degradation products when it is formed from ascorbic acid (Gresswell, 1974).

The results of this study emphasize the need for nutritional labeling to aid the consumer in choice of foods. Most of the processed fruit products analyzed were relatively poor sources of vitamin C compared with levels which might be expected, based on the vitamin C content of the corresponding fresh fruit. As more tropical fruit are processed to open new markets (de Arriola et al., 1980), additional information will be needed on the vitamin content of such products.

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Philip E. Shaw*
Charles W. Wilson, III

U.S. Citrus and Subtropical Products Laboratory
Southern Region
Agricultural Research Service
U.S. Department of Agriculture
Winter Haven, Florida 33880

Synthesis and Mass Spectra of Hexachlorocyclopentadiene-$^{37}Cl_6$ and Derived Organochlorine Insecticides

The chemical syntheses of heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene-*4,5,6,7,8,8*-$^{37}Cl_6$), heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene-*4,5,6,7,8,8*-$^{37}Cl_6$), and *trans*-nonachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ (1-*exo*,2-*endo*,3-*exo*,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan-*4,5,6,7,8,8*-$^{37}Cl_6$) are described. The key intermediate in the synthesis is hexachlorocyclopentadiene-$^{37}Cl_6$, enriched to the >92% ^{37}Cl level. The mass spectra of these compounds show simplified molecular ion clusters which are clearly resolvable from those of the unlabeled analogues, thus illustrating their potential value as stable isotope labeled internal standards for analysis.

In recent years stable isotope labeled compounds have seen increasingly wide use as internal standards for mass spectrometric analysis in biomedical and environmental areas (Klein and Klein, 1979; Pohl and Nelson, 1977). In many cases advances in the use of such analytical techniques are limited by the availability of appropriately labeled standards. The most useful such compounds are labeled in nonexchangeable positions in such a way that they give clearly defined mass spectral molecular ions or fragment ions distinctly separate from those of the natural abundance species. For compounds containing multiple chlorine atoms, the latter requirement takes on unusual importance because the natural isotopic distribution of chlorine gives rise to complicated mass spectral ion patterns. *Substantial* enrichment of such compounds with ^{37}Cl would in many cases provide extremely useful labeled standard materials.

Among the organochlorine insecticides which are important environmental contaminants are a large number derived from hexachlorocyclopentadiene (Brooks, 1974).

These include mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene), Kepone (1,1a,3,3a,4,5,5,5a,5b,6-decachloroocta-hydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one), heptachlor epoxide, *trans*-chlordane (1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*exo*-5,8-5,8-dimethanonaphthalene), dieldrin (1,2,3,4,10,10-hexachloro-6,7-*exo*-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene), endrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene), Kelevan [ethyl 5-(1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-2-hydroxy-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalen-2-yl)levulinate], and endosulfan (isomers of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide).

The present paper describes the synthesis of hexachlorocyclopentadiene-$^{37}Cl_6$ of 92.9% ^{37}Cl isotopic abundance, illustrates its use in the synthesis of some ^{37}Cl-la-

Communications

J. Agric. Food Chem., Vol. 30, No. 2, 1982 **397**

beled organochlorine insecticides, and compares their mass spectra to those of the corresponding unlabeled species.

EXPERIMENTAL SECTION

Materials and Apparatus. Na^{37}Cl with a reported ^{37}Cl content of 92.1 mol % was purchased from Monsanto Research Corporation, Mound Facility, Miamisburg, OH. Samples of heptachlor, heptachlor epoxide, and *trans*-nonachlor were generous gifts of the Velsicol Chemical Corp., Chicago, IL. Mass spectra (70 eV) were obtained with a Varian/MAT 311-A interfaced with a Varian 620/i MS data system. Gas chromatographic analyses were performed by using a Hewlett-Packard Model 5730A gas chromatograph equipped with a flame ionization detector. Columns used were either (a) a 1.8 m × 2.0 mm i.d. silylated glass column packed with 3% Dexsil 400 on 80/100 Supelcoport or (b) a 1.8 m × 4.0 mm i.d. silylated glass column packed with 3% SP-2250 on 100/120 Supelcoport. Melting points were obtained with a Hoover Thomas capillary melting point apparatus and are uncorrected.

