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COMPONENT ANALYSIS OF THE URUSHIOL CONTENT OF POISON IVY AND POISON OAK

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Key Word Index—*Toxicodendron radicans*; *T. diversilobum*; Anacardiaceae; poison ivy; poison oak; urushiol; quantitation of homologs.

Abstract—The partial separation and quantitation of the components of the urushiol fraction of poison ivy and poison oak are discussed. The urushiol fraction of poison ivy is primarily composed of C_{15} side-chain catechol, while the urushiol extract of poison oak is principally the C_{17} homolog. The presence of a C_{17} homolog in ivy and a C_{15} homolog in poison oak urushiol is also detected. Each of these catechol derivatives contain a mixture of congeners which are partially separated by the GLC system used. In each case the tri-olefinic component occurs in greatest abundance and the mono-olefinic congener is least abundant; no saturated material was detected. The compounds were analyzed as trimethylsilyl derivatives and a qualitative analysis was accomplished by GC-MS.

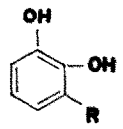
INTRODUCTION

Plants of the genus *Toxicodendron* have long been known for their ability to produce allergic contact dermatitis in susceptible individuals [1,2]. In the United States, the best known species of this genus are *T. radicans* (poison ivy), *T. diversilobum* (poison oak) and *T. vernix* (poison sumac). The systematics of this genus have been well characterized by Gillis [3].

Extracts of poison ivy leaves have been used as a diagnosis of sensitivity as well as for prophylactic hyposensitization treatment [4,5]. The dermatitis produced by the poison ivy plant has been shown to be due to the presence of a urushiol fraction, which is primarily composed of 3-pentadecylcatechol and three of its unsaturated congeners [6,7] (see Fig. 1). Extracts of poison oak also contain a urushiol fraction which is composed of 3-heptadecylcatechol and three of its unsaturated congeners [8,9]. In naturally-sensitized individuals, the unsaturated congeners appeared to exhibit a more pronounced allergenic response than did the fully saturated compound [4].

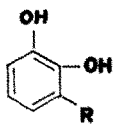
Since urushiol preparations are used to desensitize susceptible individuals, a convenient method to identify and quantitate the urushiol components is required. An assay method for the identification of the pentadecylcatechols in poison ivy *via* GLC has been published [10,11]. In those studies, the saturated C_{15} side-chain catechol congener was identified by comparison with an authentic sample, while the other peaks in the chromatogram were assigned by inference. The present study was undertaken to demonstrate techniques for the quantitative and qualitative analysis of these components as well as to enhance the chromatographic conditions for separation.

The separation of the silylated derivatives of the urushiol extract by GLC is intended to satisfy this requirement. A report on the allergic response as a function of alkyl side-chain length indicates very little activity difference between the saturated C_{15} and C_{17} homologs [12]. A significant finding in this report is the identification and quantitation of C_{15} catechol in poison oak and C_{17} catechol in poison ivy. A marked difference in the concentration ratio of the unsaturated congeners in young and mature poison ivy plants is also presented.



Poison ivy urushiol

R	double bonds
$C_{15}H_{31}$	0
$C_{15}H_{29}$	1
$C_{15}H_{27}$	2
$C_{15}H_{25}$	3



Poison oak urushiol

R	double bonds
$C_{17}H_{35}$	0
$C_{17}H_{33}$	1
$C_{17}H_{31}$	2
$C_{17}H_{29}$	3

Fig. 1. Structures of poison ivy and poison oak urushiols.

RESULTS AND DISCUSSION

The GLC behavior of non-derivatized or acetate derivatized pentadecylcatechols from poison ivy [10,11] was characterised by non-specific column absorption and poor peak shapes. We found the preparation of trimethylsilyl derivatives to be a more convenient technique from ease of derivative formation, reduced GLC retention times and improved peak shapes.

The retention times for the silylated C_{15} and C_{17} saturated catechol homologs differ by nearly a factor of two. The poison oak extract was found to contain detectable amounts of the C_{15} homolog, (Table 1) and the poison ivy to contain detectable amounts of the C_{17} catechol homolog (Table 2). This finding was verified both by spiking the respective extracts with the appropriate homolog as well as by GC–MS.

Neither the mass spectral nor GLC procedure showed any detectable presence of the saturated catechol congener from either poison ivy or oak. The composition

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Table 1. Composition of mature plants of poison ivy

M (%)	DT (%)	ΣMDT (%)	C_{17} (%)	$\frac{\Sigma MDT}{C_{17}}$	$\frac{DT}{M}$
6·20	89·65	95·85	4·15	23·10	14·46
6·28	89·44	95·72	4·28	22·36	14·24
6·19	89·82	96·01	3·99	24·06	14·51
6·14	89·98	96·12	3·88	24·77	14·65
6·29	89·65	95·94	4·06	23·63	14·25
6·26	89·71	95·97	4·03	23·81	14·33

Key: M = mono-olefin; DT = di-olefin and tri-olefin; ΣMDT = Sum of mono- di- and tri-olefin; C_{15} = catechols with C_{15} side-chain; C_{17} = catechols with C_{17} side-chain; $\Sigma MDT/C_{17(15)}$ = ratio of sum of respective mono, di, and tri-olefins to corresponding catechol $C_{15(17)}$; DT/M = ratio of di and tri-olefin to mono-olefin.

of poison ivy is presented in Table 1. The C_{15} di- and tri-olefin content comprises about 90% of the urushiol fraction of the extract. Although no GLC separation was made for the di- and tri-olefins, a visual analysis of the mass spectral data indicates the tri-olefin to be the dominant species. The amount of C_{17} homolog comprised about 4% of the ivy urushiol content. This was shown to be primarily the tri-olefin congener by GC–MS analysis of the TMS derivatized sample.

