SEPARATION AND CHARACTERIZATION OF POISON IVY AND POISON OAK URUSHIOL COMPONENTS

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ABSTRACT.—A procedure was developed for the isolation and purification of urushiol (≅95%) from ethanolic extracts of poison ivy and poison oak. To seperate the different urushiol congeners, reversed phase chromatography was performed with prep LC-500A. All components were characterized by ^{1}H nmr, ^{13}C nmr, gc and gc/ms. Comparison of the spectral data of poison ivy and poison oak urushiol congeners (3-*n*-pentadecyl and 3-*n*-heptadecylcatechols, respectively) indicated that the double bonds were in the same positions in both urushiols, i.e., 8, 11 and 14.

Poison ivy (*Toxicodendron radicans* (L.) Kuntze), poison oak (*T. diversilobum* (T. and G.) Greene, and several other plants of the family Anacardiaceae are known to cause irritation, inflammation, and blistering of the skin of sensitive individuals (1). Active compounds of these plants, commonly referred to as urushiol, are mixtures of 3-*n*-alk-(en)-yl catechols with zero, one, two and three double bonds in the C_{15} side chain of poison ivy (2, 3) and the C_{17} side chain of poison oak (4, 5), as shown in fig. 1. These compounds are highly sensitive to

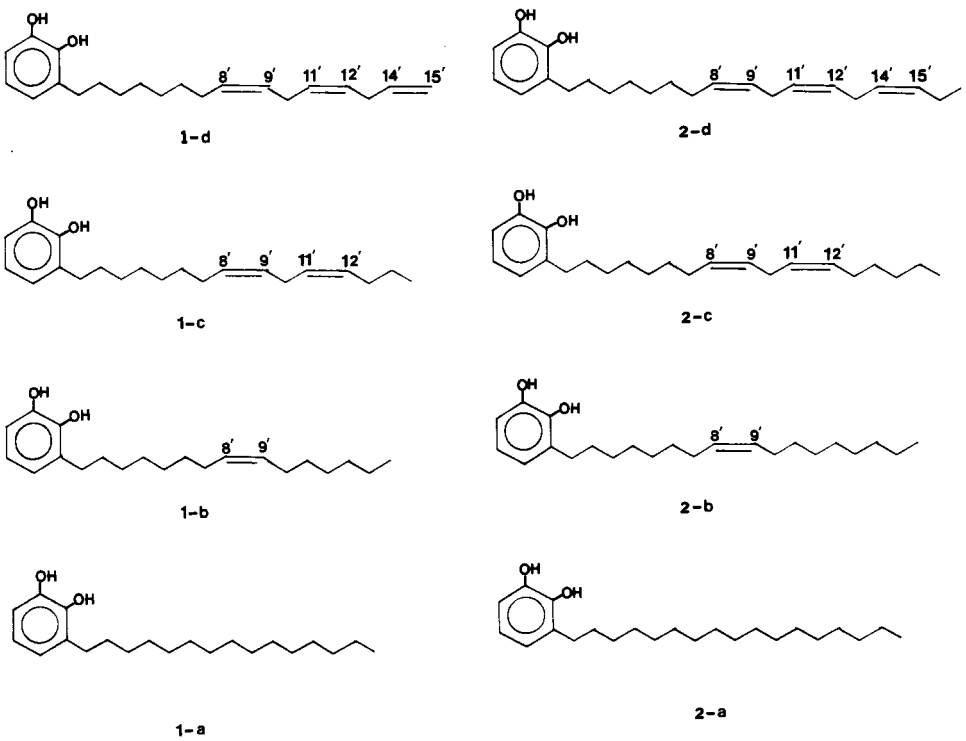


FIG. 1. Structures of poison ivy and poison oak urushiol congeners.

air oxidation and polymerization, which makes their isolation by conventional gravity column chromatography difficult. Dawson *et al.* (2, 3, 6) prepared the dimethyl ether of urushiol and separated the different congeners on an alumina column. The dimethyl ethers do not have allergenic activity. Demethylation of these compounds under acidic conditions results in extensive polymerization

(3). In 1980, Kumanotani *et al.* (7) used hplc on 10% silver nitrate-coated LiChrosorb Si-60 and LiChroprep Si-60 columns to separate urushiol diacetate congeners. From our laboratory, we published a hplc chromatography procedure which separates the individual urushiol congeners in their underivatized form (8).