Hexachlorocyclopentadiene-^{37}Cl$_6$. The procedure for a typical run is as follows. To a solution of 4.30 g of silver nitrate and 50 mL of water was added a solution of 1.177 g of Na^{37}Cl (19.6 mmol) in 10 mL of water. The white precipitate of Ag^{37}Cl was filtered, washed with water, and vacuum dried. A mixture of this Ag^{37}Cl and 5.0 g of manganese dioxide was added to a three-neck round-bottom flask equipped with dropping funnel, reflux condenser, and a short length of Tygon tubing leading to a capillary bubbler in a 25-mL pear-shaped flask containing 12 mL of a 10% sodium hydroxide solution cooled to 0 °C. Twenty milliliters of concentrated sulfuric acid was added to the Ag^{37}Cl–MnO$_2$, and the mixture was stirred and warmed to 70 °C for 2 h. A slow stream of nitrogen helped sweep the evolved ^{37}Cl$_2$ into the sodium hydroxide solution. A 100-μL portion of the resulting NaO^{37}Cl solution was diluted with 2 mL of 50% aqueous acetic acid. An ca. 50-mg portion of KI was added, and the liberated iodine was titrated against 0.10 N sodium thiosulfate, indicating a NaO^{37}Cl concentration in the original solution of 0.49 M (total 5.88 mmol).

To this was added ca. 2 mg of sodium sulfamate and 148 μL (1.80 mmol) of freshly distilled cyclopentadiene in 0.5 mL of ethanol. The reaction mixture was stirred 16 h at room temperature; then the product was extracted with methylene chloride. The organic solution was dried and evaporated at <0 °C in vacuo, and the crude product was purified by chromatography on silica gel eluted with hexane to give 120 mg of hexachlorocyclopentadiene-^{37}Cl$_6$ as a colorless oil.

After three or four such runs, the aqueous solutions from which the organic products had been extracted were combined, acidified with nitric acid, and treated with excess aqueous silver nitrate to precipitate ^{37}Cl$^-$ as Ag^{37}Cl, which was collected, dried, and used in further chlorinations. By means of such procedures 14 g of Na^{37}Cl was converted to 2.70 g (24.6% yield) of hexachlorocyclopentadiene-^{37}Cl$_6$. Gas chromatographic analysis (column a, 100–200 °C at 8 °C/min, R_T = 5.0 min) showed a purity of >90%: mass spectrum m/e (rel intensity) 282 (12.1), 6.3), 245 (100.0), 243 (42.3), 209 (39.7), 171 (26.6), 147 (36.5), 134 (42.8), 123 (30.3), 110 (17.0), 98 (22.0), 97 (61.2), 73 (24.6), 60 (48.0). Calculations from the molecular ion cluster (m/e 273–282) indicate a ^{37}Cl:^{35}Cl ratio of 92.9:7.1 (theoretical 92.1:7.9).

Heptachlor-$4,5,6,7,8,8$-^{37}Cl$_6$. Hexachlorocyclopentadiene-^{37}Cl$_6$ (0.75 g, 3.0 mmol) was reacted with cyclopentadiene (375 μL, 4.5 mmol) as previously reported (Heys et al., 1979) to give chlordene-$4,5,6,7,8,8$-^{37}Cl$_6$, which was subjected without purification to selenium dioxide
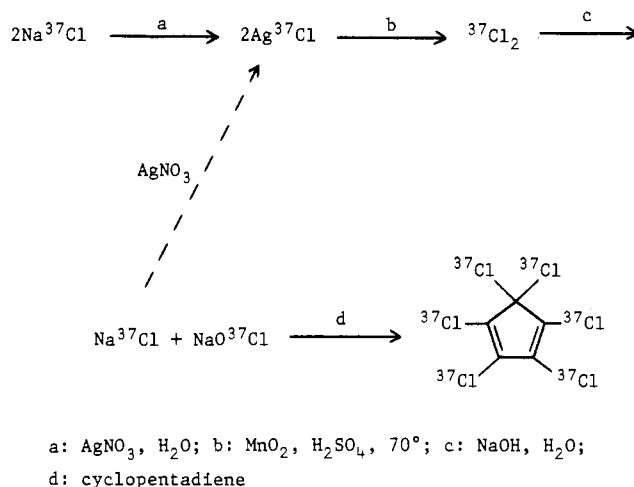

