now considered subspecies or synonyms of *S. acaule* (Hawkes and Hjerting, 1969). Whereas one group of workers report tomatine and in some cases solacauline as the major compounds in this group (Schreiber, 1963), another laboratory (Prokoshev et al., 1952) reports only demissine as the major glycoalkaloid. Our results indicate that tomatine and/or demissine are present in *S. acaule*, but the relative amounts in which they are found will vary between clones; in one clone, tomatine was not present at a detectable level. The possibility exists that either the clones examined by these laboratories were distinctly different in glycoalkaloid composition by chance or that the chromatographic or other techniques used to characterize the compounds could not easily distinguish tomatine from demissine. Also purification procedures necessary for compound characterization may have removed one or the other glycoalkaloid.

Toxicity studies (Nishie et al., 1976) of the glycoalkaloids found in the species listed in Table I indicate that they are of the same order of toxicity as α-solanine and α-chaconine (Nishie et al., 1971, 1975). Therefore, they present no greater hazard than glycoalkaloids now found in commercial potatoes.

Additional glycoalkaloid studies are planned for the analysis of leaves and roots of the species whose tuber tissue was examined in this investigation. Even though these parts of the plant are not consumed as food, the possible role of glycoalkaloids in resistance to insects and diseases and the potential use of potato foliage for animal feed justify the acquisition of this information. Although there can be variation in glycoalkaloid composition in different parts of the plant (Schreiber, 1968), our experience has shown that the difference is usually quantitative rather than qualitative.

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COMMUNICATIONS

Analysis by Gas–Liquid and Thin-Layer Chromatography of Residual [^{14}C]Diethylstilbestrol Ear Implants

The unabsorbed parts of 15 [^{14}C]diethylstilbestrol (DES) ear implants were retrieved at slaughter and were analyzed for total radiocarbon and for DES by gas–liquid chromatography (GLC) and were qualitatively evaluated for DES by three different thin-layer chromatography (TLC) systems. Time in situ and time that [^{14}C]DES was in the implant form varied among implants. The amount of DES measured by GLC averaged 98.3% of the amount of DES calculated on the basis of total radiocarbon analysis and original specific activity. This percentage did not vary with time and was similar to the percent radiopurity of the [^{14}C]DES before implantation. These results and the TLC evaluations suggested that the crystalline [^{14}C]DES, which was 88% of each implant, did not self-decompose during the implant study. The differences between TLC systems demonstrated limitations of TLC for radiopurity evaluation of [^{14}C]DES.

Radiocarbon-labeled diethylstilbestrol ([^{14}C]DES) ear implants from the same supplier and formulation were divided between Aschbacher et al. (1975) and Rumsey et al. (1975) for recent studies on the fate of implanted DES in beef steers. These studies related the absorption of [^{14}C]DES from the implants to the amounts of DES and radiocarbon in tissues, blood, and excreta of the implanted steers. However, interpretation of the radiocarbon data was limited by a small amount of impurities in the administered [^{14}C]DES and the possibility that the highly concentrated [^{14}C]DES self-decomposed while in the implant form (Rochlin, 1965).

Results from three separate analyses indicated that the [^{14}C]DES implants used by Aschbacher et al. (1975) and Rumsey et al. (1975) contained a small amount of radiocarbon not associated with DES. The supplier of crystalline [^{14}C]DES for the implants determined by paper chromatography and by thin-layer chromatography (TLC)

that radiopurity of the synthesized material was 98%. Tennent et al. (1976) determined by TLC that radiopurity in some of the unpelleted [^{14}C]DES implant formulation was 94%. Aschbacher et al. (1975) determined by isotope dilution that radiopurity in an initial [^{14}C]DES implant was 97%. The latter workers also reported that radiopurity in a second implant stored for 1 year was 89%, which suggested that [^{14}C]DES self-decomposed when it was kept in the implant form.

The objective of the present research was to use gas–liquid and thin-layer chromatography methods to obtain evidence on whether the initial radiopurity of the [^{14}C]DES implants was maintained or changed during the implant study of Rumsey et al. (1975).

