Gas Chromatographic, Infrared, Proton Magnetic Resonance, Mass Spectral, and Threshold Analyses of All Pentyl Acetates

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The eight pentyl acetate isomers and cyclopentyl acetate, obtained from commercial sources or by synthesis, were purified by preparative gas chromatography, and purity of each sample was determined by high resolution analytical gas chromatography. The infrared, proton magnetic resonance, and mass spectra of each of the nine compounds were recorded, and samples of each were subjected to organoleptic evaluation for threshold and discrimination determinations.

THE ubiquity of esters in fruit volatiles, the difficulty of separation of some isomers, and the lack of spectroscopic data and of organoleptic description have prompted us to investigate in detail the separation and analysis of all the pentyl acetates. PMR spectra are especially valuable in confirming structures and indicating purity, and probably such data on the complete pentyl acetate series have not been published because spectroscopically pure samples have not been available. Some mass spectra data have been presented (*5*) [especially the elegant high resolution work by Beynon (*1*) should be mentioned], but not all of the isomers have been investigated. Therefore, the spectroscopic data of all the pentyl acetate isomers presented here should be useful for identification purposes in the composition studies of plant volatiles.

Experimental

Pentyl Acetates. Because of the difficulty of separating 2-methyl-1-butyl from 3-methyl-1-butyl, either as alcohols or as acetates, these acetates were synthesized from the corresponding acids. The *p*-bromophenacyl derivatives of the commercially available acids were recrystallized at least three times before they were reduced to the corresponding alcohols with lithium aluminum hydride. All acetates were formed by the acetic anhydride–pyridine method. Thus, with the crystallization purification of the acid derivatives, spectroscopically pure 2-methyl-1-butyl and 3-methyl-1-butyl acetates were obtained. All other commercially available alcohols were pure enough to yield spectroscopically, greater than 98%, pure acetates after preparative GC of the alcohols, then the acetates. The sources of the acetates are listed in Table I.

Gas Chromatography. For crude separations, preparative gas chromatographs made in our laboratory were used, with 30-foot, 0.5-inch i.d. aluminum tubing columns packed with 60- to 70-mesh Chromosorb G impregnated with 4% SF 96(50) silicone oil or with 4% Carbowax 20M. For final difficult separations and for retention time analyses, large-bore open stainless steel tubes, 500-foot, 0.02-inch i.d., and 1000-foot, 0.03-inch i.d. coated with SF 96(50) silicone oil or with Carbowax 20M, were used (*6*).

Infrared Spectrometry. The infrared spectra were obtained with a Beckman spectrophotometer, Model IR-5. Samples were 10% solutions in carbon tetrachloride in standard 0.1-mm. cells.

Proton Magnetic Resonance. The PMR spectra were obtained with a Varian Associates instrument, Model A-60. Samples were 5 to 10% solutions in carbon tetrachloride in glass spherical microcells (*3*).

Mass Spectrometry. The MS data were obtained with a Bendix Time-of-Flight mass spectrometer, Model 12. Collected samples were analyzed batchwise.

Table I. Sources of the Pentyl Acetates

Acetates	*Sources*
1-Pentyl	Eastman Kodak Co.
2-Pentyl	2-Pentanone, Eastman, reduced, esterified
3-Pentyl	Alcohol, Eastman, esterified
2-Methyl-1-butyl (active amyl)	2-Methylbutyric acid, Eastman → *p*-bromophenacyl derivative, recrystallized 3 times, reduced to 2-methyl-1-butyl alcohol with lithium aluminum hydride → esterified
3-Methyl-1-butyl (isoamyl)	3-Methylbutyric acid, Eastman, treated similarly to 2-methylbutyric acid
3-Methyl-2-butyl	Alcohol, Eastman, esterified
1,1-Dimethyl-1-propyl (*tert*-amyl)	Alcohol, Eastman, esterified
2,2-Dimethyl-1-propyl (neopentyl)	Alcohol, Eastman, esterified
Cyclopentyl	Alcohol, Eastman, esterified

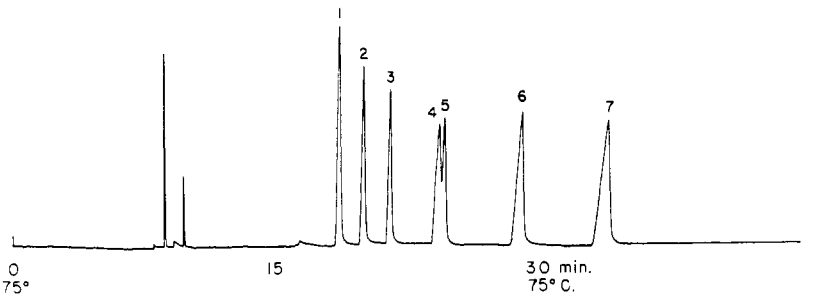

Figure 1. Gas chromatographic analysis of pentyl acetate isomers

Column. 500-foot, 0.02-inch i.d., open tubular, coated with SF 96(50)
Sample volume. 0.2 μl.
Temperatures of injection port and column. 200° and 75° C.
Carrier gas (He) inlet pressure. 20 p.s.i. (gage)
Flow. 6 ml./min.
Peaks (all acetates).
1. 1-Butyl, 2,2-dimethyl-1-propyl, and 1,1-dimethyl-1-propyl
2. 3-Methyl-2-butyl
3. 2-Pentyl and 3-pentyl
4. 3-Methyl-1-butyl
5. 2-Methyl-1-butyl
6. 1-Pentyl
7. Cyclopentyl

