Table II—Minimum Effective Concentrations (MEC) in Goldfish, Minimum Blocking Concentrations (MBC) in Isolated Fibers, and Partition Coefficients of Local Anesthetics

Local Anesthetic	MEC, m*M*	Log MBC, m*M*[a]	Partition Coefficient[b]
Procaine hydrochloride	0.136	−1.67	19
Lidocaine hydrochloride	0.049	−1.96	41
Tetracaine hydrochloride	0.0012	−2.9	800
Dibucaine hydrochloride	0.00013	−4.2	2200

[a] Data obtained from Ref. 6. [b] Between chloroform and pH 8 buffer.

procaine, lidocaine, tetracaine, and dibucaine are listed in Table II. A comparison of the results of the present study with those of Agin *et al.* (6) are presented in Fig. 2; the agreement between the two sets of data is good, and the results of present experiments utilizing the goldfish would give a good prediction as to the effect of the local anesthetics on isolated nerve and muscle fibers.

The correlation of biological activity with physical–chemical properties of the drug has been well documented (8). To determine the role of lipid solubility on the effect of the local anesthetic agent on overturn time in goldfish, partition coefficients between chloroform and pH 8 buffer were measured (Table II). The order of increasing lipid solubility of the agents between chloroform and pH 8 buffer is as follows: procaine < lidocaine < tetracaine < dibucaine. The relationship between partition coefficient and the apparent MEC is illustrated in Fig. 3. The fit is rather good and demonstrates that the activity of the local anesthetics in producing overturn in goldfish is due, in part, to the lipophilicity of the drug molecule.

Experimental results also appear to indicate that the nonionized drug molecule is responsible for the observed effect of the anesthetics on overturn time. In an experiment with lidocaine where the pH of the buffer solution was varied, the mean overturn time in goldfish for a 0.4 m*M* solution was 2.6 min at pH 8 and 16.8 min at pH 7; there was no response at pH 6. Lidocaine, with a pKa of approximately 7.9 (9), is 55.7% unionized at pH 8, 11.2% unionized at pH 7, and 1.2% unionized at pH 6. Thus, with decreasing pH and the subsequent decrease in in the concentration of unionized drug and the ability of the drug to penetrate biological membranes, there is a reduction in the pharmacological activity of lidocaine in the goldfish. A similar effect of pH on the overturn of goldfish in the presence of the other anesthetics investigated was noted.

The results of the present study illustrated the potential usefulness of goldfish as a model for the prediction of drug activity. Investigations are currently underway concerning the structure–activity relationships involved in producing overturn and death in goldfish for a series of lidocaine derivatives.

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Characterization of Poison Oak Urushiol

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Abstract □ Procedures are described that were used in the isolation and characterization of urushiol components reported to be the allergenic constituents of poison oak, *Toxicodendron diversilobum*. Characterization of these components by spectral techniques indicated they are unsaturated congeners of 3-heptadecylcatechol, possessing one, two, or three double bonds in an unbranched C_{17} side chain. These components are shown to differ from those isolated from poison ivy, *Toxicodendron radicans*, by a $—CH_2CH_2—$ unit in the unbranched alkyl side chain.

Keyphrases □ Poison oak—isolation and characterization of urushiol components □ *Toxicodendron diversilobum*—isolation and characterization of urushiol components □ Urushiol components of poison oak (*Toxicodendron diversilobum*)—isolation and characterization

Plants of the genus *Toxicodendron* (Anacardiaceae) have long been known for their ability to produce contact dermatitis in susceptible individuals. The best known species of this genus in the United States are *Toxicodendron radicans* (poison ivy), *Toxicodendron diversilobum* (western poison oak), and *Toxicodendron vernix* (poison sumac). The systematics of this genus have been well characterized (1).