MATERIALS AND METHODS

Implant Preparation. The unabsorbed parts of the 15 [^{14}C]DES ear implants that were used for this study were from the animal study of Rumsey et al. (1975). Monoethyl-*1*-^{14}*C*-diethylstilbestrol [3,4-bis(*p*-hydroxyphenyl-3-hexene] was obtained in crystalline form from Amersham-Searle Corp., Arlington Heights, Ill. Radiochemical purity was determined at manufacture by paper chromatography in benzene–petrol (80 to 100 °C)–methanol–water (5:5:7:3 by volume) and by thin-layer chromatography on silica gel in chloroform–acetone (3:2 by volume). Crystalline [^{14}C]DES was formulated into implants (88.24% DES, 10.29% hydrogenated peanut oil, and 1.47% calcium stearate) by the Chemical Research Laboratory of Hess and Clark; the specified weight and thickness tolerances established for Hess and Clark's commercial DiBESTrol-C implants (17 mg ± 10% and 0.267 to 0.29 cm) were used. [^{14}C]DES added to five of the implants had a specific activity of 59 mCi/mM and [^{14}C]DES added to ten of the implants had a specific activity of 49 mCi/mM. The unabsorbed part of each implant was retrieved at slaughter by careful dissection of the implant area of the ears of eight steers. Two steers had been implanted with two implants each for 30 days, two steers had been implanted with two implants each for 60 days, and two steers had been implanted with two implants each for 90 days. One steer had been implanted with two implants for 120 days and one steer had been implanted with one implant for 120 days. Each retrieved implant was cleaned under a magnifying glass. Forceps were used to remove tissue debris and a film of tissue that usually encapsulated each implant. Each implant was lightly rinsed with distilled water, blotted, dried to a constant weight at 60 °C, weighed to the nearest tenth of a milligram, and dissolved in 50 mL of benzene. The dissolved implants were stored in a sealed container at 10 °C until all implants were retrieved, and then they were analyzed as a group. Exposure of the implants and implant solutions to light during preparation and analysis was minimal, and the dissolved implants were stored in the dark.

Radiocarbon Analysis. Aliquots of the benzene solutions that contained the dissolved [^{14}C]DES implants were diluted with equal amounts of benzene, so the theoretical amount of radioactivity in the counting vials did not exceed 15 000 disintegrations/min (dpm). Thus, the counts per minute (cpm) ranged from 1300 to 13 000 for the diluted aliquots, depending on the size of the dissolved implant. The diluted aliquots were compared with standards that contained known amounts of radioactivity within the range of the samples. Standards of [^{14}C]DES for the radiocarbon and TLC analyses were from Amersham-Searle Corp., Arlington Heights, Ill., and were maintained in dilute benzene solution from the time of synthesis. Duplicates of the implant solutions and standards were added to a scintillation cocktail that contained 8.25 g of 2,5-diphenyloxazole/L of toluene and analyzed by scintillation spectrophotometry. Efficiency determined by the external standardization method for converting cpm to dpm approximated 80%.

GLC Analysis. Aliquots of the benzene solutions that contained the dissolved [^{14}C]DES implants were diluted uniformly with benzene so the theoretical concentration of DES did not exceed 0.250 μg/mL. Analysis of these dilute solutions started with the derivatization step of the GLC method used by Rumsey et al. (1974) with a non-radiolabeled DES standard used for quantitation.

TLC Analysis. Aliquots of the benzene solutions that contained the dissolved [^{14}C]DES implants were diluted with benzene, so about the same amount of radioactivity (50 000 dpm) in the same sample volume could be applied to each TLC plate. The plates were 20 cm × 20 cm glass coated with a 250-μm layer of silica gel from Analabs, Inc., North Haven, Conn.

Three two-dimensional TLC analyses were used. All implants were analyzed by the first two TLC systems, and nine of the 15 implants were analyzed by the third system. The mobile phases for the first two-dimensional analysis were a 19:1 solvent mixture of benzene–ethyl ether followed by a 19:1 mixture of methylene chloride–ethanol. Mobile phases for the second analysis were a 4:3:2 mixture of hexane–ethyl ether–methylene chloride, followed by a 19:1 mixture of chloroform–ethanol; and the mobile phases for the third analysis were a 16:3:1 mixture of ethyl acetate–aqueous hexane–ethanol, followed by the initial phase of the second analysis. The aqueous hexane was prepared by saturation of hexane with water. The mobile phases were similar to those used previously for DES and for estrogens (Kircher, 1967; Schuller, 1967; Jeffus and Kenner, 1972; and Tennent et al., 1976). All solvents except ethyl ether were distilled and mixed on the basis of volume. Standard [^{14}C]DES in benzene solution was cochromatographed with each phase on the same plate with the diluted aliquots of dissolved implants (Kircher, 1976) and also on separate two-dimensional plates.