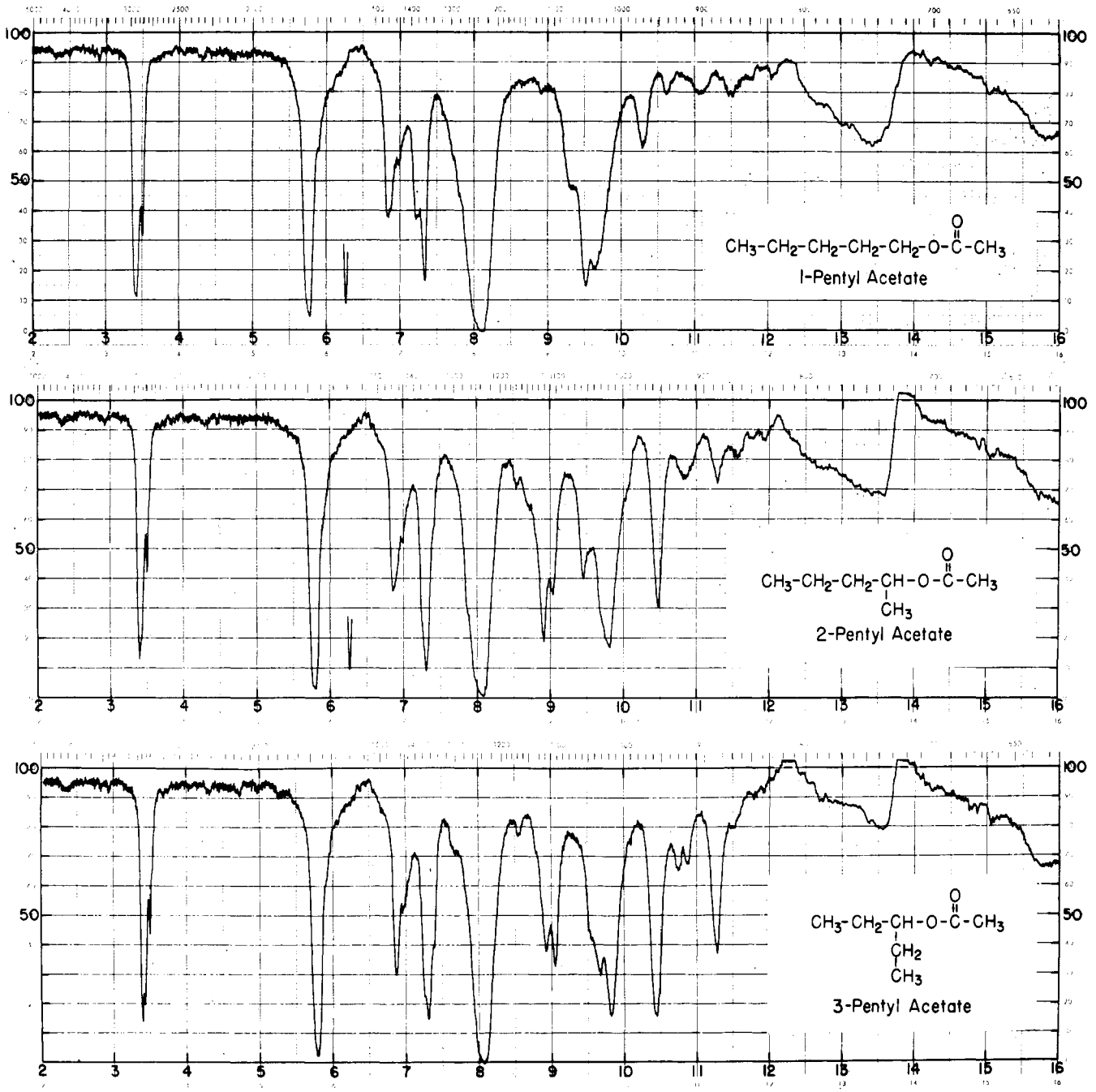

Figure 2. Infrared spectra of pentyl acetate isomers

Odor Discrimination and Threshold Values. Odor discrimination was tested by the well known triangle method. Threshold measurements were made by the same panel and by panel techniques already described (*2*). Each threshold value represents the average of three to six separate determinations, and each determination was made on the basis of 50 to 100 judgments.

Results and Discussion

Figure 1 shows a GC analysis of a mixture of the pentyl acetates with an open tubular column 500-foot, 0.02-inch i.d., coated with SF 96(50) silicone oil. This column has over 100,000 theoretical plates at 125° C. (*6*); at 75° C., over 30,000 theoretical plates. The separation of 3-methyl-1-butyl (isoamyl) acetate (peak 4) from 2-methyl-1-butyl (active amyl) acetate (peak 5) is difficult, even with these high efficiency columns with great resolving power. The resolution was no better with a Carbowax 20M column. Webb and Kepner (*7*) were able to separate the alcohols with a packed column, employing a special

