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, hexachlorocyclopentadiene 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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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.

METHODS

Several branches (3–5-mm diameter) were sampled from guayule plants for analyses. Hand-defoliated branches were dried overnight in a vacuum oven at 50 °C. Sample drying is not essential for rubber analysis but was utilized only for ease of calculations. Small portions of these dried branches were shaved off and chopped with a scalpel to produce a granular-type material. About 0.30 g of this material was placed in a 10-mm NMR tube. This weight of ground guayule was usually found to be sufficient to fill the NMR tube just to the height of the receiver coil. A coaxial tube (stem o.d. 4 mm; Wilmad, Inc.) containing 8% poly(ethylene glycol) (an internal standard) in D_2O (to provide a deuterium lock for the instrument) was placed in the NMR tube and pushed through the sample. The use of the coaxial tube was one of the major departures from Shoolery's method. It allowed a simpler method of calibration which was less dependent on instrumental variations. Any changes to the sensitivity of the detection of the sample (differences in the physical forms of standards and samples included) would equally effect the poly(ethylene glycol) internal standard and the ratio of the two would remain constant. Also, a large cost savings was realized since it was no longer necessary to add expensive D_2O to each sample. A ^{13}C Fourier transform NMR spectrometer (JEOL FX-60) operating at 15 MHz was used. Sample accumulation (8K) was performed for 1 h at a 90° pulse width at a 0.2-s pulse repetition.

As described previously (Shoolery, 1978), both hevea rubber and rubber-containing guayule yield spectra containing two peaks in the 120–130-ppm region (A and B peaks) and three peaks in the 20–35-ppm region [see spectra in NAS (1977)]. In this study, the sum of the integrals of the two downfield peaks was divided by the integral of the internal standard, and this was used for quantification. A standard curve of this integral ratio vs. the weight of rubber was constructed by two methods. The lower end of the curve was constructed by repeatedly dipping the coaxial tube into a methylene chloride solution of rubber. The solvent was evaporated after each dipping with a hair dryer. When the last layer of rubber had been dried, excess rubber film was cut to conform to the upper limit of the receiver coil (as determined by observing the response of the instrument approach zero as a band of rubber tubing is moved up to coaxial tube). For construction of the upper end of the curve, small strips of rubber were cut from a sheet of pure rubber (a gift from Goodyear Rubber Co.) and attached to the coaxial tube. As in the first method, the strips of rubber were weighed after cutting to conform to the height of the receiver coil. A linear standard curve of seven points was thus obtained.

RESULTS AND DISCUSSION

As described earlier, the standard method for the analysis of guayule rubber is by Soxhlet extraction. For evaluation of the accuracy of the NMR method, a comparison was made of the NMR and extraction methods by using the same samples. These results are detailed in Table I (values are derived from two subsamples and are typical of replicates and other samples not reported). The ^{13}C NMR and extraction methods give reasonably similar values. Moreover, there seems to be less variability in the NMR method as evidenced by a comparison of standard deviations. Results by our method are consistently higher than those by extraction. This is partially explained by the fact that NMR analysis of the plant residue after extraction indicated 3–5% rubber still remains. Increasing the extraction time from 16 to 48 h did not increase the amount of rubber extracted. The rubber polymer undergoes cross-linking easily. The cross-linked polymer is much less soluble than the normal rubber. Thus, the rubber left in the residue after extraction may well be cross-linked rubber caused by the mere physical action of grinding the sample prior to extraction. The values from line IV are higher than those of line I because the extraction probably removed more than just rubber.

Table I. Comparison of Methods of Analysis of Guayule Rubber (Percent Rubber in Oven-Dried Samples ± One Standard Deviation)

no.	method or operation	sample no. 1	sample no. 2	sample no. 3
I	NMR	14.7 ± 0.2	16.3 ± 0.4	12.7 ± 0.2
II	Soxhlet extraction with CH_2Cl_2	10.7 ± 0.9	14.5 ± 1.7	9.8 ± 0.5
III	NMR on residue of II	5.5 ± 1.5	2.8 ± 0	3.1 ± 0.5
IV	II + III	16.2 ± 0.6	17.3 ± 1.7	12.9 ± 0.0

The success of the ^{13}C NMR determination of rubber in guayule is dependent on the elimination of the terpenoid background. These compounds will give nearly identical chemical shifts as rubber and are present in concentrations in the same order of magnitude as rubber. Rubber, however, exhibits much shorter T_1's than do the interfering background materials (Shoolery lists a T_1 of 0.45 and 0.05 s for the A and B peaks, respectively). The pulse repetition time (0.20 s in this study) was thus chosen to be quick enough to saturate the background, and consequently no signal is observed from the terpenoids. As described by Martin et al. (1980), it is desirable in quantitative NMR studies to have a pulse repetition time 5 times longer than the T_1 of the peak under consideration. Obviously this requirement is nearly met for peak B but not for peak A. Yet inspection of Table I indicates the NMR method is certainly accurate. Furthermore, increasing the pulse repetition time to 2.2 s did not change the A:B ratio. This, together with the fact that the areas of the A and B peaks are the same as would be predicted from the stoichiometry of the molecule, indicates that peak A was fortunately not saturated. This may be explained by the natural inclusion of some relaxation agent in the sample. Levy et al. (1980) report that the ^{13}C relaxation process can be facilitated by as little as 10^{-7} M concentrations of metallic paramagnetic ions.