Figure 1. Synthesis of hexachlorocyclopentadiene-^{37}Cl.

oxidation (Kleinmann and Goldman, 1954). This crude 3-hydroxychlordene-$4,5,6,7,8,8$-^{37}Cl$_6$ was treated with thionyl chloride (Cochrane et al., 1970) to yield, after purification by column chromatography, 450 mg of heptachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ (39% overall yield): mp 108–110 °C [lit. 109–110 °C (Cochrane et al., 1970)]; TLC (SiO$_2$, benzene) R_f = 0.67; TLC (SiO$_2$, petroleum ether) R_f = 0.50; GLC, column b, 150–300 °C at 8 °C/min, R_T = 15.0 min, purity ≥ 97%; mass spectrum m/e (rel intensity) 382 (9.5), 347 (17.4), 309 (5.2), 282 (98.2), 272 (14.3), 245 (30.9), 236 (21.6), 198 (12.4), 162 (10.6), 137 (17.6), 100 (100.0), 65 (31.4). Calculations from the molecular ion cluster (m/e 448–456) indicate an overall ^{37}Cl:^{35}Cl ratio of 83.0:17.0 (theoretical 82.4:17.6).

Heptachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ Epoxide. Heptachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ (450 mg, 1.18 mmol) was converted by the method of Heys et al. (1979) to 138 mg (29.5% yield) of heptachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ epoxide: mp 156–157 °C [lit. 157–158 °C (Carlson, 1964)]; TLC (SiO$_2$, benzene) R_f = 0.50; TLC (SiO$_2$, heptane-acetone, 99:1) R_f = 0.20; GLC, column b, 150–250 °C at 10 °C/min, R_T = 9.5 min, purity ≥ 99%; mass spectrum m/e (rel intensity) 398 (9.3), 361 (100.0), 325 (11.1), 271 (25.5), 245 (24.6), 223 (15.3), 153 (22.2), 111 (17.6), 81 (85.1). Calculations from the molecular ion cluster (m/e 394–400) indicate an overall ^{37}Cl:^{35}Cl ratio of 82.7:17.3 (theoretical 82.4:17.6).

trans-**Nonachlor-$4,5,6,7,8,8$-^{37}Cl$_6$.** To a solution of 519 mg (1.45 mmol) of heptachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ in 20 mL of carbon tetrachloride cooled to 0 °C was added 5 mg of 2,6-di(*tert*-butyl)-4-methylphenol, and a stream of chlorine gas, purified by passage through a column of calcium oxide, was bubbled through for 30 min. The resulting yellowish solution was stirred at room temperature for 24 h, and the evaporation of the solvent under reduced pressure yielded a white semisolid. Purification was accomplished by chromatography on a silica gel column, the fraction consisting mainly of *trans*-nonachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ being eluted with heptane–acetone (99:1). Removal of solvent in vacuo and recrystallization frm hexane afforded 105 mg (16% yield) of *trans*-nonachlor-$4,5,6,7,8,8$-^{37}Cl$_6$ as large needles: mp 126–127 °C [lit. 125.5–128 °C (Lawrence et al., 1970)]; TLC (SiO$_2$, hexane) R_f = 0.30; TLC (SiO$_2$, heptane–acetone, 99:1) R_f = 0.35; GC, column b, 200–250 °C at 8 °C/min, R_t = 9.0 min, purity ≥ 98%; mass spectrum m/e (rel intensity) 452 (4.1), 419 (22.6), 417 (76.3), 415 (100.0), 413 (34.6), 282 (23.5), 271 (17.0), 245 (33.8), 147 (10.7), 123 (15.4), 99 (12.6). Calculations from the molecular ion cluster (m/e 448–456) indicate an overall ^{37}Cl:^{35}Cl ratio of 69.9:30.1 (theoretical 69.6:30.4).