The composition of the allergenic urushiol components (2–4) of poison oak (*T. diversilobum*) was studied. A prior study of the urushiol content of poison

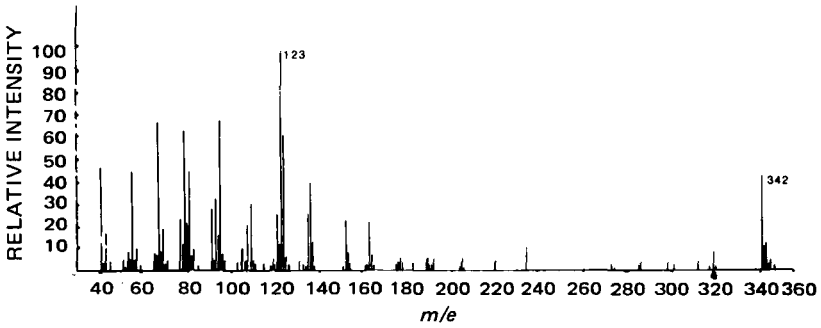


Figure 1—*Mass spectrum of isolated urushiol fraction. Molecular ions at 342, 344, 346, and 348 are for a series of unsaturated C_{17} catechol congeners.*

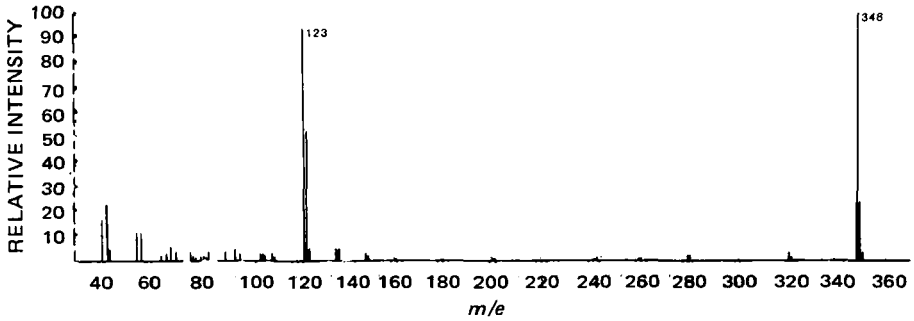


Figure 2—*Mass spectrum of hydrogenated urushiol extract. Molecular ion of heptadecylcatechol is at* m/e *348.*

ivy (*T. radicans*) indicated that it consists of four components (5, 6) having the carbon skeleton of 3-pentadecylcatechol (I), with the components differing from each other only in the degree of unsaturation in the C_{15} side chain. An early investigation (4) of the active component of poison oak indicated the presence of a catechol nucleus; however, a complete structural identification was not made.

This report demonstrates that the urushiol of poison oak is composed principally of a similar series of catechol components with a C_{17} side chain (II). The structural characterization of poison oak urushiol was made by mass spectrometry and UV and NMR analyses.

DISCUSSION

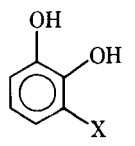
Pure urushiol was isolated as 0.18% of the fresh weight of *T. diversilobum*, employing two chromatographic steps. Additional urushiol fractions, totaling 0.10% of the fresh weight, were contaminated by nonphenolic materials and were not used for characterization of poison oak urushiol. The pure urushiol was a pale-amber-colored oil which solidified below 0°. The double bond number showed that most of the urushiol is the triunsaturated component.

The UV absorption spectrum of the hydrogenated urushiol was virtually identical with that of the urushiol etract with regard to the position and magnitude of their absorption maxima and minima. This observation parallels that reported for the poison ivy analysis (6) and thus precludes the existence of any conjugation in either the diolefinic or triolefinic component. In addition, the maximum absorption peak at 275 nm compares favorably with that of free catechol (λ_{max} 274 nm), which also indicates that there is no interaction between the centers of unsaturation in the side chain and the aromatic ring.

A direct probe mass spectral analysis of the isolated urushiol fraction shows a series of intense ions characteristic of an olefinic homologous series, ranging in mass from *m/e* 342 to 348 (Fig. 1). Although the relative intensities of these ions vary slightly with probe and source temperature, the ion at *m/e* 342 is always the most abundant; this ion is due to the molecular species of the triolefin. The most intense fragment ion occurs at *m/e* 123 and is probably a dihydroxytropylium ion, as might be expected from alkyl–aromatic compounds of this type.

