6	*p*-(heptyloxy)aniline	207	7	50	86
7	*p*-(dodecyloxy)aniline	277	9	50	82
8	*p*-phenoxyaniline	185	11	43	74
9	*o*-phenoxyaniline	185	23	43	47
10	*o*-methoxyaniline	123	20	41	51
11	*o*-ethoxyaniline	137	16	41	61
12	*o*-*n*-butoxyaniline	165	15	41	63
13	*p*-methoxy-2-methylaniline	137	<3 (2)	18	89
14	*p*-*sec*-butoxyaniline	165	<3 (1)	18	94
15	*m*-methoxyaniline	123	5	18	71
16	*o*,*p*-dimethoxyaniline	153	3	23	87

[a] * Detection limit about 3 μmol × 10^{-2}/kg.

Table II. Comparison of *p*-(Propyloxy)aniline with *p*-(Dodecyloxy)aniline

substance	mol wt	mmol/kg of bacon	ppm	nitrosamines, μmol × 10^{-2}/kg: test	control	redn, %
p-(propyloxy)-aniline	151	0.181	27.4	11	33	67
p-(dodecyloxy)-aniline	277	0.181	50.0	9	33	73

RESULTS AND DISCUSSION

The effect of addition of various primary aromatic amines in soybean oil to bacon slices at 100 ppm on the nitrosamine content of cooked bacon is summarized in Table I. The data show that, as postulated from mechanistic considerations (Bharucha et al., 1985) for ethoxyquin and derivatives, all of the *p*-alkoxyanilines provide excellent reduction of NA formation in comparison with the controls. *o*,*p*-Dimethoxyaniline (16) is also highly effective in contrast to *o*-methoxyaniline (10), which shows only 51% inhibition, demonstrating that the alkoxy group in the para position is an important requirement for good antinitrosamine activity. The results also show that the blockage of one of the two available ortho positions in the benzene ring is not detrimental to activity, the view being further buttressed by the effectiveness of *p*-methoxy-2-methylaniline (13) in reducing the NA content of bacon below our detection limit of 3 ppb. A phenoxy is not as effective as an alkoxy substituent as reflected by the performance (74% reduction) of *p*-phenoxyaniline (8) as compared to *p*-(hexyloxy)aniline (5; 91% reduction) of nearly equal molecular weight. Likewise an alkoxy group in the meta position is inferior to that in the para position

Table III. Effect of Concentration on the Antinitrosamine Action of p-(Dodecyloxy)aniline

sample	ppm additive	nitrosamines in cook-out fat,[a] μmol × 10^{-2}/kg	sample	ppm additive	nitrosamines in cook-out fat,[a] μmol × 10^{-2}/kg
1	0	33	4	125	4
2	25	14	5	250	<3 (nd)
3	50	9			

[a] Detection limit about 3 μmol × 10^{-2}/kg.

Table IV. Effect of Secondary Aromatic Amines at 100 ppm on the Nitrosamine Level Found in Fried Bacon