After the TLC plates were developed, X-ray film was exposed to the plates for 5 days. The visualized zones of radioactivity were scraped from the TLC plates into scintillation vials. The previously described scintillation cocktail was added to the vials and they were then shaken; radioactivity was determined after the silica gel had settled at the bottom of the vial. For each TLC plate, the cpm for the various zones of radioactivity were totaled, and each zone was then expressed as a percentage of the total. The appearance of new zones of radioactivity or consistent shifts in the size of radioactive zones relative to time would indicate the possibility of autodegradation.

For some implants, the length of time in situ did not parallel the length of time in the implant form. This difference is shown in the first two lines of Tables I and II and is a result of the implant schedule that was used by Rumsey et al. (1975). Therefore, the analytical data were summarized on the basis of both time in situ and time in implant form. The data for each summary were analyzed statistically by analysis of variance with time, animals within time and implants within animals as the sources of variation (Steel and Torrie, 1960). If time was significant, then Duncan's (1955) multiple range test was used to indicate differences between times.

RESULTS AND DISCUSSION

GLC Analysis. Figure 1 shows the total weight of the unabsorbed implants, the unabsorbed DES, and the un-

Table I. Composition of Unabsorbed [^{14}C]DES Ear Implants Retrieved after 30, 60, 90, and 120 Days in situ

item	days in situ 30	60	90	120	av	$S\bar{x}$
no. of implants	4	4	4	3		
av no. of days [^{14}C]DES was in implant form[a]	85	67	111	137		
theoret. amount[b] of DES/initial implant, mg	13.2	14.1	13.9	13.4	13.7	0.6
DES/retrieved implant						
theoret. amount,[b] mg	7.7	7.8	7.0	3.0	6.6	0.9
GLC-determined amount, mg	5.8	6.3	5.9	2.1	5.2	1.1
% of theoretical	72.9	81.2	84.7	65.6	76.8	8.0
^{14}C-determined amount, mg	5.9	6.5	5.7	2.2	5.2	1.2
% of theoretical	74.0	83.1	82.1	71.8	78.1	8.4
DES by GLC/DES by ^{14}C × 100[c]	98.8	98.1	103.2	91.5	98.3	4.1

[a] [^{14}C]DES was in implant form for approximately 68 days in two of the 30-day implants, 102 days in the other two 30-day implants, 67 days in two of the 60-day implants, 66 days in the other two 60-day implants, 101 days in two of the 90-day implants, 121 days in the other two 90-day implants, 158 days in one of the 120-day implants, and 126 days in the other two 120-day implants. [b] Theoretical amount equals total weight of each initial or retrieved implant multiplied by 0.882 [proportion of implant formulation as DES (Hess and Clark Laboratories)] and by 0.968 [radiopurity of [^{14}C]DES in prepared implants as determined by Aschbacher et al. (1975)]. [c] Represents average of individual ratios; thus, somewhat different than the ratio of the GLC and ^{14}C means.

Table II. Composition of Unabsorbed [^{14}C]DES Implants on the Basis of Number of Days DES Was in Implant Form

item	av days in implant form 67	102	130	av	$S\bar{x}$
no. of implants	6[a]	4[b]	5[c]		
av no. of days in situ	50	60	108		
theoret. amount[d] of DES/initial implant, mg	14.3	12.9	13.5	13.7	0.6
DES/retrieved implant					
theoret. amount,[d,e] mg	7.4	8.6[f]	4.1[g]	6.6	0.6
GLC-determined amount,[e] mg	5.6[f,g]	7.4[f]	3.0[g]	5.2	0.8
% of theoretical	74.8	86.1	71.7	76.8	6.6
^{14}C-determined amount, mg	5.7	7.3	3.2	5.2	0.9
% of theoretical	75.9	84.1	76.1	78.1	7.2
DES by GLC/DES by ^{14}C × 100[h]	99.1	102.5	94.0	98.3	3.7

