Table I. Recovery of Methyl Parathion by Acetone-o-Xylene (19:1) Extraction from Samples of Honeybees, Beeswax, and Pollen Spiked with Penncap-M

	mean % recovery (N = 3)		
concn, ppm	honeybees	beeswax	pollen
0.1	97.3	95.4	91.7
0.5	95.4	102.1	94.3
1.0	98.8	98.7	93.5
5.0	95.6	98.9	98.6
10.0	99.3	96.7	95.1
25.0	101.3	95.1	91.9
mean	98.0	97.8	94.2

by comparison to an external standard on a Hewlett-Packard 3380A integrator.

RESULTS AND DISCUSSION

The mean percentage recoveries of methyl parathion from triplicate analyses of honeybees, beeswax, and pollen spiked with Penncap-M and measured by the acetone–o-xylene (19:1) extraction procedure are listed in Table I. Mean recoveries for the six concentrations of Penncap-M were 98.0, 97.8, and 94.2% from honeybees, beeswax, and pollen, respectively. Triplicate honey samples were seeded at 0.1, 0.5, 1.0, 5.0, and 10.0 ppm of Penncap-M, and mean methyl parathion recovery utilizing this method was 91.7, 85.6, 87.2, 86.7, and 85.2%, respectively. When this method was used for analyses of honeybees, beeswax, and pollen exposed to Penncap-M in the field, quantitation at 1.0 ppb was achieved (Ross and Harvey, 1981).

This improved method for analysis of methyl parathion residues can be used for quantitating Penncap-M in contaminated honeybees, beeswax, and pollen.

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A Comparison of High-Performance Liquid Chromatography and Proton Nuclear Magnetic Resonance in Determining the Phosphatidylcholine Content in Soy Lecithin

A study was conducted comparing HPLC and ^{1}H NMR as methods for determining the phosphatidylcholine content in soy lecithin. The HPLC method employs detection of the phosphatidylcholine at 210 nm while the ^{1}H NMR utilizes the resonance of the choline at δ 3.3. Both methods gave similar precision data with good correlation between methods.

Lecithin is a generic name for an emulsifier and surface active agent derived from many sources. The highest lecithin content is in egg yolk with 8–10% generic lecithin while soybean oil contains ~2.5% generic lecithin. Soy lecithin consists of three major phosphatides (Minifie, 1980): ~20% phosphatidylcholine, 20% phosphatidylethanolamine, and ~20% phosphatidylinositol.

The analysis of lecithin has usually been performed by the method of acetone insolubles (Horwitz, 1975).

This paper reports the comparative analysis of one of the phosphatides in soy lecithin using HPLC and ^{1}H NMR.

EXPERIMENTAL PROCEDURES

HPLC Analysis. The HPLC conditions were those specified in earlier studies (Hurst and Martin, 1980).

^{1}H NMR Analysis. The ^{1}H NMR consisted of a Varian T-60 NMR. Fifty milligrams of sample or standard was dissolved in 500 μL of acetic acid-d_4. Figures 1 and 2 show the ^{1}H NMR spectra of phosphatidylcholine standard and lecithin extract. The peak at δ 3.3 is the phosphatidylcholine peak, and the integration of the standard vs. in-

Table I. Percent Phosphatidylcholine in Different Lecithin Lots[a]

lot no.	HPLC	^{1}H NMR
1	27.88	25.84
2	24.70	23.03
3	23.91	22.47
4	27.31	24.72
5	26.43	24.16
6	28.67	25.84
$\bar{x}$	26.49	24.34
Cv	6.9	6.7

[a] That is, same type but differing lot numbers.

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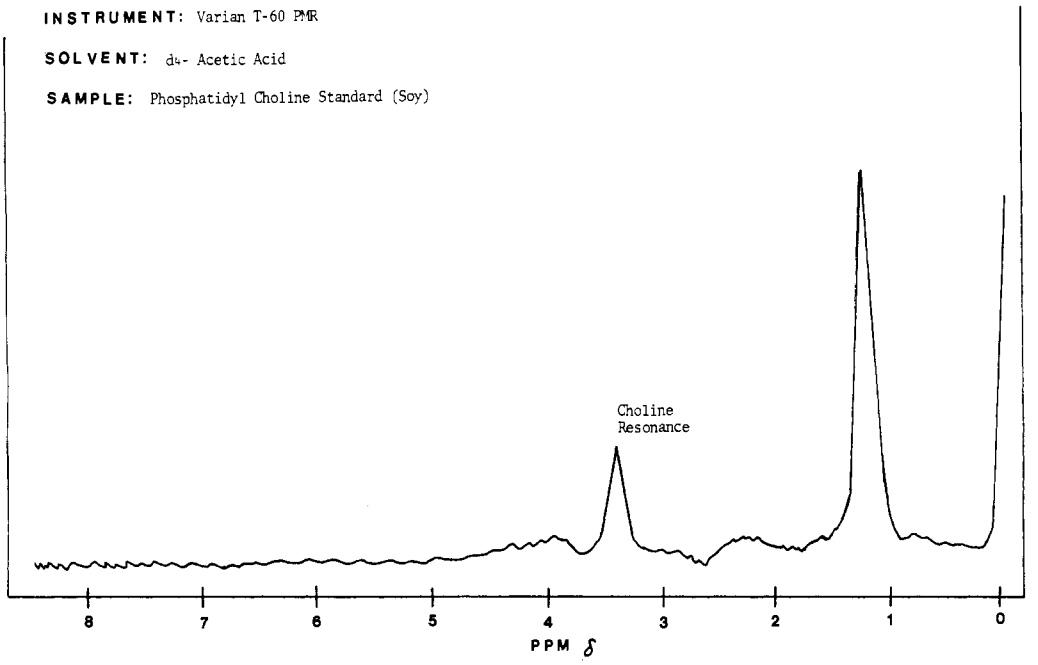