sample	additive at 100 ppm	nitrosamines,[a] μmol × 10^{-2}/kg		redn, %
		test	control	
1	p-methoxy-N-dodecylaniline	<3 (2)	76	96
2	p-ethoxy-N-dodecylaniline	3	60	95
3	p-(dodecyloxy)-N-dodecylaniline	<3 (2)	29	90
4	p-methoxy-N-ethylaniline	<3 (nd)	43	100
5	p-ethoxy-N-ethylaniline	<3 (nd)	43	100
6	p-methoxy-N-methylaniline	<3 (nd)	33	100
7	p-methoxy-N-butylaniline	<3 (nd)	28	100
8	p-methoxy-N-hexylaniline	<3 (nd)	28	100
9	p-ethoxy-N-methylaniline	<3 (nd)	28	100
10	p-ethoxy-N-butylaniline	<3 (nd)	28	100
11	p-ethoxy-N-hexylaniline	<3 (nd)	28	100
12	p-methoxy-N-(2,2-dimethylpropyl)aniline	<3 (1)	18	94
13	p-sec-butoxy-N-dodecylaniline	<3 (1)	20	95
14	p-methoxy-N-tert-butylaniline	<3 (2)	19	89
15	p-methoxy-N-benzylaniline	<3	34	91
16	p-methoxy-N-(2-phenylbutyl)aniline	<3	34	91
17	o,p-dimethoxy-N-dodecylaniline	<3	23	87
18	m-methoxy-N-dodecylaniline	7	34	79
19	N-dodecylaniline	16	76	79
20	p-phenoxy-N-hexylaniline	9	41	78
21	o-phenoxy-N-hexylaniline	26	41	37
22	o-methoxy-N-hexylaniline	11	36	69
23	o-butoxy-N-dodecylaniline	11	36	69
24	o-methoxy-N-ethylaniline	7	36	69
25	o-dodecyloxy-N-dodecylaniline	47	51	8
26	o-methoxy-N-butylaniline	11	36	69
27	o-methoxy-N-dodecylaniline	11	36	69
28	o-methoxy-N-dodecylaniline (200 ppm)	6	32	81
29	o-methoxy-N-dodecylaniline (300 ppm)	6	32	81

[a] Detection limit about 3 μmol × 10^{-2}/kg. nd = no visible color developed.

as shown by the performance of m-methoxyaniline (15; 71% reduction) vis-a-vis the corresponding para isomer (1; 93% reduction). An alkoxy group in the ortho, unlike that in the meta, position can provide stabilization but not as effectively as the one in the para position. Also the o-alkoxy moiety tends to increase the volatility of the compounds due to intramolecular hydrogen bonding with the proton on nitrogen. The cumulative effect could be considerably decreased activities for o-alkoxyanilines (9–12) as given in Table I. The same table also shows substantially no differences between compounds having straight-chain and branched-chain alkoxy substituents, as exemplified by p-sec-butoxyaniline (14) and p-n-butoxyaniline (4), both of which show excellent inhibition of NA formation.

A priori it would appear from the results in Table I that, in the homologous series of p-alkoxyanilines, the activity peaks at p-(propyloxy)aniline (3). However, this does not take into account the differences in molecular weights. Thus, for example p-(dodecyloxy)aniline (7) with nearly double the molecular weight of p-(propyloxy)aniline (3) shows only 82% inhibition vs. 95% for the latter. However, when both the substances are incorporated in bacon at the same molar levels (Table II), it becomes obvious that the dodecyl compound (7) is, if anything, slightly more effective than the propyl analogue (3). Table III shows that as the concentration of p-dodecyloxyaniline is raised from 25 to 250 ppm, the antinitrosamine activity also increases roughly proportionately.

The effect of secondary aromatic amines at the 100 ppm level on the NA content of fried bacon is shown in Table IV. The results parallel very closely those obtained with the corresponding primary amines. As expected, without exception, all of the p-alkoxyanilines virtually eliminated the formation of undesirable nitrosamines in cooked bacon. As with the primary amine series and for the same reasons, the ortho-, meta-, and phenoxyanilines were somewhat less effective than their corresponding p-alkoxy counterparts. While appreciable inhibitory effect is manifested in ortho-substituted secondary amines where the substituent is the methoxyl group, with the higher homologues in the ortho position, e.g. o-(dodecyloxy)-N-dodecylaniline (25), the activity more or less disappears, presumably due to the shielding of the nitrogen atom by the long alkoxy chain. With the p-alkoxyanilines, it is immaterial whether the alkoxy chain is primary or secondary, both of them showing excellent antinitrosamine activity. The same is also true for the alkyl side chain on nitrogen, with primary, secondary, and tertiary N-substituted p-alkoxyanilines again exhibiting excellent inhibition of NA formation. The presence of an aromatic substituent in the N-alkyl side chain (16) is also compatible with excellent activity. In general, the secondary amines have been found to be about twice as effective as the corresponding primary amines. Thus, for example, as results in Table V show, increasing the concentration of p-methoxy-N-dodecylaniline (1) from 0 to 80 ppm virtually eliminates NA formation in bacon. In the primary amine series, on the other hand, amounts greater than 125 ppm of p-(dodecyloxy)aniline, of nearly the same molecular weight, are required to achieve the same objective (Table III).