[a] [^{14}C]DES was in situ for 30 days and in implant form for 68 days in two implants, in situ for 60 days and in implant form for 66 days in two implants, and in situ for 60 days and in implant form for 67 days in two implants. [b] [^{14}C]DES was in situ for 30 days and in implant form for 102 days in two implants and in situ for 90 days and in implant form for 101 days in two implants. [c] [^{14}C]DES was in situ for 90 days and in implant form for 121 days in two implants, in situ for 120 days and in implant form for 126 days in two implants, and in situ for 120 days and in implant form for 158 days in one implant. [d] See footnote *b*, Table I. [e] Significantly different among days, $P < 0.05$. [f,g] Time means that do not have a common letter in their superscript are different at $P < 0.05$. [h] Represents average of individual ratios; thus, somewhat different than the ratio of the GLC and ^{14}C means.

absorbed radiocarbon all expressed as percentage of the original implant dose. The rapid implant absorption during the first 30 days, the more rapid relative absorption initially of DES and radiocarbon relative to the total implant, and the presence of unabsorbed implants at 120 days agree with earlier implant absorption results from a different trial (Rumsey et al., 1974) and with the patterns of radiocarbon in blood and excreta of the steers implanted with these [^{14}C]DES implants (Rumsey et al., 1975). At the end of each implant period, the amount of radiocarbon in the unabsorbed implants expressed as a percentage of the original dose was about equal to the amount of DES expressed as a percentage of the original dose as would be expected of a highly radiopure material.

Table I shows the analysis of the unabsorbed [^{14}C]DES implants summarized on the basis of the number of days in situ. The theoretical amount of DES per initial implant, calculated on the basis of the implant formulation, averaged 13.7 mg, and the calculated theoretical amount recovered in unabsorbed implants averaged 6.6 mg. On the basis of GLC analysis, the average amount of DES unabsorbed was 5.2 mg or 76.8% of theoretical. The fact that this amount varied with time in situ suggested a slight difference in the absorption of DES relative to the total implant, but the variation was not significant ($P > 0.05$). For all the analyses, the variation among animals within

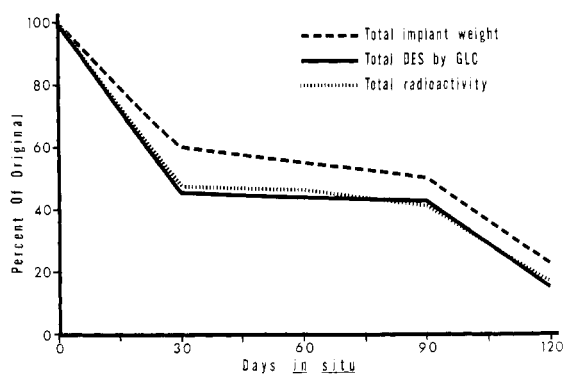

Figure 1. The percentage of original dose of [^{14}C]DES implants in unabsorbed implants retrieved at different times after implantation.

time was considerable. The average amount of unabsorbed DES as determined by GLC was about the same as the amount determined on the basis of radiocarbon analysis. The average amount of unabsorbed DES determined by GLC averaged 98.3% of the amount determined by radiocarbon analysis.

The composition of unabsorbed implants summarized on the basis of the number of days DES was in the implant form is shown in Table II. Results were similar to those

Table III. R_f Values for Visualized Zones of Radioactivity for Two-Dimensional TLC Systems Used to Evaluate ^{14}C-Labeled Diethylstilbestrol

visualized radiocarbon zones	R_f values					
	system 1[a]		system 2		system 3	
	first dimension	second dimension	first dimension	second dimension	first dimension	second dimension
origin						
1	0	0	0	0	0	0
2	0.06	0	0.31	0	0.68	0
3	0.23	0	0.47	0		
DES, major zones						
4	0.06	0.44	0.31	0.18	0.68	0.34
5	0.23	0.44	0.47	0.18	0.68	0.50
6	0.06	0.61	0.31	0.37		
7	0.23	0.61	0.47	0.37		
R_f < *cis*-DES						
8	0.01	0.29	0.11	0.11	0.68	0.18
R_f between *cis*- and *trans*-DES						
9	0.07	0.50	0.32	0.26		
R_f > *trans*-DES						
10	0.05	0.87	0.33	0.47	0.68	0.61
11	0.22	0.87	0.48	0.48		
12	0.42	0.68	0.33	0.56		
13	0.52	0.80	0.48	0.56		

[a] Systems 1, 2, and 3 correspond to those of Tables IV, V, and VI, respectively.