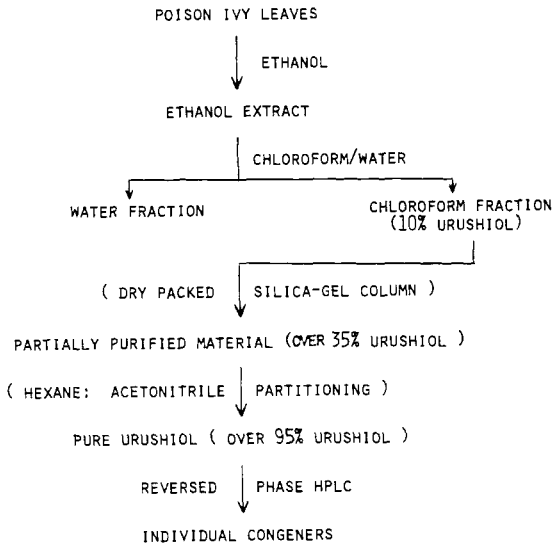
This investigation reports a simple procedure to prepare purified urushiol from crude extracts of poison ivy and poison oak. In addition, the idividual congeners of both urushiols were separated in their underivatized form by reversed phase chromatography on a Waters LC–500A instrument. Spectral data were collected on the purified congeners.

EXPERIMENTAL[1]

PLANT MATERIAL.—Poison ivy [*Toxicodendron radicans* (L.) Kuntze] leaves were collected in October 1975 at Oxford, Mississippi, and extracted immediately after collection. The plants were authenticated by Professor Maynard W. Quimby, and herbarium specimens are stored in the drug plant herbarium, Department of Pharmacognosy, School of Pharmacy, University of Mississippi. Poison oak [*Toxicodendron diversilobum* (T. and G.) Greene] extract was provided by Hollister-Stier Laboratories, Spokane, Washington, and was found by gc analysis (9) to contain 10% w/w urushiol.

EXTRACTIONS.—Poison ivy (*T. radicans*) leaves (fresh weight, 2 kg) were extracted by percolation with 95% ethanol at room temperature. The residue obtained after removal of the ethanol was partitioned between water (150 ml) and chloroform (3 x 300 ml). Evaporation of the chloroform afforded a residue which was shown to contain 10% w/w urushiol by gc analysis.

INITIAL PURIFICATION.—Poison oak extract (40.0 g) was dissolved in 50 ml of chloroform and chromatographed over 110.0 g of dry-packed silica gel-60 in a column 14 cm x 4 cm and



SCHEME 1. Purification and separation of urushiol congeners.

[1]Nmr spectra were recorded on a Varian EM–390 90 MHz instrument. Chemical shifts were measured relative to TMS as internal standard. ^{13}C nmr spectra were obtained on a JEOL–FX60 Fourier transform instrument. Chemical shifts were reported in ppm, with TMS as the internal standard. Deuterated chloroform was used as the solvent in all cases. For gc/ms, a Finnigan 3200 GC/MS/DS instrument was used at 70 ev. The TMS derivatives were prepared with BSTFA/pyridine and chromatographed an a 3% OV–225 column at 210° on a Beckman GC–65 instrument. Ir spectra were recorded on a Perkin Elmer 281 B instrument; samples were placed in sodium chloride cells as neat liquids. Uv spectra were recorded in MeOH on a Beckman Acta III spectrophotometer.