To establish that the mechanism of inhibition of NA formation by the alkoxyanilines followed the same pattern as with ethoxyquin, described in an earlier paper (Bharucha et al., 1985), in vitro nitrosation of p-methoxy-N-dodecylaniline (1, Table IV) was undertaken. The crude product was an orange oil, which on the basis of its NMR

Table V. Effect of Concentration on the Antinitrosamine Action of p-Methoxy-N-dodecylaniline

sample	ppm additive	nitrosamines,[a] μmol × 10^{-2}/kg	sample	ppm additive	nitrosamines,[a] μmol × 10^{-2}/kg
1	0	87	4	20	16
2	5	32	5	40	4
3	10	20	6	80	<3 (2.5)

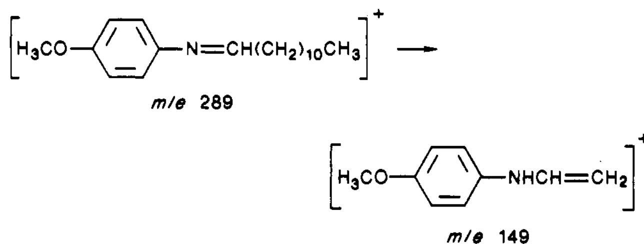
[a] Detection limit about 3 μmol × 10^{-2}/kg.

Table VI. Nitrosation of *p*-Methoxy-*N*-dodecylaniline Mass Spectrometric Data

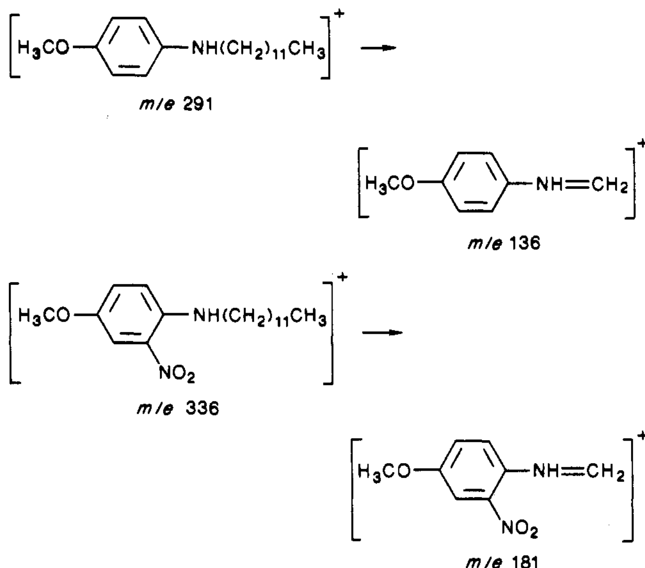
peak no.	(mol ion)$^+$	other main frag ions, m/e >70[a]
1	(289)	162, 149,* 134
2	(291)	276, 162, 149, 136*
3	(336)	306, 301, 181,* 175, 162, 149

[a] Asterisk indicates base peak in the mass spectrum.

spectrum (AB pattern with J = 9 Hz in the aromatic region) and analysis for *N*-nitroso group appears to be essentially *N*-nitroso-*p*-methoxy-*N*-dodecylaniline. Gas chromatography of the crude nitrosated product, however, showed three peaks reminiscent of crude nitrosated ethoxyquin. From the mass spectral data of the peaks given in Table VI, it appears that, in the case of peak 1, the molecular ion m/e 289 is an imine, shown below, which then fragments at the C–C bond β to the double bond (cleavage with rearrangement of a hydrogen atom) to give the ion with m/e 149.