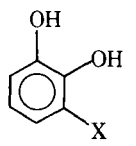
To confirm that the spectrum represents a homologous series of molecular ions having a varying number of double bonds, a sample of the extract was reduced by hydrogenation and again analyzed by mass spectrometry. The results in Fig. 2 show a single intense peak in the molecular ion region at *m/e* 348. A compound of this molecular weight would be the product of a reduction reaction involving mono-, di-, and triolefinic precursors. The most intense fragment ion again occurs at *m/e* 123, which is consistent with the structure previously postulated since the aromatic nucleus is not reduced by the mild hydrogenation process.

Confirmation of the molecular formula of these species was made by high-resolution mass spectrometry (Table I). A weak intensity ion at *m/e* 320 was detected in the hydrogenated sample;



I

Ia: X = $C_{15}H_{25}$
Ib: X = $C_{15}H_{27}$
Ic: X = $C_{15}H_{29}$
Id: X = $C_{15}H_{31}$



II

IIa: X = $C_{17}H_{29}$
IIb: X = $C_{17}H_{31}$
IIc: X = $C_{17}H_{33}$
IId: X = $C_{17}H_{35}$

Table I—High-Resolution Analysis of Molecular Ions

Ion	Theoretical Mass	Experimental Mass	Formula	Difference
320	320.2715	320.2704	$C_{21}H_{36}O_2$	0.0011
342	342.2559	342.2547	$C_{23}H_{34}O_2$	0.0012
348	348.3028	348.3016	$C_{23}H_{40}O_2$	0.0012
123	123.0446	123.0452	$C_7H_7O_2$	0.0006

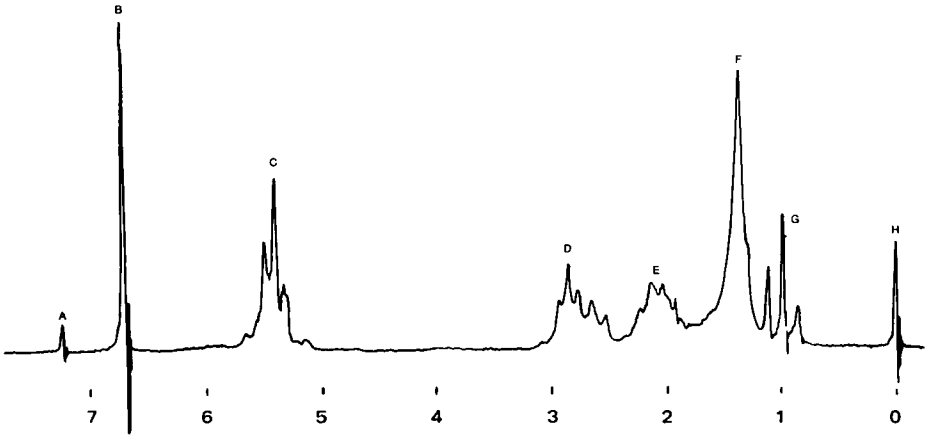


Figure 3—*NMR spectrum of isolated urushiol fraction. Key: A, chloroform; B, phenyl; C, vinyl; D, benzyl; E, allyl; F, methylene; G, methyl; and H, internal standard.*