398 *J. Agric. Food Chem.*, Vol. 30, No. 2, 1982

Communications
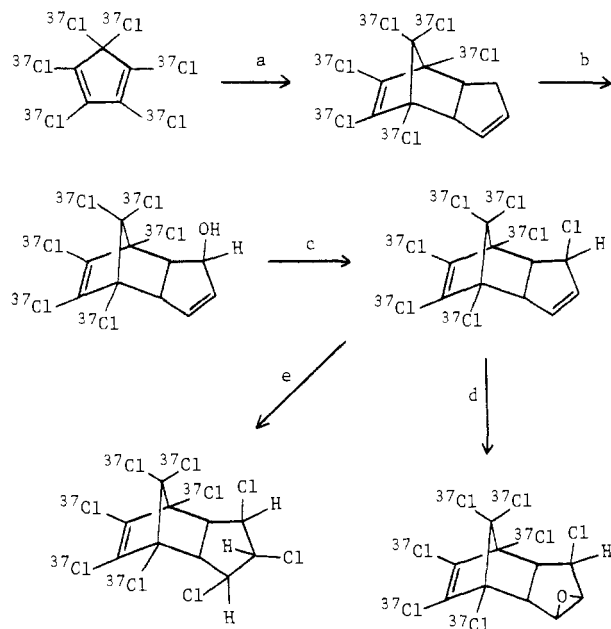
a: cyclopentadiene, Δ; b: SeO₂, dioxane, 95°, 5d;

c: SOCl₂, 80°, 24 hr; d: CrO₃, H₂SO₄, HOAc, H₂O;

e: Cl₂, CCl₄, BHT

Figure 2. Synthesis of ^{37}Cl-labeled polycyclic pesticides.

In unlabeled runs in the absence of 2,6-di(*tert*-butyl)-4-methylphenol, the major product obtained was tentatively identified as 2-chlorononachlor (1-*exo*,2,2,3-*exo*,4,5,6,7,8,8-decachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan): mp 179–183 °C; TLC (SiO₂, hexane–acetone, 99:1) R_f = 0.43; TLC (SiO₂, hexane) R_f = 0.50; IR

1600 cm⁻¹; NMR (CCl₄) δ 3.37–3.94 (symmetrical multiplet with satellites at ±23, 43.5, and 60 Hz from center, apparent AA'BB' system); mass spectrum m/e (rel intensity) 478 (44.3), 443 (100.0), 407 (27.8), 371 (18.1), 334 (23.6), 300 (26.6), 272 (69.0), 263 (63.2), 237 (69.1), 203 (28.2), 169 (52.4), 143 (79.7), 133 (53.8), 119 (60.0).

RESULTS AND DISCUSSION

The synthesis of hexachlorocyclopentadiene-$^{37}Cl_6$ is outlined in Figure 1. The hexachlorination of cyclopentadiene using NaO³⁷Cl solution [based on the procedure of Kleinmann and Goldman (1954) using unlabeled material] was chosen because of its relatively efficient use of labeled chlorine and because of NaO³⁷Cl could conveniently be prepared from Na³⁷Cl, commercially available at enrichments of greater than 90%. In our hands the manganese dioxide oxidation of Ag³⁷Cl resulted in a higher yield of ³⁷Cl₂ than with Na³⁷Cl (Townes and Aamodt, 1949). In several runs yields of 55–65% of NaO³⁷Cl were obtained, starting with 20–40 mmol of Ag³⁷Cl and bubbling the evolved ³⁷Cl₂ into 10% NaOH solutions cooled to ≤5 °C.