Figure 1. ^{1}H NMR spectrum of phosphatidylcholine standard (soy).

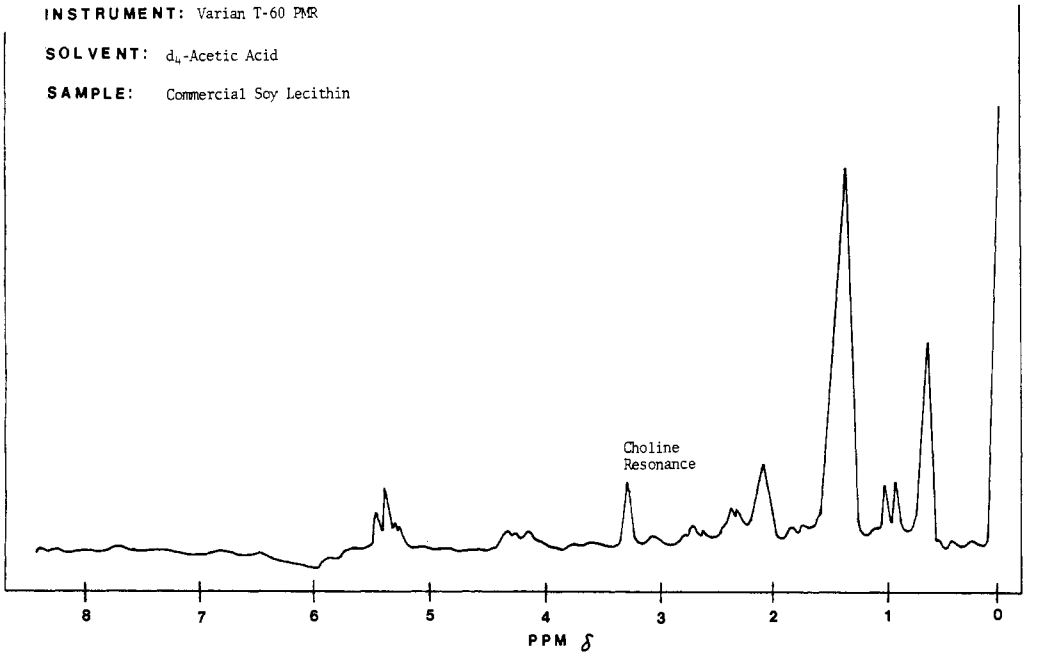

Figure 2. ^{1}H NMR spectrum of commercial soy lecithin.

tegration of the sample was used to determine percent phosphatidylcholine in various soy lecithins. Instrument settings were the same for all portions of the analytical run.

Samples and Standard. The standard was L-α-lecithin from soybeans (Sigma Chemical Co. or Calbiochem-Behring). Different lots of soy lecithin were obtained compliments of A. E. Staley Manufacturing Co. and Central Soya Manufacturing Co.

RESULTS

Precision studies for the HPLC method indicate a coefficient of variation (Cv) of less than 7% (Hurst and Martin, 1980) as do precision studies for ^{1}H NMR. Table I shows the choline content for six different lots of soy lecithin when HPLC and ^{1}H NMR are used. Table II shows the phosphatidylcholine content for five different varieties of lecithin by using the two techniques. The correlation between the two techniques can be seen by regression coefficients of 0.98 in Table I and 0.99 in Table II.

Table II. Percent Phosphatidylcholine in Different Lecithin Types

lecithin type	HPLC	^{1}H NMR
A	15.03	15.30
B	28.16	25.00
C	23.91	19.30
D	34.41	30.68
E	22.95	20.45

CONCLUSION

This study shows the comparison of two independent techniques for the analysis of phosphatidylcholine in soy

lecithin. Good agreement can be seen between the two techniques.