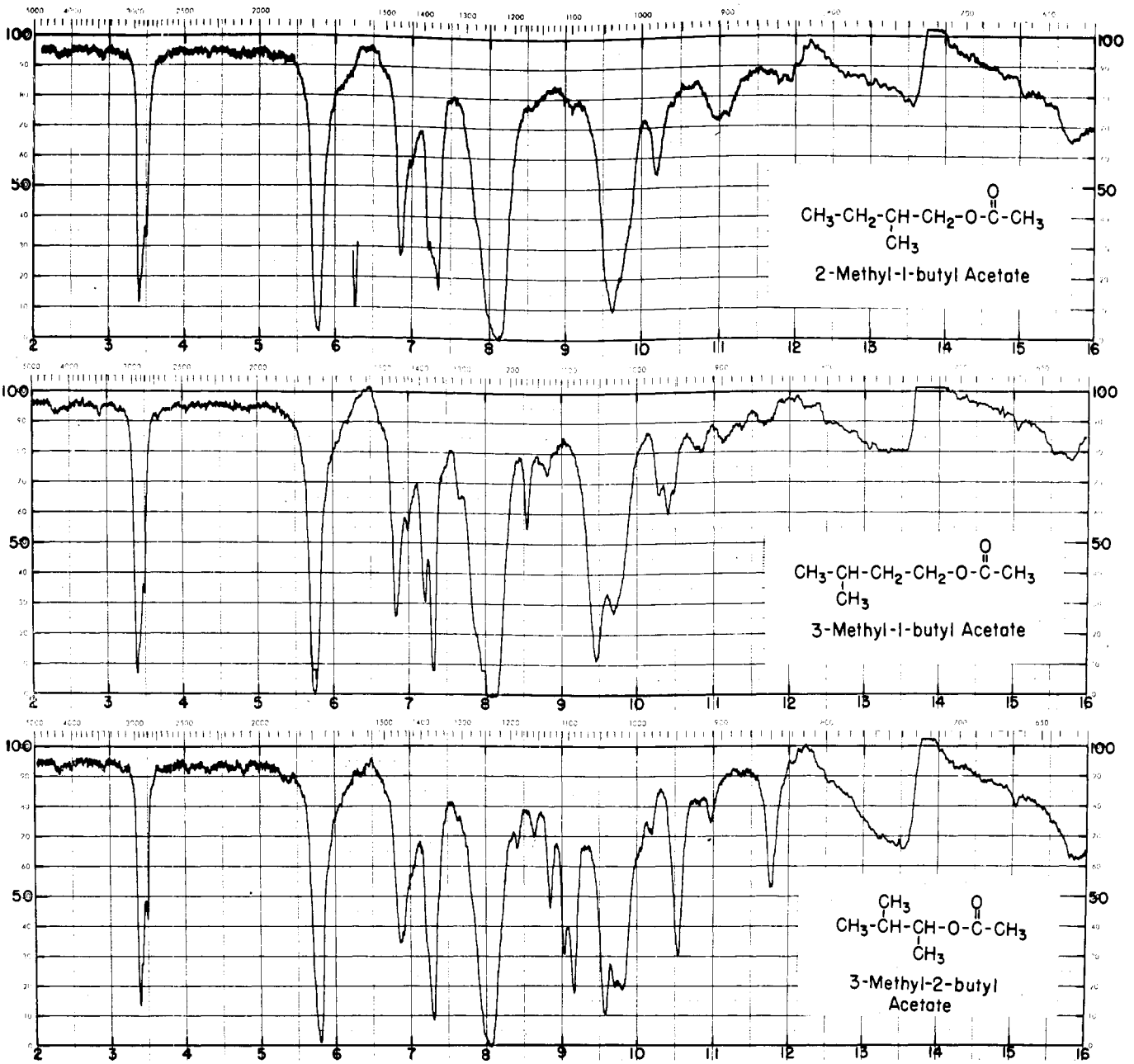

Figure 3. Infrared spectra of pentyl acetate isomers

mixture for the partitioning liquid. This example illustrates the need for different partitioning liquids even with columns having many theoretical plates. Peak 3 represents both 2-pentyl and 3-pentyl acetates. If the temperature of the column is lowered to 50° C., a partial separation of these two isomers is observed. Three acetates constitute peak 1: 1-butyl, 1,1-dimethyl-1-propyl (*tert*-amyl), and 2,2-dimethyl-1-propyl (neopentyl). The difficulty encountered in separating such simple compounds as these acetates illustrates the danger of relying solely upon GC retention time data for structure or purity assignments.

Spectral data of the purified samples are given in Figures 2 to 10. The degree of certainty of identification is good if all spectral data agree.

Figure 11 shows the PMR spectra of mixtures which are predominantly 2-pentyl and 3-pentyl acetates: 2% of 2-pentyl in 3-pentyl, and 20% of 3-pentyl in 2-pentyl. In the 3-pentyl acetate spectrum, the doublet at 8.82 tau, indicated by arrows, from the methyl res-