Table IV. Distribution of Radiocarbon from Unabsorbed [^{14}C]DES Ear Implants on Two-Dimensional TLC Plates with Benzene–Ethyl Ether and Methylene Chloride–Ethanol as Mobile Phases

conditions	no. of implants	visualized radiocarbon zones[a]				
		origin	DES	R_f < *cis*-DES	R_f between *cis*- and *trans*-DES	R_f > *trans*-DES
days in situ						
30	4	0.3	88.5	0.5	2.8	8.0
60	4	0.7	90.0	0.6	2.5	6.3
90	4	0.9	92.4	0.9	2.0	3.9
120	3	0.7	91.4	1.2	2.0	4.8
$S\bar{x}$		0.3	2.6	0.2	0.3	2.4
days in implant form						
67	6	0.6	87.8	0.6[b]	3.0	8.1
102	4	0.3	92.4	0.5[b]	1.7	5.1
130	5	0.9	92.2	1.2[c]	2.1	3.6
$S\bar{x}$		0.2	1.6	0.1	.3	1.5
av of all implants		0.6	90.5	0.8	2.3	5.8
standard		0.8	91.2	0.8	5.4	1.8

[a] Data are expressed as the percentage of total radioactivity on the TLC plates. [b,c] Time means that do not have a common letter in their superscript are different at $P < 0.05$.

shown in Table I. There was no indication from the GLC and radiocarbon analyses that the radiopurity of the [^{14}C]DES implant decreased with time. One implant that was included in the 130-day implant form group was actually maintained in implant form for 158 days. The amount of DES measured in this implant by GLC was equal to the amount of DES calculated on the basis of radiocarbon analysis and specific activity of the [^{14}C]DES used in the implant formulation. The average radiopurity of 98.3% was similar to the radiopurity determined by the supplier of the crystalline [^{14}C]DES and by Aschbacher et al. (1975).

TLC Analysis. A summary of the R_f values for the various radioactive zones of the TLC plates for the three TLC systems is shown in Table III. In all cases, a similar array of zones were obtained for the [^{14}C]DES standard that was maintained from the time of synthesis in dilute benzene solution as for the dissolved implants.

Some of the radioactivity data were combined for the purpose of clarity in summarizing the data by combining data of the individual zones that were not significantly or consistently affected by days in situ or days in the implant form. Results for the first TLC system are shown in Table IV. Data identified as origin in the table include radioactivity from three zones, that which remained at the original zone of sample application plus radioactivity that appeared to move as *cis*- and *trans*-DES zones during the first phase and remained at the origin during the second phase. In all the systems, *cis*-DES migrated slower than *trans*-DES. Four major zones of radioactivity for the first system were those that moved like the major zones of standard DES during both phases. The four major zones probably result from the isomerization of DES in solvent (Ferrando and Renard, 1968; Winkler et al., 1971); the cis and trans zones separated during the first TLC phase each isomerized to cis and trans during the second TLC phase. The radioactivity from these four major zones was combined as DES for summary purposed. A small zone of radioactivity migrated slower than *cis*-DES and this zone was larger at 130 days in situ than at 102 and 67 days. This difference may represent a product of slow autodegradation. One distinct zone migrated between *cis*- and *trans*-DES and may correspond to the zone identified as pseudodiethylstilbestrol by Tennent et al. (1976). Four zones of radioactivity migrated faster than *trans*-DES and these were combined.

Table V. Distribution of Radiocarbon from Unabsorbed [^{14}C]DES Ear Implants on Two-Dimensional TLC Plates with Hexane-Ethyl Ether-Methylene Chloride and Chloroform-Ethanol as Mobile Phases

conditions	no. of implants	visualized radiocarbon zones[a] origin	DES	$R_f <$ *cis*-DES	R_f between *cis*- and *trans*-DES	$R_f >$ *trans*-DES
days in situ						
30	4	16.0	49.4	0.6	17.3	16.7
60	4	3.8	61.1	0.8	22.2	12.3
90	4	15.1	55.1	0.8	14.4	14.7
120	3	18.2	47.7	0.8	15.0	18.1
$S\bar{x}$		6.1	2.4	0.2	2.3	1.8
days in implant form						
67	6	6.7	56.7	0.8	21.2[b]	14.7
102	4	15.7	53.6	0.7	16.1[b,c]	13.9
130	5	18.2	51.2	0.9	13.7[c]	17.1
$S\bar{x}$		4.4	4.4	0.2	1.6	2.2
av of all implants		12.9	53.7	0.8	17.4	15.3
standard		11.9	57.9	0.5	11.8	17.9