A Waters Associates Prep LC/system 500A liquid chromatograph with a Prep PAK–500/C_{18} cartridge and a refractive index detector was used for preparative separations. For analytical studies, a Waters Associates chromatograph equipped with a U6K injector, a model 6000 pump and a model 440 uv detector at 254 nm was used; a 30 cm x 3.9 mm id reversed phase column (μ Bondapak C_{18}) with a 10 μ particle size was used. Silica gel 60 (E. Merck, Dramstadt, Germany) was used for column chromatography and Whatman $KC_{18}F$ plates (Whatman Inc., Clifton, N. J.)were used for tlc with methanol-water (95:5) as the solvent system.

eluted with chloroform at 5 ml per minute; 50 ml fractions were collected. All fractions were analyzed by the (silica gel 60 F-254); 1% methanol in chloroform was the eluant. Fractions number 2 through 14, which showed a positive ferric chloride test, were combined, and the solvent was removed to obtain an oily residue. The above procedure was repeated twice; 11.0 g of brownish oily product containing 38% w/w urushiol (scheme 1) was obtained. When a similar chromatographic procedure was used with the chloroform fraction (10% urushiol) of the poison ivy extract, the product showed 35% w/w urushiol by gc analysis.

FURTHER PURIFICATION BY SOLVENT PARTITIONING.—Purified poison oak extract (11.0 g, 38% urushiol) was dissolved in 150 ml of hexane and extracted 3 times with 125 ml portions of acetonitrile (10). The acetonitrile fraction was evaporated to give 4.0 g of light brown oil. Gas chromatographic analysis of this product showed 99% w/w urushiol. The same procedure was used for further purification of purified poison ivy extract (10 g, 35% urushiol); a product (3.5 g) containing 95% w/w urushiol was obtained.

SEPARATION OF URUSHIOL CONGENERS.—Preparative separation of urushiol components of the further purified poison oak and poison ivy extracts was carried out by reversed phase chromatography. Poison oak urushiol (3.0 g) was dissolved in 5 ml of methanol, and the solution was loaded on the Prep LC-500A column. Methanol-water mixture (92:8) was used for elution at 200 ml/min; 1300 ml were collected before any component started to appear. Fractions of 150 ml each were then collected and analyzed by tlc performed with reversed phase (C-18) silica gel plates and with methanol-water (95:5) as the solvent. The plates were sprayed with ethanolic ferric chloride (1%) and fractions were combined according to their tlc similarities. Fractions 1 and 2 contained the pure triolefinic component of poison oak urushiol; 3 and 4 showed a mixture of triene and diene; 5 and 6 had diene with little impurity; 7 was a mixture of diene and monoene; 8, 9 and 10 showed pure monoene; 11 had monoene and the saturated component of poison ivy urushiol. The latter compound was separated in pure form from fractions 12–16. The slightly impure mixture of diene was concentrated and rechromato-

TABLE 1. Analytical and spectral data of poison ivy and poison oak urushiol congenres.[a]

	Poison Ivy Urushiol Congeners				Poison Oak Urushiol Congeners			
	1-a	**1-b**	**1-c**	**1-d**	**2-a**	**2-b**	**2-c**	**2-d**
Tlc R_f value	0.30	0.37	0.42	0.46	0.22	0.29	0.35	0.40
Gc Retention time in minutes	4.38	4.56	5.03	5.56	7.30	8.06	9.06	10.00
Hplc Retention time in minutes	23.84	13.16	10.20	7.84	42.98	24.63	16.63	12.24
ir: ν(Max) cm^{-1}	3400	3460	3460	3470	3380	3440	3460	3450
	1615	1618	1618	1618	1615	1620	1630	1620
	1475	1470	1470	1470	1475	1472	1482	1480
	1590	1590	1590	1590	1590	1590	1605	1595
uv: λ Max (log ϵ)nm	278	275	275	275	275	275	275	276
	(3.23)	(3.42)	(3.38)	(3.40)	(3.21)	(3.58)	(3.55)	(3.56)
	230	218	218	218	230	218	218	218
	(3.19)	(3.99)	(4.02)	(4.03)	(3.19)	(3.92)	(4.23)	(4.17)

[a]See experimental section for details of conditions and instrumentation.