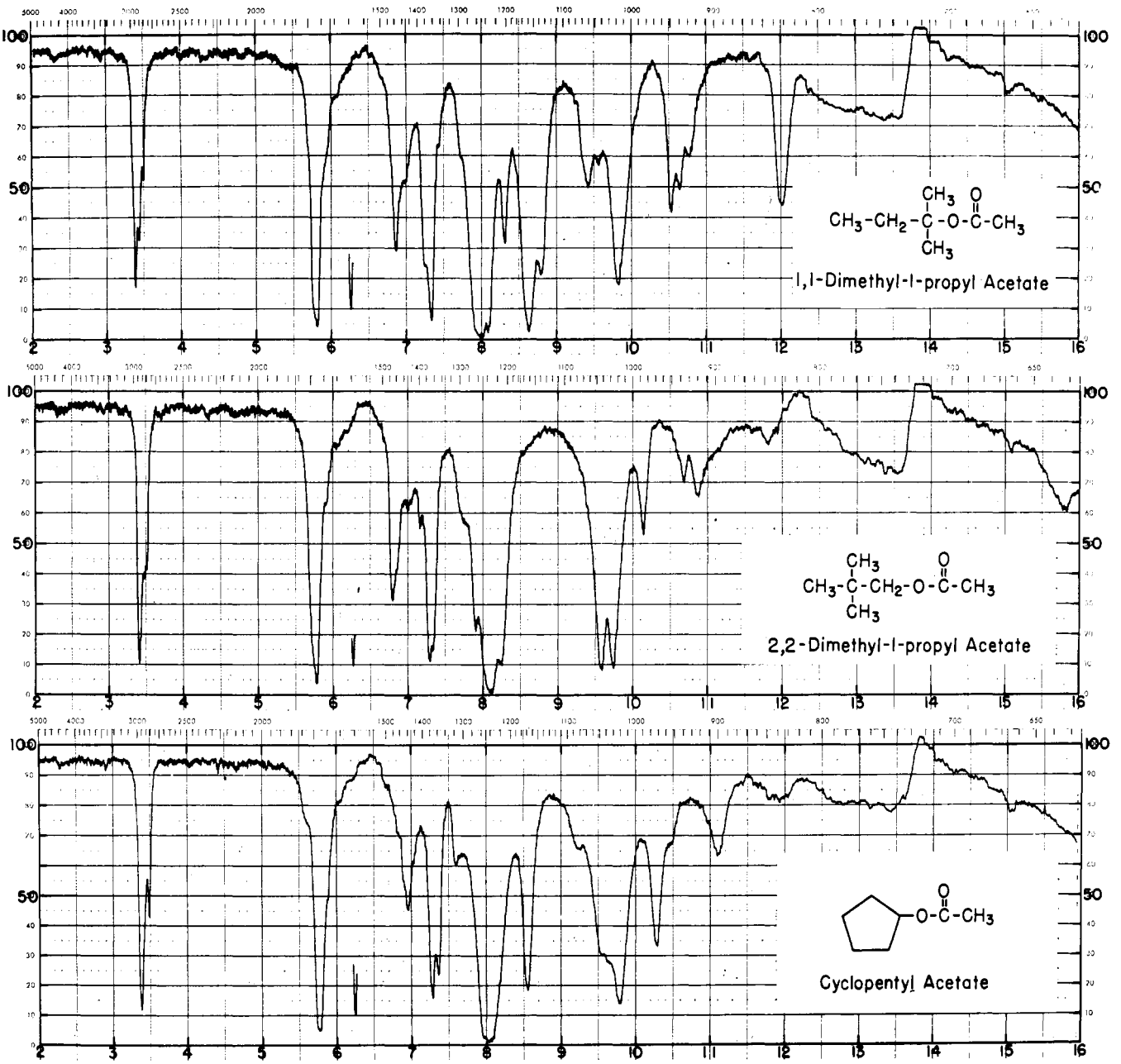

Figure 4. Infrared spectra of pentyl acetate isomers

onance split by the carbinol proton of the 2-pentyl acetate isomer, is clearly seen even as low as 2%, and the various resonances from 3-pentyl acetate are indicated by the arrows in the 2-pentyl acetate spectrum. If the 3-pentyl acetate spectrum were obtained by sweeping in the other direction than is shown, the acetate methyl resonance at 8.05 tau from the 2-pentyl acetate would not be hidden in the "ringing" and would be more prominently shown than the doublet at 8.82 tau. The acetate methyl resonances of these two pentyl acetates differ by 2 cycles (2-pentyl acetate, 117 cycles from TMS; 3-pentyl acetate, 119 cycles from TMS), and these are clearly shown at 8.05 tau in the 2-pentyl acetate spectrum. Inspection of the acetate methyl resonances will permit detection of less than 2% of cross contamination of these two isomers, especially if we consider that the acetate methyl is a singlet, and doublet at 8.82 tau is clearly discernible at the 2% level. Since the combination of these two isomers is the most difficult in which to show cross