[a] Data are expressed as the percentage of total radioactivity on the TLC plates. [b,c] Time means that do not have a common letter in their superscript are different at $P < 0.05$.

Table VI. Distribution of Radiocarbon from Unabsorbed [^{14}C]DES Ear Implants on Two-Dimensional TLC Plates with Ethyl Acetate-Aqueous Hexane-Ethanol and Hexane-Ethyl Ether-Methylene Chloride as Mobile Phases

conditions	no. of implants	visualized radiocarbon zones[a] origin	DES	$R_f <$ *cis*-DES	$R_f >$ *trans*-DES
days implanted					
30	2	5.0	82.3	7.6	5.2
60	3	2.8	86.6	6.6	4.0
90	2	1.4	79.7	8.0	8.6
120	2	2.7	83.0	8.7	5.7
$S\bar{x}$		1.3	3.7	0.7	1.6
days in implant form					
67	4	3.9	85.0	7.1	4.1
102	2	2.4	78.0	8.0	9.2
130	3	2.0	84.6	8.0	5.4
$S\bar{x}$		1.2	2.8	0.7	1.1
av of all implants		2.9	83.3	7.6	5.6
standard		5.7	78.9	11.3	4.1

[a] Data are expressed as the percentage of total radioactivity on the TLC plates.

Thirteen visible zones of radioactivity were found in this study for the first system. Only nine zones were obtained by Tennent et al. (1976) who used a similar system. This inconsistency in the number of zones may be caused by factors that affect method sensitivity such as the amount of radioactivity applied to TLC plates and the length of time that X-ray film is exposed to TLC plates. Tennent et al. (1976) did not state the amount of radioactivity applied but stated that exposure time was variable (2 to 5 days). In our laboratory, shortening the exposure time and, particularly, reducing the amount of radioactivity applied to the TLC plates reduced the number of radioactive zones that were visible in the autoradiogram.

The results of the second TLC system, which was hexane–ethyl ether–methylene chloride and chloroform–ethanol are shown in Table V. Except for overall differences in migration rates of radioactive zones, the array of zones visible with this system was similar to that with the first system. The major difference between the first and second systems for both implants and standards was that with the second system, less radioactivity was associated with the DES zones and more with the zones that moved intermediate to and faster than *cis*- and *trans*-DES and more radioactivity remained at the origin. The zone of radioactivity that moved more slowly than *cis*-DES moved did not increase as days in implant form increased; however, there appeared to be an increase in the radioactivity at the origin as days in implant form increased. The zone intermediate to *cis*- and *trans*-DES decreased ($P < 0.05$) as days in implant form increased.

The results of the third TLC system, which was ethyl acetate–aqueous hexane–ethanol and hexane–ethyl ether–methylene chloride, are shown in Table VI. This system gave a pattern of radioactive zones dissimilar to the patterns for both the first and second systems. Except for a small zone of radioactivity that remained at the zone of sample application, all the radioactivity moved as one zone during the first phase and then as four zones during the second phase. There was no relationship between the amount of radioactivity in any zone and the number of days in situ or days in implant form.

In general, the three different TLC analyses and the GLC analysis gave evidence, based on no apparent analytical changes with time, that initial radiopurity of the [^{14}C]DES implants was maintained during the implant study of Rumsey et al. (1975). Self-decomposition of the [^{14}C]DES was not apparent unless self-decomposition was followed by immediate absorption of the labeled decomposition product while in situ. The TLC analyses showed marked differences in the qualitative results obtained by different TLC systems. It is not known whether the appearance of several radioactive zones was due solely to initial impurities or to the interaction of DES with the TLC systems. However, these differences demonstrate limitations in the use of TLC for radiopurity evaluation of [^{14}C]DES.