Mass spectral scans of the GLC peaks in the silylated urushiol fractions allowed for a qualitative analysis of the components. The major GLC peak from the poison ivy extract provides a simple mass spectral analysis. The molecular ion region of the spectrum is characteristic of a mixture of double bond congeners. The most intense of the molecular ions in that region is that of the silylated tri-olefin *m/e* 458 (RI-65·6%). The most intense of fragment ion at *m/e* 267 (RI-15·6%) was shown by high resolution analysis to be formed by cleavage of the alkyl side-chain, α to the aromatic ring; probably leading to the formation of a tropylium-like ion. The base peak at *m/e* 73 is characteristic of trimethylsilyl derivatives.

The composition of poison oak urushiol is shown in Table 2. The major catechol homolog possesses a C_{17} side-chain and comprises over 98% of the urushiol content. The detectable amount of C_{15} side-chain homolog was slightly more than 1% of the urushiol content. As with the ivy urushiol, the tri-olefin component was most abundant. The ratio of total C_{17} urushiol to C_{15} homolog in oak is more than three times that of the corresponding ratio from ivy where the major C_{15} fraction is compared to the amount of C_{17} homolog present. The ratio of di- and tri-olefin to mono-olefin is roughly equivalent in both plants.

Table 2. Composition of poison oak

M (%)	DT (%)	ΣMDT (%)	C_{15} (%)	$\frac{\Sigma MDT}{C_{15}}$	$\frac{DT}{M}$
9·06	89·64	98·70	1·30	75·92	9·89
8·48	90·34	98·82	1·18	83·75	10·65
8·36	90·03	98·40	1·60	61·50	10·77
8·39	90·45	98·84	1·16	85·20	10·78
8·21	90·37	98·59	1·41	69·92	11·00
8·36	90·17	98·53	1·47	67·03	10·79

For key see Table 1.

Table 3. Congener and homolog ratio of young plants of poison ivy

$\frac{\Sigma MDT}{C_{17}}$	$\frac{DT}{M}$	$\frac{\Sigma MDT}{C_{17}}$	$\frac{DT}{M}$
28·49	2·59	31·48	1·66
38·51	2·05	26·14	1·34
26·82	2·48	21·48	1·44

The poison ivy, *T. radicans*, samples discussed above were collected from older well-established plants around the campus of the University of Mississippi. A second collection of poison ivy, one year later, was made from this general locale; however, most of these plants were younger. The samples from the second collection of poison ivy were extracted and treated in the same manner as were the previous samples, but the results were slightly different. The analysis shows only slightly less C_{17} homolog in the younger plants; consequently, the ratio of total C_{15} urushiol to total C_{17} homolog was approximately the same as that found in the mature plants. However, within the C_{15} fraction of congeners, there is a distinct difference in that the amount of mono-olefin is much more abundant in the younger plants than in the mature plants.

The ratio between C_{15} and C_{17} congeners as well as that between the olefinic components of the C_{15} homolog in the younger plants is listed in Table 3. There appears to be a seven fold difference in the concentration ratio of mono-olefin to di- and tri-olefin in the younger plants than in the first group discussed. The area of collection for both groups of poison ivy were comparable but the major difference between these two collections was the age of the plant. Since the allergenic activity of the congeners is known to be different [4] this observation would indicate some variation in allergic potential as the plant matures. Such differences may be of importance in the preparation of extracts to be used for the induction of hyposensitivity.

EXPERIMENTAL

The isolation procedure used for the ivy and oak urushiol homologs has been previously described [8]. A Beckman GC-45 gas chromatograph equipped with a hydrogen flame ionization detector and interfaced to a digital computer was used in the analysis. A glass column 244 cm × 6·4 mm packed with 5% OV-25 on 80–100 mesh Gas Chrom Q was used in the quantitation study. The GC operating temperatures were: column 215°; detector 260°; and injection port 260°. N_2 was used as the carrier gas at a flow rate of 21 ml/min. For GC–MS, a Varian 1400 GC was used with the same 5% OV-25 packing, but in a 183 cm × 3·2 mm stainless steel column. This instrument is interfaced to a Dupont 21–492 mass spectrometer. Gas Chromatograph temperature conditions were comparable, but He was used as the carrier gas. *Bis*-(trimethylsilyl)-trifluoroacetamide (BSTFA) catalyzed with 1% trimethylchlorosilane was used as the silylating agent. 4-Androstene-3-17-dione was used as the internal standard. *T. diversilobum* was supplied by Hollister-Stier laboratories of Spokane, Washington. Fresh leaves, berries and green stems were collected in Northern California, packed in dry ice and shipped air freight to the University of Mississippi. Samples of *T. radicans* were collected from areas near the campus of the University of Mississippi. Plant samples were authenticated as *T. diversilobum* and *T. radicans* by Dr. Maynard W. Quimby. Voucher species are stored in the drug plant herbarium at the University of Mississippi.

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