In the case of peaks 2 and 3, cleavage of the side chain gives the base peak in both instances.



Peak 2, with the same retention time as the starting amine, is therefore *p*-methoxy-*N*-dodecylaniline while peak 3, as expected by analogy with nitrosated ethoxyquin (Bharucha et al., 1985), is *o*-nitro-*p*-methoxy-*N*-dodecylaniline. None of the major peaks in the crude nitrosated product was an N-nitrosated compound, although the former was shown by colorimetric test to contain approximately the theoretical amount of *N*-nitroso group. It thus appears that the gas chromatographic peaks do not represent the mixture as it exists, but rather show the breakdown products formed in the injection port; only the nitro compound may be present as such in the crude mixture.

When the crude nitrosated product was heated at 160, 180, 200, and 220 °C for 10 min and then analyzed by gas chromatography, as the results in Table VII show there is no polymerization or extensive degradation until sample 3 when the recovery (last column) drops to 49 from about 70%. If one assumes from the above work that the imine and *p*-methoxy-*N*-dodecylaniline are measures of the amount of *N*-nitroso compound, there again does not appear to be much change in the composition of the heated samples until sample 3. On the other hand, the fourth column showing the amount of measurable N–NO present indicates that a considerable change has occurred. Separation by TLC on alumina was resorted to in order to identify the changes occurring on heating. The unheated crude nitrosated product (sample 0) on TLC showed a pink spot with R_f 0.73, which was identified by GC–MS after extraction to be a nitro derivative of *p*-methoxy-*N*-dodecylaniline, very likely the *o*-nitro derivative by analogy with our work on ethoxyquin. On irradiation of the TLC plate with both long- and short-wave UV light, two more spots became visible: a purple spot at R_f 0.49 and an orange spot at R_f 0.19. These were tentatively identified as *N*-nitroso-*p*-methoxy-*N*-dodecylaniline and *o*-*C*-nitroso-*N*-nitroso-*p*-methoxy-*N*-dodecylaniline for the following reasons. When the crude nitrosated product was streaked on an alumina plate and the streak corresponding to R_f 0.19 extracted and analyzed by the colorimetric method (Cross et al., 1978), it showed a very strong positive response, indicating the presence of *N*-nitroso or *C*-nitroso-*N*-nitroso compound. Since *C*-nitroso compounds are known not to undergo splitting with HBr in acetic acid (Johnson and Walters, 1971), the split product from the former would appear as *p*-methoxy-*N*-dodecylaniline and *o*-nitro-*p*-methoxy-*N*-dodecylaniline from the latter, since aromatic nitroso compounds have a propensity to undergo oxidation to the corresponding nitro compound. In the unlikely event that the *C*-nitroso groups were cleaved with HBr–acetic acid, the resultant product in both instances would be *p*-methoxy-*N*-dodecylaniline. Both TLC and GC analysis of the split product showed it to be *o*-nitro-*p*-methoxy-*N*-dodecylaniline. It can therefore be concluded that the orange spot with R_f 0.19 is *o*-nitroso-*N*-nitroso-*p*-methoxy-*N*-dodecylaniline. When the streak with R_f 0.49 was eluted after irradiation with UV light for 15 min and analyzed by GC, it was found to contain anisidine, dodecanal, the imine, and the parent *p*-methoxy-*N*-dodecylaniline, with the latter predominating. The presence of the aldehyde and anisidine indicates cleavage of the imine