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Determination of *N*-Nitrosoproline in Meat Samples

A liquid chromatographic method using a thermal energy analyzer detector for the determination of *N*-nitrosoproline in processed meat samples was studied. The method was shown to have sufficient accuracy and moderate precision in spiked meat samples containing 100 ppb *N*-nitrosoproline. A number of commercial meat samples were also examined and were found to contain very low levels of *N*-nitrosoproline in some samples.

It has long been known that simple nitroso amino acids such as *N*-nitrosoproline (NPRO) can be decarboxylated by heating in dilute alkali to produce the corresponding nitrosamine (Lijinsky et al., 1970). It has also been shown that raw bacon contains only very small quantities of the extremely potent carcinogen, *N*-nitrosopyrrolidine, but when it is fried, considerably higher levels of *N*-nitrosopyrrolidine can be formed from the decarboxylation of NPRO (Pensabene et al., 1974; Kushnir et al., 1975; Warthensen et al., 1976). NPRO has been isolated and identified in bacon at an estimated level of 1 μg/g (Kushnir et al., 1975).

Though the formation of NPRO in nitrite cured meat products is of considerable interest, work in the area has been hampered by a lack of an analytical method that is sufficiently sensitive (ca. 10–100 ng/g) and selective. The gas chromatographic determination of NPRO as a methyl ester derivative (Wolfram et al., 1977) and as a trimethylsilyl derivative (Eisenbrand et al., 1975) have been reported; however, the methods have not been applied to meat samples or other complex systems. A semiquantitative TLC method has also been demonstrated to be useful for commercial bacon containing NPRO (Sen et al., 1977); however, the method requires a lengthy clean-up procedure and has a detection limit of only 100 ng/g.

The analysis of nonvolatile nitrosamines in food products using a thermal energy analyzer (TEA) and high-pressure liquid chromatography has recently been demonstrated (Fine et al., 1976). The TEA detector is useful for analysis of nitrosamines in the 10 μg/mL range (prior to sample concentration), and it is fairly selective for compounds containing the *N*-nitroso group.

The major efforts of the work described in this report were directed toward the development of a high-pressure liquid chromatographic method using the TEA detector for the determination of NPRO in the 10–100 ng/g range in meat samples. In addition to demonstrating the selectivity, precision, and accuracy of the method with spiked beef samples, the method was also used for the analysis of several commercial nitrite preserved meat samples.

MATERIALS AND METHODS

LC and Detector Operating Conditions. The principle of operation of the TEA detector (Fine et al., 1975) and application as a detector in LC analysis (Fine et al., 1976) has been previously reported. The detector unit used in this study was a prototype to Model TEA 502/LC produced by the Thermo Electron Corporation, and it was essentially the same as the units described in the earlier literature. The furnace consisted of a nonglazed ceramic tube ($^1/_8$ in. i.d. × 22 in.); however, it did not contain tungsten oxide powder as described in the earlier report (Fine et al., 1975). The response of the detector varied with pyrolysis temperature and with the condition of the apparatus. The largest response was obtained at temperatures over the range 350–400 °C, but was variable. The flow of oxygen to the ozone generator was adjusted to increase the pressure in the chemiluminescence chamber by 1.0 Torr. Nitrogen was used to purge the solvent cold traps at a flow rate of 50 mL/min (STP), and the cold traps were maintained at 0 °C.

A Waters Associates Model 202 LC equipped with a U6K injector was used. The reverse-phase chromatograms were obtained using a 3.9 × 300 mm μ-Bondapak-C18 column, and 5% v/v glacial acetic acid in water was used as the mobile phase (2.0 mL/min).

Chemicals. *N*-Nitroso-L-proline (mp 104–106 °C, lit. 97.5–98 °C) and *N*-nitrosopipecolic acid (mp 92–93 °C, lit. 91–92 °C) were prepared through the nitrosation of L-proline and racemic pipecolic acid with sodium nitrite (Lijinsky et al., 1970). Vitamin-free casein was used as supplied by Fisher Scientific Co. and the acetonitrile was freshly distilled. All other chemicals and solvents were of standard reagent grade.

General Extraction Procedure. A 20-g meat sample was weighed (20 g of vitamin-free casein for control sample) and transferred to a Waring blender containing 20 mL of water, 0.2 g of sulfamic acid, and 20 mL of 25%

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