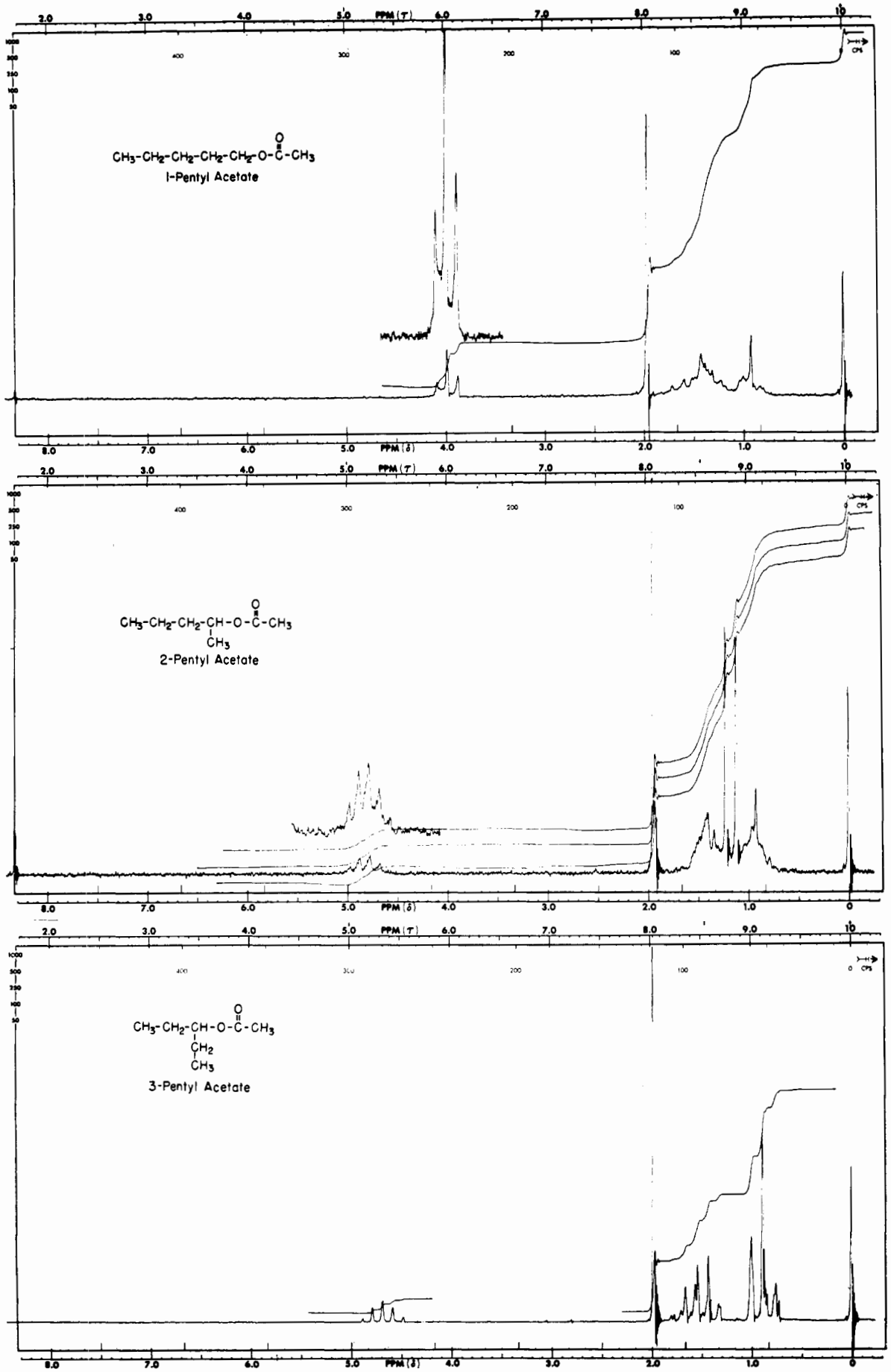

Figure 5. Proton magnetic resonance spectra of pentyl acetate isomers

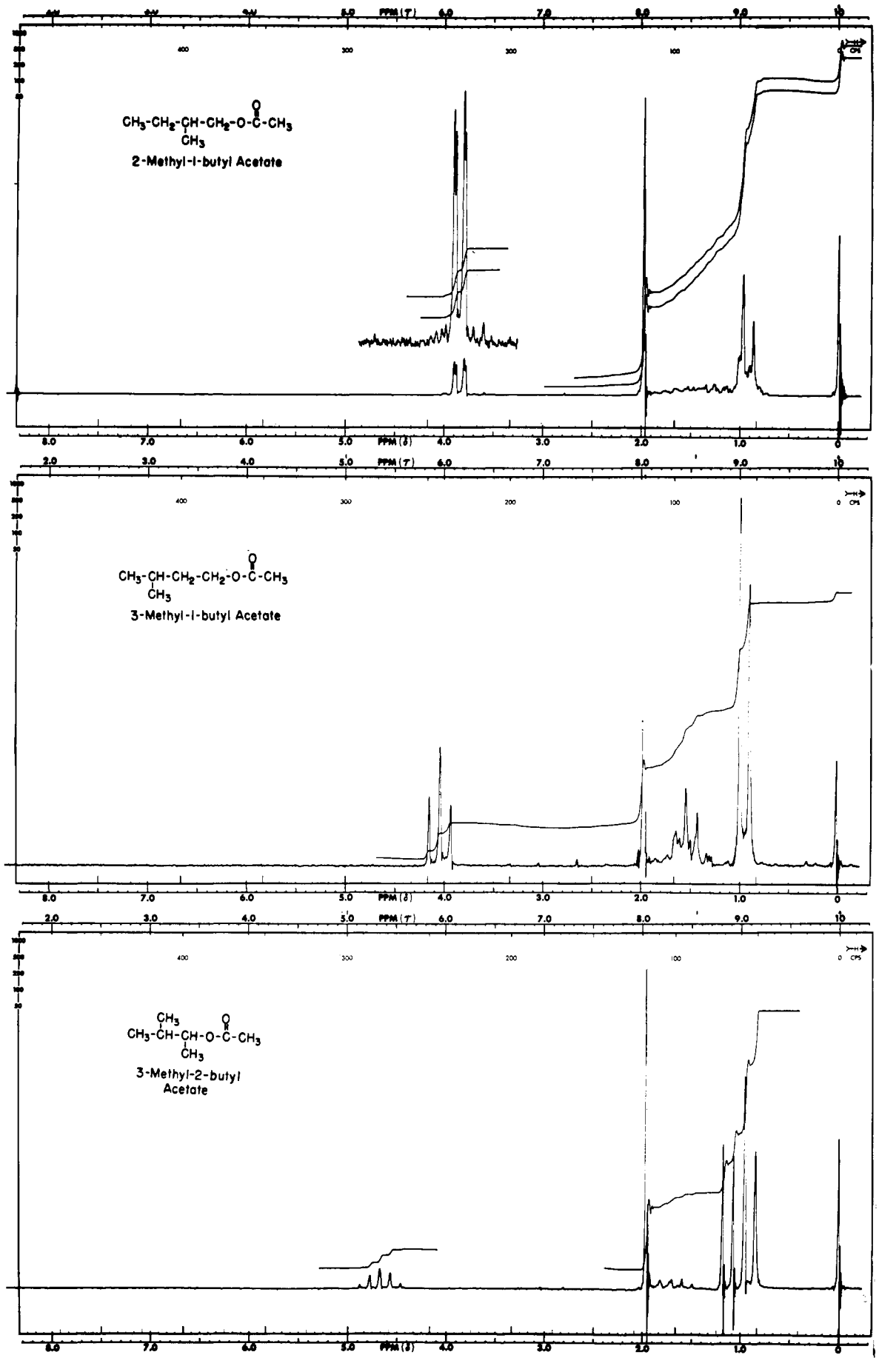

Figure 6. Proton magnetic resonance spectra of pentyl acetate isomers

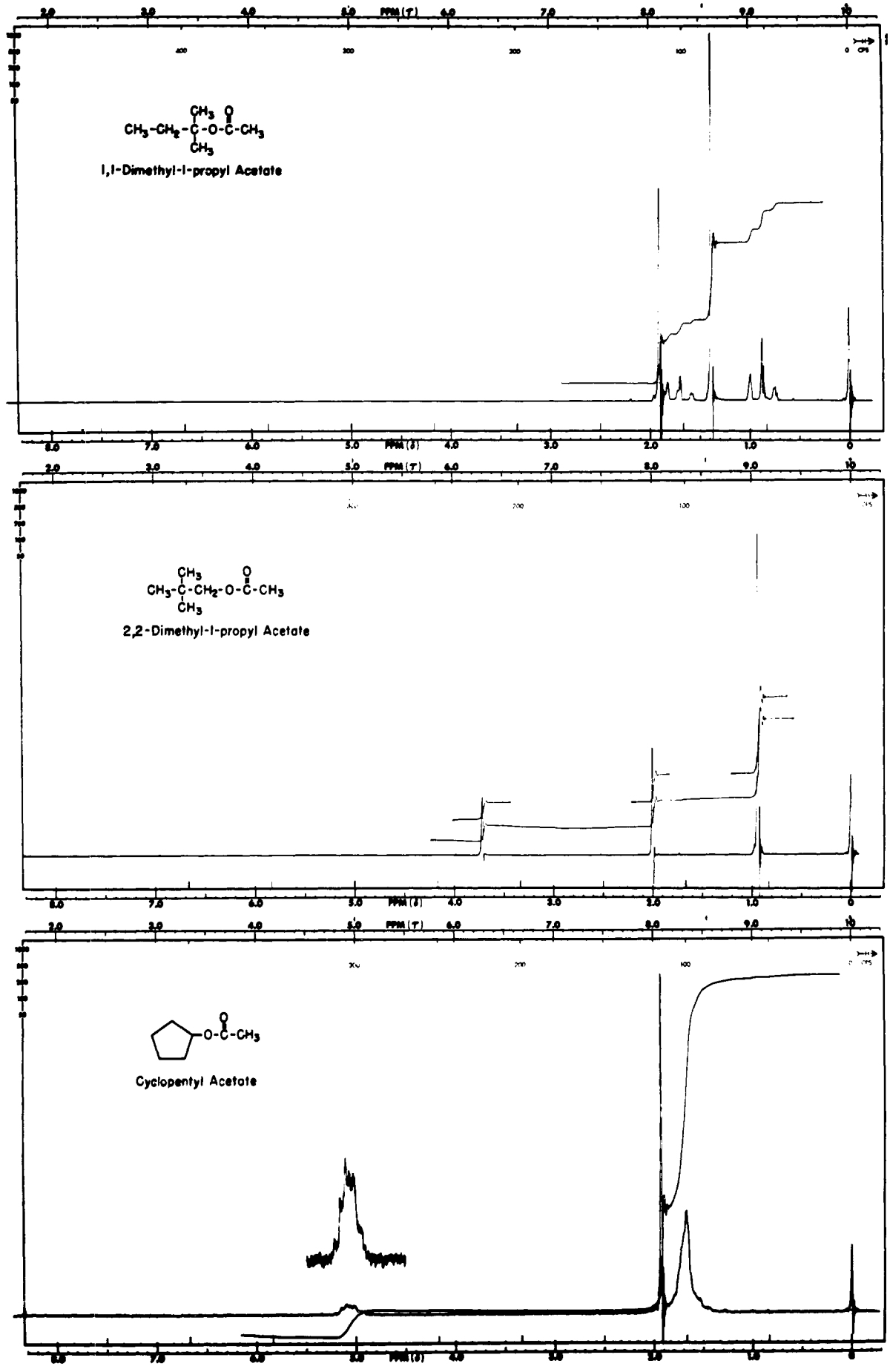

Figure 7. Proton magnetic resonance spectra of pentyl acetate isomers

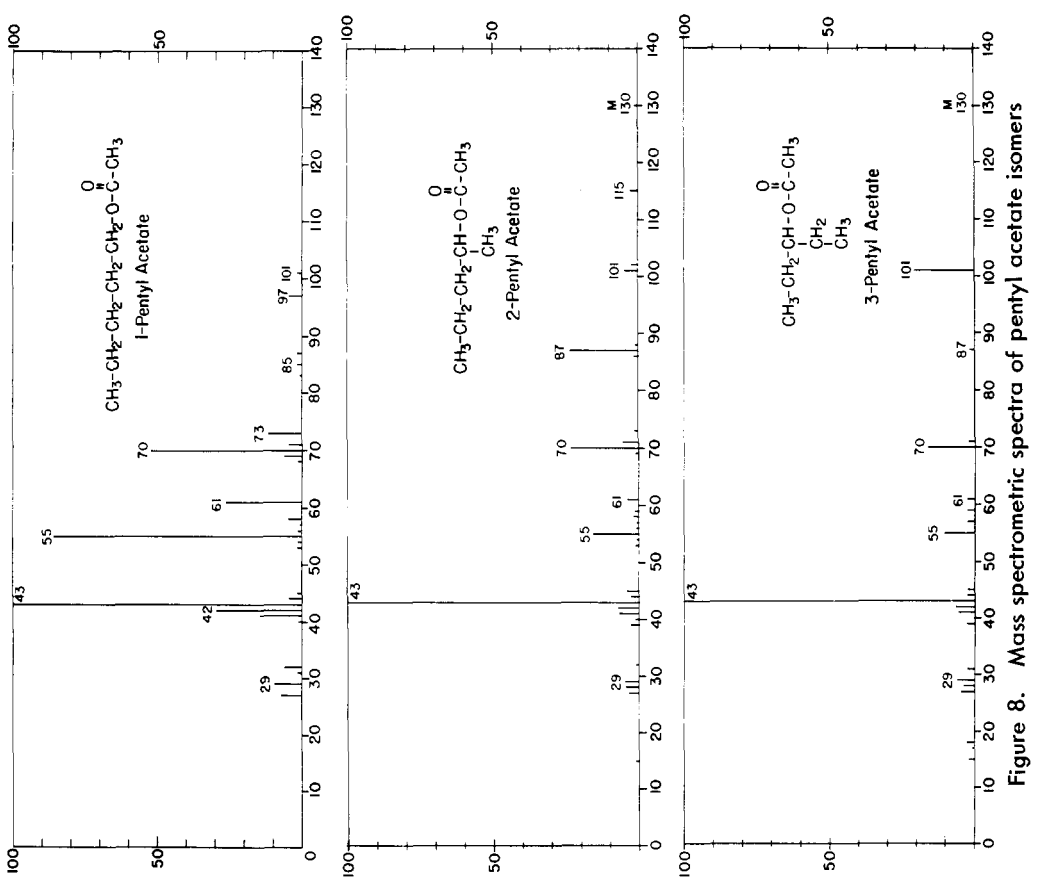

Figure 8. Mass spectrometric spectra of pentyl acetate isomers

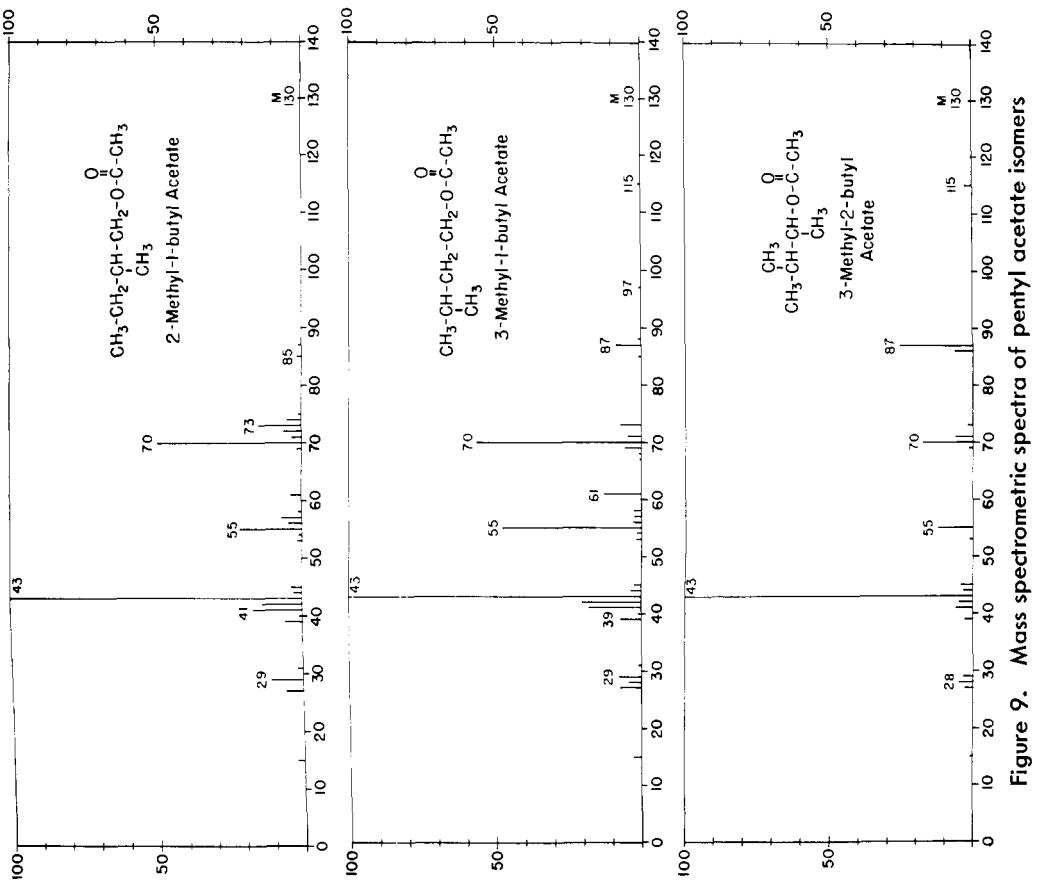

Figure 9. Mass spectrometric spectra of pentyl acetate isomers

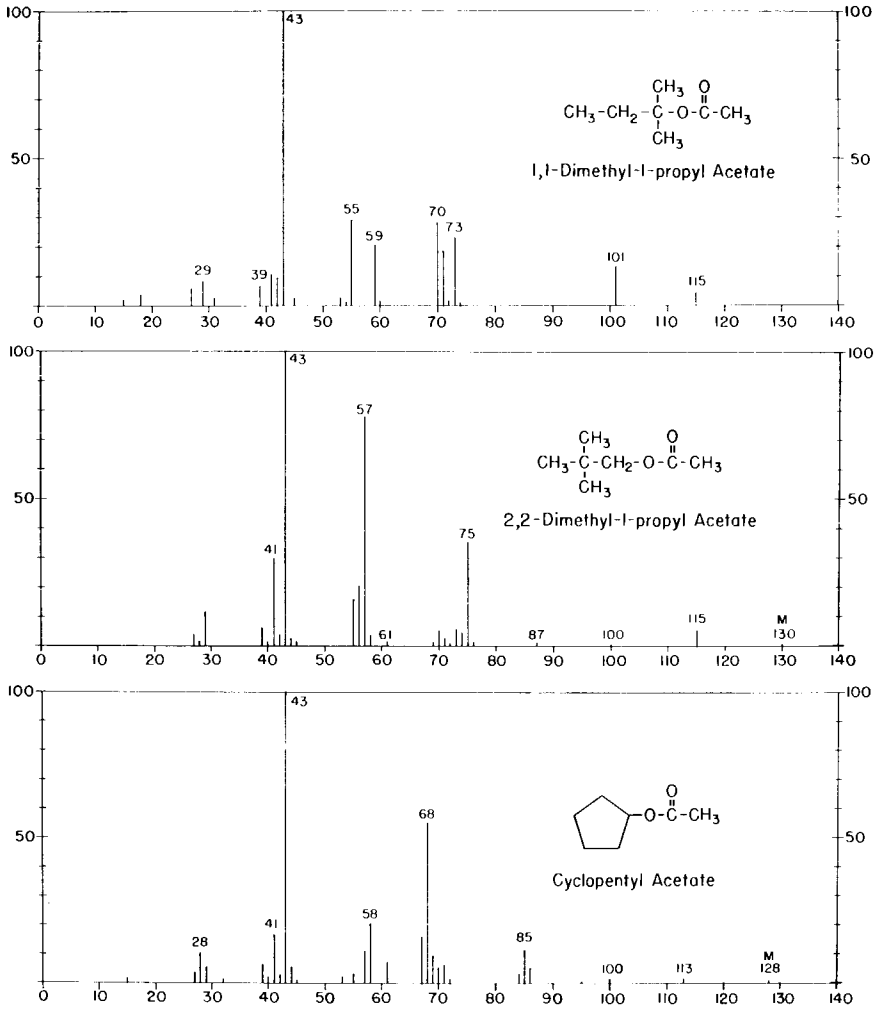

Figure 10. Mass spectrometric spectra of pentyl acetate isomers

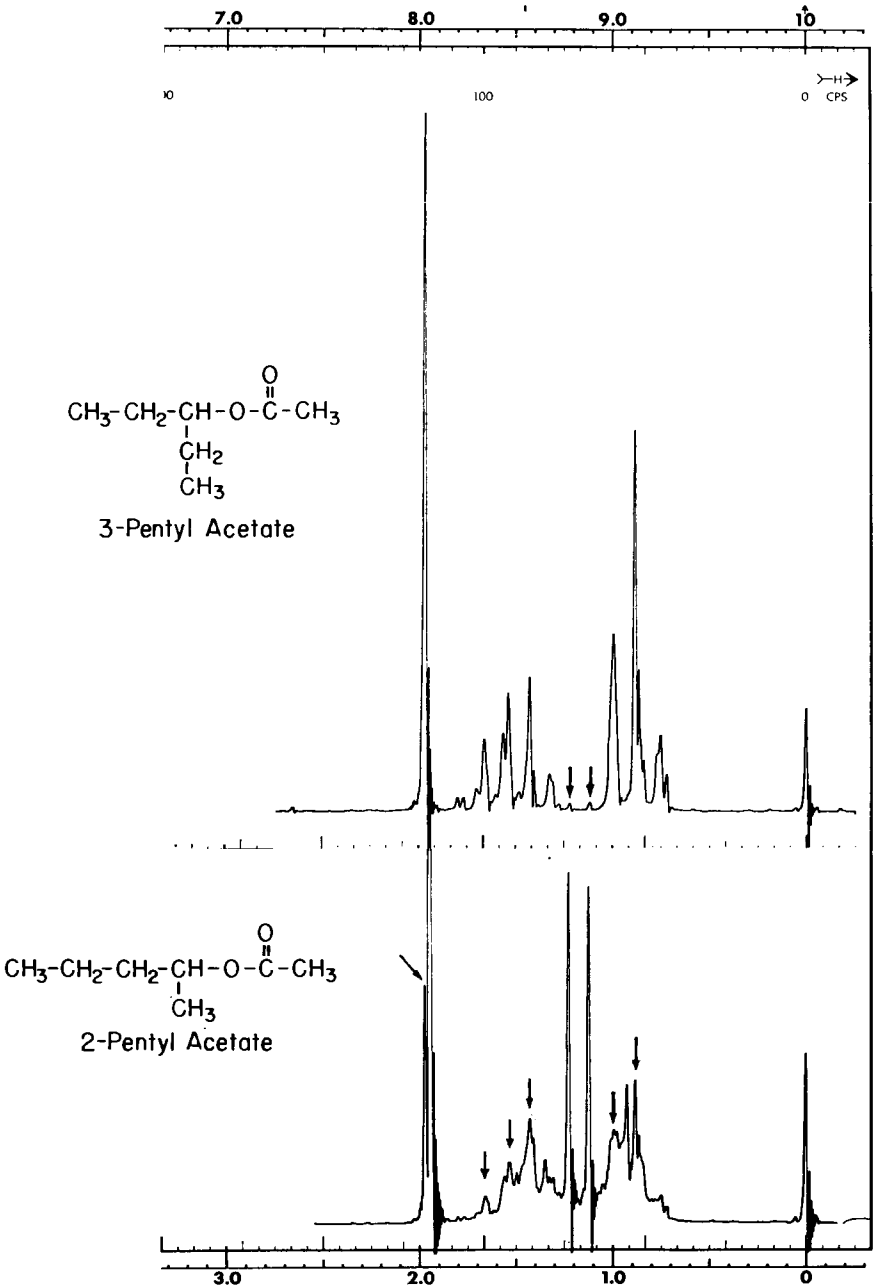

Figure 11. Proton magnetic resonance spectra of 2-pentyl and 3-pentyl acetates

contamination, it is obvious that other cross contaminations can be detected more easily and to lower levels.

High resolution mass spectra of 1-pentyl and 3-methyl-1-butyl acetates have been reported by Beynon (*1*). The specific rearrangements shown in mass spectral analyses of 2,2-dimethyl-1-propyl esters have been reported in detail (*4*).

Table II summarizes the data on threshold values. While definite differences in threshold values or odor pervasiveness were found among some of the acetates, the total range of values is not particularly large. This is especially apparent in comparing these differences with those obtained with different classes of compounds, which