Table VII. Analysis of Heated Nitrosated *p*-Methoxy-*N*-dodecylaniline by GC

				imine		*p*-methoxy-*N*-dodecylaniline		*p*-methoxy-*o*-nitro-*N*-dodecylaniline			
sample	aliquot wt, μg	bath temp, °C	% theor N–NO	found, μg	% recd wt	found, μg	% recd wt	found, μg	% recd wt	total wt recd, μg	% rec
0	129.6		111.3	10.2	11.4	67.1	74.7	12.5	13.9	89.8	68
1	121.5	160	97.4	9.4	11.2	59.3	71.0	14.8	17.7	83.5	69
2	149.8	180	30.8	10.7	10.1	76.9	73.0	17.7	16.8	105.3	70
3	152.1	200	27.4	2.7	2.7	54.6	72.6	18.6	24.7	75.2	49
4	137.7	220	0	0	0	42.9	73.3	15.6	26.7	58.5	43

on the plate. The results show that the purple spot with R_f 0.49 is *N*-nitroso-*p*-methoxy-*N*-dodecylaniline, which on irradiation undergoes denitrosation to the parent amine and dehydronitrosation to the imine, with the latter undergoing further cleavage to anisidine and dodecanal. When solutions 0–4 (Table VII) were analyzed by TLC on an alumina plate as above, a pink spot now known to be *o*-nitro-*p*-methoxy-*N*-dodecylaniline was clearly visible and increased in intensity on going from sample 0 to sample 3. Irradiation of the plate for 15 min under UV light made three more spots visible. The first (R_f 0.61) was purple and coincident with *p*-methoxy-*N*-dodecylaniline. This spot did not appear until sample 3. The purple spot (R_f 0.49), identified as described above as *N*-nitroso-*p*-methoxy-*N*-dodecylaniline, was relatively constant in samples 0–2 but decreased dramatically in sample 3 and was absent in sample 4. The orange spot (R_f 0.19), representing as proven above the *N*-nitroso-*C*-nitroso compound, decreased steadily through the series and was practically absent in sample 4. It would thus appear that the crude nitrosated product from *p*-methoxy-*N*-dodecylaniline on heating to about 200 °C decomposes extensively to give the parent amine and *o*-nitro-*p*-methoxy-*N*-dodecylaniline, presumably via rearrangement of the *N*-nitroso to the *o*-*C*-nitroso compound followed by air oxidation. In this respect, the inhibitory effect of *p*-methoxy-*N*-dodecylaniline in NA formation in bacon is very reminiscent, mechanistically speaking, of that of ethoxyquin described in an earlier paper from this laboratory (Bharucha et al., 1985).

In summary, *p*-alkoxyanilines, both primary and secondary, are excellent antinitrosamine agents in bacon, with the latter more efficient than the former. Ortho-, meta-, and phenoxy-substituted anilines also inhibit NA formation, but somewhat less efficiently. The mechanism of inhibition appears similar to that of ethoxyquin, viz. initial N-nitrosation followed by rearrangement and oxidation to the *o*-nitro compound in the case of secondary amines.