After assay of the NaO³⁷Cl concentration by treatment of a small aliquot with potassium iodide and titration of the liberated iodine with standard sodium thiosulfate solution, the appropriate amount (30 mol % based on NaO³⁷Cl) of freshly prepared cyclopentadiene was added. After completion of reaction after several hours, workup and purification generally resulted in 25–30% yields of hexachlorocyclopentadiene-$^{37}Cl_6$. The Na³⁷Cl remaining in the aqueous solution was recovered as Ag³⁷Cl by acidification with nitric acid and addition of silver nitrate. By repetition of the above procedure with recycling of the Na³⁷Cl, an overall yield of 2.7 g of hexachlorocyclopentadiene-$^{37}Cl_6$ (25% isotopic yield) was obtained from 14 g of Na³⁷Cl. The 92.9% ³⁷Cl enrichment as calculated

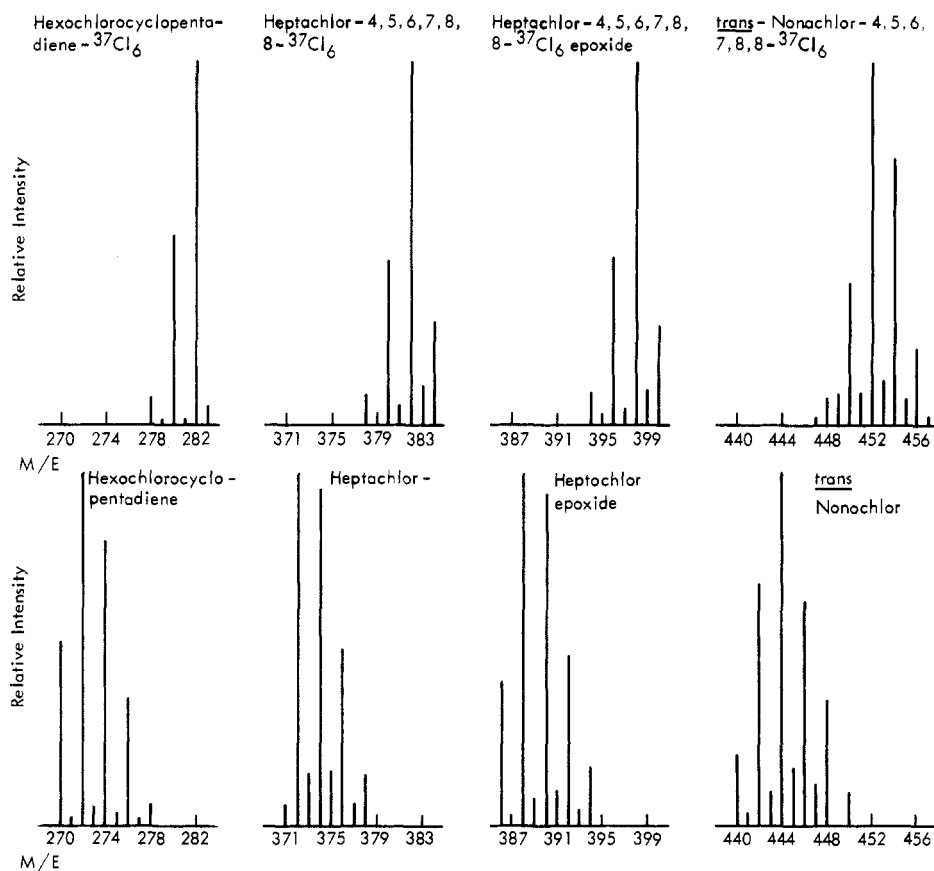**Figure 3.** Mass spectral molecular ion clusters of ^{37}Cl-labeled compounds.

from the mass spectral molecular ion cluster agrees well with the reported 92.1% ^{37}Cl enrichment of the starting $Na^{37}Cl$.