TABLE 2. ^1H nmr chemical shifts in ppm for poison ivy urushiol congeners.

	1-a	**1-b**	**1-c**	**1-d**
4,5,6	6.72 s	6.67 s	6.70 s	6.67 s
OH	5.00 br	5.56 br	5.53 br	5.67 br
1'	2.60 t(J=8Hz)	2.60 t(J=8Hz)	2.60 t(J=8Hz)	2.60 t(J=8Hz)
2'-6'	1.24	2.19	1.30	1.30
7'	1.24	2.04	2.06	2.04
8'	1.24	5.37 t	5.37 t	5.40
9'	1.24	5.37 t	5.37 t	5.40
10'	1.24	2.04	2.80	2.83
11'	1.24	1.29	5.37 t	5.40
12'	1.24	1.29	5.37 t	5.40
13'	1.24	1.29	2.06	2.83
14'	1.24	1.29	1.30	5.77
15'	0.89 t(J=5Hz)	0.89 t(J=5Hz)	0.90 t(J=5Hz)	4.93 s(H cis to $C_{14'}$ proton) 5.07 d(J=9Hz) (H trans to $C_{14'}$ rpoton)

TABLE 3. ^{1}H nmr chemical shifts in ppm for poison oak urushiol congeners.

	2-a	2-b	2-c	2-d
4,5,6	6.70 s	6.70 s	6.70 s	6.70 s
OH	5.23 br	5.17 br	5.40 br	5.56 br
1'	2.57 t(J=8Hz)	2.60 t(J=8Hz)	2.60 t(J=8Hz)	2.61 t(J=8Hz)
2'-6'	1.23	1.24	1.31	1.30
7'	1.23	2.00	2.06	2.11
8'	1.23	5.34 t	5.37 t	5.37 t
9'	1.23	5.34 t	5.37 t	5.37 t
10'	1.23	2.00	2.79 t	2.83 t
11'	1.23	1.24	5.37 t	5.37 t
12'	1.23	1.24	5.37 t	5.37 t
13'	1.23	1.24	2.06	2.83 t
14'	1.23	1.24	1.31	5.37 t
15'	1.23	1.24	1.31	5.37 t
16'	1.23	1.24	1.31	2.11
17'	0.87 t(J=5Hz)	0.87 t(J=5Hz)	0.89 t(J=5Hz)	0.93 t(J=5Hz)

TABLE 4. ^{13}C Chemical shifts in ppm for poison ivy urushiol congeners.[a]

	1-a	1-b	1-c	1-d
1	143.3	143.4	143.3	143.3
2	142.2	142.3	142.2	142.2
3	129.7	129.9	129.8	129.4
4	122.3	122.2	122.3	122.3
5	120.3	120.2	120.3	120.3
6	113.1	113.2	113.2	113.2
1'	29.8	29.8	29.8	29.8
2'		29.8[e]	29.8[e]	29.8[e]
|	29.8	29.6[e]	29.6[e]	29.5[e]
6'		29.4[e]		29.3[e]
7'	29.8	27.3	27.4	27.3
8'	29.8	130.0	130.2[a]	130.5
9'	29.8	130.0	128.4[b]	127.7[b]
10'	29.8	27.3	25.8	25.7
11'	29.8[d]	29.8[d]	128.2[b]	127.0[b]
12'	29.4[d]	29.4[d]	130.0[a]	129.8[b]
13'	32.0[d]	32.1[d]	27.4	25.7
14'	22.7[d]	22.7[d]	22.9[d]	114.7[e]
15'	14.1[d]	14.0[d]	13.8[d]	136.9[e]

[a]Superscripts a, b and c on the chemical shift data indicate that the assignments could be interchanged within the respective group, while d and e mean the assignments are made with reference to methyl myristate and 1-octene respectively.

graphed as above to give a pure sample of diene. Poison ivy urushiol mixture (1.0 g) was similarily chromatographed with methanol-water (90:10) as the solvent. Fractions were collected to give the saturated, mono-, di- and triolefinic components in pure form. The purity of each component was established by tlc, hplc, gc and gc/ms. Spectral data on all components are presented in tables 1-6.