Registry No. *p*-MeOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 54574-77-5; *p*-EtOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-08-3; *p*-Me(CH$_2$)$_{11}$OC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-10-7; *p*-MeOC$_6$H$_4$NHEt, 104-48-3; *p*-EtOC$_6$H$_4$NHEt, 65570-13-0; *p*-MeOC$_6$H$_4$NHMe, 5961-59-1; *p*-MeOC$_6$H$_4$NHBu, 61829-43-4; *p*-MeOC$_6$H$_4$NH(CH$_2$)$_5$Me, 16664-54-3; *p*-EtOC$_6$H$_4$NHMe, 3154-18-5; *p*-EtOC$_6$H$_4$NHBu, 15498-39-2; *p*-EtOC$_6$H$_4$NH(CH$_2$)$_5$Me, 16663-90-4; *p*-MeOC$_6$H$_4$NHCH$_2$C(Me)$_3$, 65570-14-1; *p*-*sec*-BuOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-15-2; *p*-MeOC$_6$H$_4$NHBu-*t*, 15408-62-5; *p*-MeOC$_6$H$_4$NHCH$_2$Ph, 17377-95-6; *p*-MeOC$_6$H$_4$NHCH$_2$CH(Ph)Et, 65570-16-3; *o*,*p*-(MeO)$_2$C$_6$H$_3$NH(CH$_2$)$_{11}$Me, 65570-17-4; *m*-MeOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-18-5; PhNH(CH$_2$)$_{11}$Me, 3007-74-7; *p*-PhOC$_6$H$_4$NH(CH$_2$)$_5$Me, 65570-11-8; *o*-PhOC$_6$H$_4$NH(CH$_2$)$_5$Me, 65570-19-6; *o*-MeOC$_6$H$_4$NH(CH$_2$)$_5$Me, 65570-21-0; *o*-BuOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65606-63-5; *o*-MeOC$_6$H$_4$NHEt, 15258-43-2; *o*-Me(CH$_2$)$_{11}$OC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 103439-76-5; *o*-MeOC$_6$H$_4$NHBu, 65570-20-9; *o*-MeOC$_6$H$_4$NH(CH$_2$)$_{11}$Me, 65570-22-1; *p*-PrOC$_6$H$_4$NH$_2$, 4469-80-1; *p*-MeOC$_6$H$_4$NH$_2$, 104-94-9; *p*-EtOC$_6$H$_4$NH$_2$, 156-43-4; *p*-BuOC$_6$H$_4$NH$_2$, 4344-55-2; *p*-Me(CH$_2$)$_5$OC$_6$H$_4$NH$_2$, 39905-57-2; *p*-Me(CH$_2$)$_6$OC$_6$H$_4$NH$_2$, 39905-44-7; *p*-Me(CH$_2$)$_{11}$OC$_6$H$_4$NH$_2$, 65039-19-2; *p*-PhOC$_6$H$_4$NH$_2$, 139-59-3; *o*-PhOC$_6$H$_4$NH$_2$, 2688-84-8; *o*-MeOC$_6$H$_4$NH$_2$, 90-04-0; *o*-EtOC$_6$H$_4$NH$_2$, 94-70-2; *o*-BuOC$_6$H$_4$NH$_2$, 4469-81-2; *p*-*sec*-BuOC$_6$H$_4$NH$_2$, 59002-72-1; *m*-MeOC$_6$H$_4$NH$_2$, 536-90-3; *o*,*p*-(MeO)$_2$C$_6$H$_3$NH, 2735-04-8; *p*-methoxy-2-methylaniline, 102-50-1; *p*-methoxy-*o*-nitro-*N*-dodecylaniline, 103439-77-6; ethoxyquin, 91-53-2.

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Valencia Orange Leaf Oil Composition

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Leaf oils both from Valencia orange trees treated with the abscission agent 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (Release) and from untreated trees were analyzed, and 36 compounds were identified; seven of them are being reported for the first time as components of orange leaf oil. 4-Vinylguaiacol was found for the first time as a natural citrus constituent. It had been reported previously as a degradation product and artifact in stored canned single-strength orange juice. Other newly found orange leaf oil components were α- and β-sinensal, geranyl acetate, *m*-cymene, *p*-cymene, and *cis*-*p*-2-menthen-1-ol.

Abscission-inducing chemicals are used to loosen citrus fruit prior to mechanical harvesting. Moshonas et al. (1976, 1977) showed that these chemicals affect the quality of processed orange juice and cold-pressed peel essential oil. Six phenolic ether compounds isolated from chemically treated fruit were identified and shown to be compounds that do not normally occur in citrus but are formed by a change in metabolic pathways brought about by the abscission agents (Moshonas and Shaw, 1978). Because the effect of these phenolic ethers was characterized as contributing an "overripe" flavor, a study was made that showed that these compounds were not formed when fruit were allowed to stay on the tree until they were extremely overripe (Moshonas and Shaw, 1979).

Since the leaves are also sprayed and are loosened by the abscission agents used to loosen fruit, the current study was made to determine whether phenolic ethers or other

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