Hexachlorocyclopentadiene-$^{37}Cl_6$ was converted by procedures analogous to those previously published (Heys et al., 1979; Kleinmann and Goldman, 1954; Cochrane et al., 1970) to heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ and heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ epoxide as outlined in Figure 2. Yields are comparable to those previously reported with the ^{14}C-labeled compounds (Heys et al., 1979).

In addition, heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ was converted to *trans*-nonachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ (along with some of the cis isomer) by direct chlorination using Cl_2 in carbon tetrachloride (Buechel et al., 1966) in the presence of a trace of 2,6-di(*tert*-butyl)-4-methylphenol as the radical scavenger. In the absence of the phenol, the major product is a decachlorinated compound, mp 179–183 °C, whose mass, infrared, and nuclear magnetic spectra are consistent with 2-chlorononachlor.

The mass spectral molecular ion clusters of the labeled compounds are compared to their unlabeled analogues in Figure 3. The rest of the spectrum for each labeled compound differs from that of its unlabeled analogue only in the mass shifts of fragment ions as expected due to their altered isotope content. The ^{37}Cl enrichments of the four compounds as calculated from their molecular ion clusters differed less than 1% from the theoretical based on the 92.1 mol % ^{37}Cl reported for starting $Na^{37}Cl$ and the natural abundance of any added unenriched chlorine. In each case, the molecular ion cluster of the enriched compound is simplified and nonoverlapping with the unenriched. Even in the case of *trans*-nonachlor, which contains three added unenriched chlorine atoms and has an overall $^{37}Cl:^{35}Cl$ ratio of only 69.9:30.1, the labeled compound's molecular ion cluster is completely resolved from the unlabeled. Such compounds are thus potentially useful as internal standards for the mass spectral analysis of their natural abundance analogues. Moreover, hexachloro-cyclopentadiene with a ^{37}Cl enrichment level such as described here could be used in the synthesis of similarly useful isotope-labeled standards of other related chlorinated insecticides.

Note Added in Proof. The 250-MHz NMR spectrum (in acetone-d_6) of the putative 2-chlorononachlor displays an AA′BB′ system [δ 3.64 (H$_{3a}$, H$_{7a}$) and 4.36 (H$_1$, H$_3$)] in which iterative spin simulation reveals that $J_{3a,7a}$ = 10.18 Hz and $J_{1,7a}$ = $J_{3,3a}$ = 9.66 Hz, consistent with the proposed structure.

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David H. T. Chien
J. Richard Heys*

BioOrganic Chemistry Department
Midwest Research Institute
Kansas City, Missouri 64110

Carbon-13 Nuclear Magnetic Resonance Determination of Rubber in Guayule (*Parthenium argentatum*)

A method is described for the ^{13}C NMR determination of rubber in guayule. Compared to older methods, the results are less variable and can be obtained more rapidly.

Guayule is a crop of considerable research interest at the present time. The worldwide demand for poly(*cis*-isoprene) rubber, the increasing labor costs of hevea rubber harvesting, and international political uncertainties all contribute to the increased interest in guayule as a source of rubber (D'Ianni et al., 1978). Whether this research is agronomic, genetic, or biochemical, an accurate and convenient method for the determination of rubber in the guayule plant is necessary.

Many methods of rubber determination have been described. The analysis of rubber has been reviewed by Wadelin and Trick (1967) and Wadelin and Morris (1975, 1977, 1979). Many of the methods first employ pyrolysis such as the infrared technique described by Osland et al.

(1978). The simplest method to date is a photometric one by Traub (1946) and its modification by Mehta et al. (1979).

The standard method used for the analysis of rubber in guayule is a tedious one, the variables of which have been studied by Holmes and Robbins (1947). The samples are dried and ground, and interfering resinous materials are removed by Soxhlet extraction with acetone (8 h). The rubber is then extracted with benzene or methylene chloride overnight and the residue weighed after evaporation of the solvent. A less time-consuming method based on ^{13}C NMR spectrum of guayule rubber has been proposed by Shoolery (1978). This paper describes a simplification of Shoolery's method.