RESULTS AND DISCUSSION

The isolation of pure urushiol ($\geqq$95%) from poison ivy or oak extracts was achieved by a simple procedure. Initial purification of the extracts was carried out by chromatography over a dry packed silica gel column. The product obtained from this step, when partitioned between hexane and acetonitrile, gave highly purified urushiol in the acetonitrile layer. The urushiol mixture was separated into the individual congeners on a Waters prep. LC 500A instrument with a C_{18} reversed phase cartridge. Scheme 1 summarizes this procedure.

Poison ivy urushiol yielded the mono-, di- and triolefinic congeners of 3-*n*-pentadecylcatechol (PDC), while poison oak urushiol gave the mono, di- and triolefinic congeners of 3-*n*-heptadecylcatechol (HDC). The individual congeners

TABLE 5. ^{13}C Chemical shifts in ppm for poison oak urushiol congeners.[a]

	2-a	2-b	2-c	2-d
1	143.2	143.3	134.2	143.3
2	142.0	142.2	142.1	142.2
3	129.7	129.7	129.7	129.7
4	122.3	122.3	122.2	122.3
5	120.3	120.3	120.3	120.3
6	113.1	113.1	113.2	113.2
1'	29.8	29.8	29.8	29.8
2'		29.8[c]	29.8[c]	29.8[c]
|	29.8	29.6[c]	29.4[c]	29.5[c]
6'		29.4[c]		29.3[c]
7'	29.8	27.3	27.3	27.3
8'	29.8	129.9	130.2	130.5[a]
9'	29.8	129.9	128.1	127.9[b]
10'	29.8	27.3	25.7	25.7
11'	29.8	29.4[c]	128.1	128.4[b]
12'	29.8	29.6[c]	130.2	128.4[b]
13'	29.8[d]	29.8[d]	27.3	25.7
14'	29.4[d]	29.4[d]	29.4[d]	127.3[b]
15'	32.0[d]	32.0[d]	31.6[d]	132.0[a]
16'	22.7[d]	22.7[d]	22.6[d]	20.6
17'	14.0[d]	14.0[d]	14.0[d]	14.2[d]

[a]Superscripts a, b and c on the chemical shift data mean that the assignments could be interchanged within the respective groups, while d and e mean that the assignments are made with reference to methyl myristate and 1-octene respectively.

TABLE 6. Major fragments and the relative intensities of the different ions in the mass spectra of urushiol congeners.

	Relative Intensity (%)							
	Poison Ivy Urushiol Congeners				Poison Oak Urushiol Congeners			
ion (m/e)	1-a	1-b	1-c	1-d	2-a	2-b	2-c	2-d
M^+	3.3	7.2	2.3	1.4	3.5	6.5	5.1	1.2
163	0.8	9.6	11.2	16.4	0.2	7.2	18.5	13.7
149	1.0	11.7	10.0	17.5	0.9	8.8	16.9	14.9
136	6.6	51.1	34.0	29.1	5.1	37.5	52.7	26.7
123	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	TMS Derivatives[a]							
M^+	27.1	20.2	10.0	6.3	26.6	21.8	11.5	48.6
M^+-15	1.1	1.8	0.7	0.3	1.5	2.2	0.9	7.4
267	13.2	10.4	8.0	11.9	18.5	13.7	9.5	53.5
193	9.3	10.5	8.5	9.0	10.2	10.9	8.8	10.0
179	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
147	18.8	23.8	18.5	22.5	27.0	23.7	18.8	16.1
133	9.9	12.9	11.0	14.3	10.3	12.3	11.9	10.3

[a]Major fragments between *m/e* 100 and 550 are reported.

separated by this procedure were analyzed by tlc, gc, hplc and gc/ms. These isolations permitted the collection of spectral data, including uv, ir, ms, ^1H nmr and ^{13}C nmr, which are presented, for the first time, in tables 1–6.