Additionally, the ^1H NMR technique could offer an important and easy check of the phosphatidylcholine moiety of commercial lecithins.

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Formation of *N*-Nitrosoproline by Reacting Nitrite with L-Citrulline and L-Arginine

It was found that *N*-nitrosoproline (NPro) was formed from either L-citrulline or L-arginine when these amino acids were reacted with nitrite under simulated human stomach conditions. The determination and confirmation of the formed NPro were carried out after converting NPro to its methyl ester. This derivative was analyzed by gas chromatography and gas chromatography–mass spectrometry. The yield of NPro from L-arginine was 0.1% and that from L-citrulline was 27.1%.

Many *N*-nitroso compounds are known to be carcinogenic to various experimental animals, while NPro has been reported to be noncarcinogenic (Magee and Barnes, 1967; Druckrey et al., 1967). However, NPro can be converted to carcinogenic *N*-nitrosopyrrolidine by decarboxylation occurring at higher cooking temperatures (Lijinsky and Epstein, 1970).

As to the formation of *N*-nitroso compounds from amino acids reacted with nitrite under acidic conditions, Warthesen et al. (1975) reported that NPro was formed from ornithine, and Mirvish (1971) reported that *N*-nitrosocitrulline and *N*-nitrosoarginine were formed from L-citrulline and noncarcinogenic to experimental animals. The present study revealed that NPro can be formed from either L-citrulline or L-arginine by nitrosation under simulated human stomach conditions.

EXPERIMENTAL SECTION

Reagents. L-Arginine and L-citrulline were of reagent grade (Wako Chemicals Co.). Both compounds were assayed for purity by using an amino acids analyzer (Hitachi Model LLA-5), and only trace quantities of less than 0.01% proline and ornithine could be detected. NPro was synthesized by nitrosation of proline according to the Lijinsky method (Lijinsky and Epstein, 1970).

Reaction Conditions and Extraction of NPro. Nitrosation of the test amino acids was carried out in a pH 1.2 acetate–hydrochloric acid buffer solution prepared by mixing 1 M sodium acetate and 1 N hydrochloric acid. L-Arginine, L-citrulline, and sodium nitrite were separately dissolved in the pH 1.2 buffer solution, and then the solutions were readjusted to pH 1.2 with 6 N hydrochloric acid. Five milliliters of each amino acid and nitrite solution was transferred to a 20-mL glass-stoppered test tube and thoroughly mixed. Then the mixture was incubated in a 37 °C water bath for 2 h. The concentration of each amino acid in the final reaction mixture was 0.03 M, and that of sodium nitrite was 0.3 M. The reaction was stopped by addition of 0.1 mL of 10% ammonium sulfamate to the mixture. The reacted mixture was transferred to a separatory funnel, and 2–3 g of sodium chloride was added. The formed NPro was extracted 2 times with 20 mL of ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and transferred to a 100-mL round-bottom flask, followed by evaporation in a rotary evaporator to dryness.

Methylation of NPro with Diazomethane. A few milliliters of diazomethane-saturated diethyl ether was added to the dried reactants containing NPro. This is allowed to stand for 30 min at room temperature, and then it was concentrated to 1 mL under a gentle stream of nitrogen (Ishibashi et al., 1980). The derivative was analyzed in a gas chromatograph equipped with an alkali flame ionization detector (GC-AFID), and it was further identified by gas chromatography–mass spectrometry (GC–MS). The fragments observed in the authentic NPro methyl ester were m/e 128, 99, 69, and 68.

Operating Conditions for GC-AFID and GC–MS. A Shimadzu GC-4BF gas chromatograph equipped with an AFID was used for the analysis of NPro methyl ester. A glass column (2 m × 3 mm i.d.) packed with 3% DEGS–0.5% H_3PO_4 on Chromosorb W (60–80 mesh) pretreated with hexamethyldisilazane was employed. The carrier gas was nitrogen at a flow rate of 40 mL/min, and the temperatures of column oven and the detector were 170 and 200 °C, respectively. A shimadzu-LKB 9000 gas chromatograph–mass spectrometer was used for the GC–MS analysis of NPro methyl ester. A glass column (2 m × 3 mm i.d.) packed with the same material as employed for the GC-AFID analysis was used. The carrier gas was helium, at a flow rate of 30 mL/min. The temperatures of the injection port and column oven were 240 and 180 °C, and those of the separator and ion source were 250 and 260 °C, respectively. The electron energy was 20–70 eV. The accelerating voltage was 3.0 kV, and the trap current was 60 μA.

RESULTS AND DISCUSSION

We found that NPro was formed when either L-arginine or L-citrulline was reacted with nitrite at pH 1.2 and 37

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