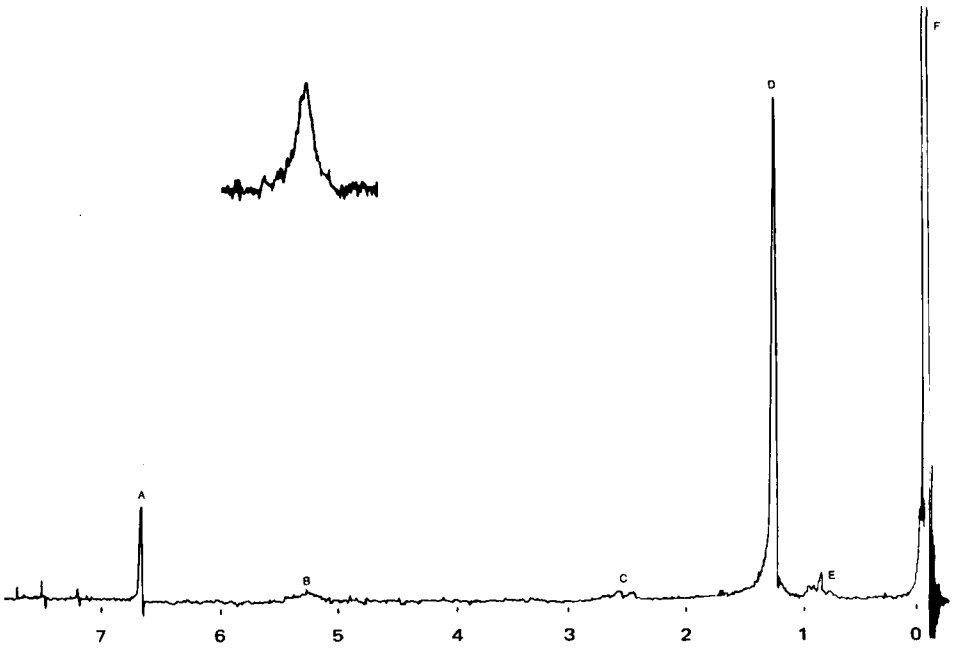


Figure 4—*NMR spectrum of hydrogenated urushiol extract. Key: A, phenyl; B, phenol (also expanded scale); C, benzyl; D, methylene; E, methyl; and F, internal standard.*

high-resolution mass spectrometry showed it to be the molecular ion of pentadecylcatechol, which is the saturated urushiol component reported in poison ivy. Subsequent analysis of the poison oak sample by mass spectrometry indicated that some of the C_{15} isomer is present in the poison oak urushiol, although the dominant component is clearly a series of C_{17} unsaturated congeners of catechol.

The complexity of the mixture and the uncertainty as to the relative rates of volatilization of each component precluded any effort to quantitate accurately the various homologs by direct insertion probe mass spectrometry. However, an approximate visual analysis indicated the triolefin to be the most abundant species and the saturated heptadecylcatechol to be the least. Use of direct probe mass spectrometry allowed for the incorporation of the molecular distillation technique which, in turn, provided sufficient control of sample volatility for analysis.

Efforts to resolve the sample components by GC so that a GC–mass spectrometric analysis could be made have been unsuccessful. The underivatized catechols or their trimethylsilyl derivatives have not been completely resolved under the GC conditions used. Efforts are underway to determine techniques to separate these olefinic catechols so that a quantitative result can be obtained. Furthermore, the double bond positions cannot be unambiguously assigned until the pure components can be analyzed. Structural information of this type was obtained from the mass spectra of various polyunsaturated fatty acids (7) and epoxy-derivatized olefins (8).

The NMR spectra of the urushiol components support the structure of a 3-*n*-alkylcatechol (Fig. 3). The benzylic, phenolic, aromatic, allylic, vinylic, methylene, and terminal methyl protons are all clearly identified. A spectrum of the reduced product (Fig. 4) retains only the aromatic, phenolic, benzylic, methylene, and methyl protons. The integrated NMR spectrum of the hydrogenated sample is easier to analyze than is the extract because of the unresolved nature and unsaturation of the urushiol prior to reduction. The integration of the methylene–methyl region (0.5–1.5) accounts for 33 protons. Two benzylic protons are observed at 2.6, and the aromatic and phenolic groups account for the remaining protons expected for this structure.

The conclusion to be drawn from the presented data is that the urushiol sample is a long chain C_{17} unbranched hydrocarbon, present as part of a catechol molecular structure. Thus, while poison ivy urushiol consists of the linear C_{15} side-chain components (9), poison oak urushiol is primarily composed of catechols with

Table II—Identification of Urushiol Fractions by TLC

Fraction	Weight, g	Purity (TLC)	R_f Solvent I	R_f Solvent II	Urushiol Test ($FeCl_3$)
A	0.3	Two major spots	0.50	0.60	— —
			0.35	0.30	+ +
B	0.9	Single spot	0.35	0.30	+ +
C	0.2	One major spot plus origin material	0.35	0.30	+ +

linear C_{17} side chains. These components, isolated from poison oak (*T. diversilobum*), are shown to differ from those reportedly found in poison ivy (*T. radicans*) by a $-CH_2CH_2-$ unit in the unbranched alkyl side chain.