The ^1H nmr and ^{13}C nmr data were helpful in making structural comparisons between the poison ivy and poison oak urushiol components to fix the double bond

positions in the latter. The data on the saturated, mono- and diolefinic congeners of ivy urushiol were identical with those of oak urushiol except for the integration of the methylenes. However, significant differences existed between the triolefinic components. The oak triene (**2-d**) showed a terminal methyl group as a triplet at 0.93 ppm in the ^1H nmr spectrum (table 3) and a peak at 14.2 ppm in the ^{13}C nmr spectrum (table 5) which was absent in the ivy triene (**1-d**). In addition, **2-d** shows a signal for two methylenes (C7 and C16) at 2.11 ppm (4H, t) compared to only one methylene (C7) in the ^1H nmr spectrum of **1-d**. Moreover, the peaks characteristic for the terminal double bond in the ^{13}C nmr spectrum of **1-d** (114.7 and 136.0 ppm, table 4) were absent in the spectrum of **2-d**. These data strongly suggest that the double bonds in poison oak urushiol congeners are in the same positions as in those of poison ivy urushiol.

When poison ivy and poison oak urushiol congeners were subjected to ms analysis with a solid probe, appropriate molecular ions were observed. All compounds gave a base peak at *m/e* 123, the dihydroxytropylium ion. When the TMS derivatives of these compounds were prepared and subjected to gc/ms analysis, the base peaks were observed at *m/e* 179. The tropylium ions with two OTMS groups at *m/e* 267 had intensities between 10 and 20%. The fragmentation patterns of both poison ivy and poison oak components were identical (fig. 2). The major ms fragments are shown in table 6.

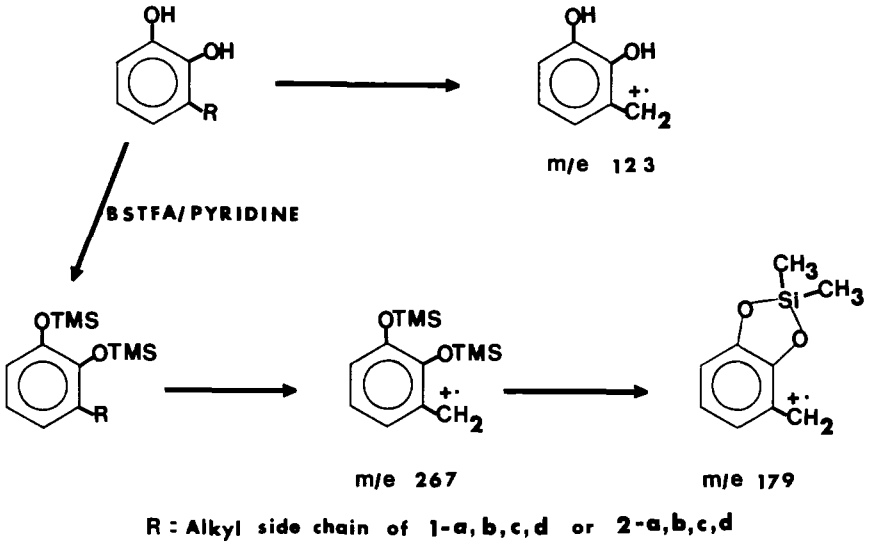


FIG. 2. Major fragments of poison ivy and poison oak urushiol congeners in ms.

This is the first reported isolation and spectral characterization of the individual congeners of these urushiols. Using guinea pigs as the animal model, Watson *et al.* (11) showed the allergenic potential of these congeners and the allergenicity increases with the increased unsaturation in the side chain (11). This was in agreement with clinical results reported by Johnson *et al.* (12).

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