can differ by several orders of magnitude. The general similarity of these values for the acetates is not too surprising, considering the fact that all these compounds have the same number of carbon atoms and the same functional group. There does not appear to be any systematic relation between structural differences and threshold values. The differences in odor pervasiveness reported here could be due to slight traces of impurities undetectable by instrumental means but registered by the olfactory system.

Odor discrimination among the purified compounds listed in Table I was determined by the triangle test ($n = 20$) for 10 of the 36 possible pairs. In all 10 comparisons, odor discrimination between samples was always highly significant ($P < 0.001$). Even though 70% or more of the panel described the characteristic odor of both 1-pentyl and cyclopentyl acetates as fruity-floral, they were able to discriminate between the two compounds at a highly significant level. While most of the compounds were given the general qualitative odor description of fruity-floral, some had other definite odor characteristics. Details of the descriptive odor analyses of these as well as other compounds are being studied and will be reported later.

Table II. Odor Thresholds of Pentyl Acetates

Acetates	Threshold and Standard Deviations, Parts per Billion Parts Water
1-Pentyl	5 ± 0.5
2-Pentyl	2 ± 0.2
3-Pentyl	9 ± 1.0
2-Methyl-1-butyl	5 ± 0.5
3-Methyl-1-butyl	2 ± 0.3
3-Methyl-2-butyl	6 ± 0.3
1,1-Dimethyl-1-propyl	30 ± 5.0
2,2-Dimethyl-1-propyl	4 ± 1.0
Cyclopentyl	21 ± 2.0

Acknowledgment

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Literature Cited

(1) Beynon, J. H., Saunders, R. A., Williams, A. E., *Anal. Chem.* **33,** 221 (1961).
(2) Guadagni, D. G., Buttery, R. G., Okano, S., *J. Sci. Food Agr.* **14** (10), 761 (1963).
(3) Lundin, R. E., Elsken, R. H., Flath, R. A., Henderson, N., Mon, T. R., Teranishi, R., *Anal. Chem.* **38,** 291 (1966).
(4) McFadden, W. H., Stevens, K. L., Meyerson, S., Karabatsos, G. J., Orzech, C. E., Jr., *J. Phys. Chem.* **69,** 1742 (1965).
(5) Sharkey, A. G., Jr., Shultz, J. L., Friedel, R. A., *Anal. Chem.* **31,** 87 (1959).
(6) Teranishi, R., Mon, T. R., *Ibid.*, **36,** 1490 (1964).
(7) Webb, A. D., Kepner, R. E., *Am. J. Enol. Viticult.* **12,** 51 (1961).

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ANIMAL METABOLISM

Further Study on the Metabolism of Labeled 3-Amino-1,2,4-triazole (ATA) and Its Plant Metabolites in Rats

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3-Amino-1,2,4-triazole-5-C^{14} (ATA) was fed to adult rats at a dosage varying from 1 to 200 mg. per rat, and the excretory pattern, tissue accumulation of C^{14}, and metabolite formation in the urine were determined. There was no significant difference in the per cent recovery of radioactivity in urine and feces in relation to the dosage fed. The formation