EXPERIMENTAL[1]

Isolation and Purification of Urushiol—Fresh leaves, berries, and green stems of *T. diversilobum*[2] were collected in northern California, packed in dry ice, and shipped air freight. The material was processed immediately upon receipt. All extractions, evaporations, and chromatography were performed under a nitrogen atmosphere to retard air oxidation of the urushiol.

Fresh plant material (500 g) was covered with 4 liters of ethanol and allowed to stand for 4 days at 5°. Then the ethanol was decanted and evaporated *in vacuo* (40°) to give a black syrupy residue. This residue was partitioned between 500 ml of benzene and 200 ml of 5% NaCl solution and evaporated *in vacuo* to give 5.2 g of a dark-green oil. All of the oil was chromatographed on 100 g of silicic acid[3] packed in a 1.5-cm i.d. column. Elution with pure benzene and combination of all fractions producing a positive phenol test with 2% ethanolic ferric chloride gave 2.3 g of a dark-brown oil after evaporation *in vacuo*. Final purification of the urushiol was achieved by use of dry column chromatography (10).

The silicic acid[3] (100 g) was deactivated by treatment with 5 ml of water and was then treated with 10 ml of 10% ether–hexane. The treated silicic acid was packed in a 1.5-cm i.d. glass column in portions with the aid of a mechanical vibrator and 4.5 kg (10 lb) gauge of nitrogen pressure. The crude urushiol was dissolved in 2 ml of 10% ether–hexane and applied to the column. Development of the column with 10% ether–hexane was assisted by the application of 2.6 kg (5 lb) of nitrogen pressure.

Recovery of materials was made by complete elution from the column, as is occasionally done with dry columns (11). Thirty 15-ml fractions were collected and analyzed by TLC on silica gel plates[4] with the following solvents: I, ethyl acetate–hexane (3:10); and II, methylene chloride. Visualization of separate plates was made with 1% ferric chloride in 0.1 *N* HCl in methanol and with 10% sodium dichromate in 30% H_2SO_4. Combination of similar fractions gave three urushiol fractions of varying purity (Table II).

Fraction B, a pale-amber-colored oil, was considered to be pure urushiol; n_D^{25} 1.5201 ± 0.0002; double bond number based on a molecular weight of 348 ($C_{23}H_{40}O_2$): 2.8. UV λ_{max} (methanol): 275 nm; IR (neat): 3450 (medium, broad), 3025 (sh 3080, medium), 2980 (medium), 2950 (strong), 2875 (medium), 1630 (weak), 1605 (weak), 1490 (strong), 730, 775, and 830 (medium) cm^{-1}.

Mass spectral data are shown in Fig. 1 and NMR data are given in Fig. 3.

Hydrogenation of Urushiol—A 100-mg sample of the urushiol was hydrogenated[5] in 20 ml of methanol, with 10 mg of 10% palladium-on-charcoal as the catalyst. After filtration and evaporation of the solvent, the residue was recrystallized (methanol) to give 70 mg of a white amorphous solid, mp 63–65°; UV λ_{max} (methanol): 277 nm.

Mass spectral data are shown in Fig. 2 and NMR data are given in Fig. 4.

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[1] Mass spectra were obtained on a high-resolution DuPont 21-492, using direct probe analysis. NMR spectra were obtained at ambient probe conditions with a Jeol C-60HL instrument; chemical shift data were measured relative to tetramethylsilane as an internal standard, and all values are presented as parts per million. Deuterated chloroform was used as the solvent in all cases. UV spectra were measured with a Beckman Acta-III in methanol solutions. IR spectra were obtained neat with a Beckman IR-33. Microhydrogenations were obtained from Schwarzkopf Laboratories, Woodside, N.Y. All solvents used in the purification were ACS reagent grade.

[2] Supplied in July 1972 by Hollister-Stier Laboratories, Spokane, Wash. The samples were authenticated by Dr. Maynard W. Quimby, and voucher specimens are stored in the drug plant herbarium at the University of Mississippi.

[3] Analytical reagent, 100 mesh, Mallinckrodt, St. Louis, Mo.

[4] GF-254, E. Merck.

[5] Parr shaker.