Experiments were conducted to determine the factors contributing to variability of the ^{13}C NMR method. One stem sample was analyzed 10 consecutive times without changing the position of the sample tube or coaxial tube (except for sample spinning). A value of 9.4 ± 0.2% rubber was obtained. Nine additional samples from different parts of the same plant were tried. A value of 10.5 ± 2.5% rubber was obtained. The preceding 10 samples were combined and sampled. After analysis each subsample was returned to the larger sample which was resampled until 10 determinations had been completed. A value of 10.7 ± 0.7% rubber was obtained. Thus, it appears that biological factors rather than the NMR method itself are responsible for most of the observed variability.

The results obtained by the ^{13}C NMR method for rubber determination in guayule are more accurate, less variable, and faster than the Soxhlet extraction method. Preparation of the samples and performance of calculations, etc. take about 5 min (not including optional oven drying). The results described in this report were obtained by using an accumulation time of 1 h. A 30-min accumulation time was tried without any significant differences in the results. Two samples were analyzed first for 30 min. Values of 5.6 and 8.1% rubber were obtained. After 60 min of analysis,

the values were 5.7 and 8.3% rubber, respectively.

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Chemistry of Toxic Range Plants. Water-Soluble Lignols of Ponderosa Pine Needles

A water-soluble fraction of the acetone extract of Ponderosa pine needles, known to cause abortions in western range cattle, was chromatographically examined. Seven lignol compounds were isolated and characterized, including two monolignols and a dilignol rhamnoside not previously reported to occur naturally. The new compounds are dihydro-*p*-coumaryl alcohol γ-*O*-acetate, dihydroconiferyl alcohol γ-*O*-acetate, and 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-(*O*-rhamnosylmethyl)-5-benzofuranpropanol. Several common flavonoids, organic acids, and pinitol were also isolated.

Ponderosa pine (*Pinus ponderosa*) needles are recognized as a toxic plant material responsible for induced abortion in range cattle consuming the needles in the late fall, winter, and early spring on western range lands (Stevenson et al., 1972). Abortions were observed to begin within 48 h after needle ingestion but may continue as long as 2 weeks after the animals are denied access to the needles. These abortions are characterized by retained placenta and may be accompanied by hemorrhaging. Complications associated with placenta retention may cause animal death.

The abortifacient principle of the pine needles has never been specifically identified. However, in vitro tests of Ponderosa pine needle extractives related to uterine growth and reproductive failure in mice (Chow et al., 1972) have indicated the toxic agent to be water soluble and thermolabile. An examination of the effect of an aqueous extract of fungal-infected Ponderosa pine needles on the uterine growth of mice (Chow et al., 1974) suggested the causative agent to be a fungal metabolite. A more recent study (Anderson and Lozano, 1977) delegated the fungal metabolite to a secondary role while suggesting the toxic constituent affecting reproduction in mice occurs in the pine needle fiber.

The observation of embryonic resorption in mice fed Ponderosa pine needle extracts established the occurrence of a heat-stable toxin soluble in many organic solvents of different polarity (Anderson and Lozano, 1979). Most recently, embryonic resorption has been observed in the uterus of mice administered a mixture of Ponderosa pine needle diterpene resin acids (Kubik and Jackson, 1981). The results of this investigation could not totally account for the earlier observed embryotoxic effects (Anderson and Lozano, 1979) of the Ponderosa pine extracts, but they did suggest that the diterpene resin acids were the principal water-insoluble, heat-stable embryotoxins in the needles. The diterpene resin acids are the first specific pine needle extractive constituents to be biologically evaluated as embryotoxins. The biological evaluation of pine needle toxicity in mammalian systems has thus far been restricted to studies in mice. The results of these studies may bear no relation to the abortion problems observed in ruminants.

The extractive constituents of the needles of some pines have been extensively examined and a large number of diverse chemical constituents have been isolated and characterized, including terpenes (Enzell and Theander, 1962; Norin et al., 1971), carbohydrates (Assarsson and Theander, 1958), aglycons and glycosides of phenylpropanes (Higuchi et al., 1977; Higuchi and Donnelly, 1977, 1978), flavonoids (Higuchi and Donnelly, 1977; Kowalska, 1977), and dilignols (Popoff and Theander, 1975, 1977). This investigation reports the first examination of specific Ponderosa pine water-soluble extractives.

EXPERIMENTAL PROCEDURES

Hammer-milled dry Ponderosa pine needles (5.3 kg), collected near John Day, OR, were sequentually hot solvent