of ATA-metabolite by rats as per cent of dose decreased as the ATA dosage increased. The rate of elimination of ATA from all tissues was slightly slower with rats fed a 200-mg. dosage as compared with those fed a 1-mg. dosage. Using a mixture of ATA-H^3 and ATA-5-C^{14} and studying the change of H^3/C^{14} ratio in rat-ATA-metabolite indicated that 5-hydrogen atoms in the ring of ATA has been substituted. One of the amino hydrogens in the ATA-metabolite from male rats may be substituted also. Similar studies were carried out in rats using C^{14}-labeled or H^3 and C^{14}-labeled ATA-metabolites isolated from bean plants. The pattern of elimination for metabolite-1 and metabolite-3 differed greatly in adult rats.

Very little study has been done on the metabolism by mammals of 3-amino-1,2,4-triazole (ATA) and its transformation products from plants. In the previous paper (*2*), a study on the excretory pattern, metabolic fate, and tissue residues of ATA-5-C^{14} in rats was reported. This communication reports a further observation on the rate of ATA elimination and metabolite formation in rats on the basis of varying dosages, the nature of ATA metabolism in rats using a mixture of ATA-H^3 and ATA-5-C^{14} as tracer, and the rates of elimination of two ATA transformation products isolated from bean plants.

Materials and Methods

Rate of Excretion and Metabolite Formation in Rat Urine as a Function of Dose. This experiment was designed