New GLC Analysis of Urushiol Congeners in Different Plant Parts of Poison Ivy, *Toxicodendron radicans*

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Abstract □ Methods are presented for the direct GLC analysis of the catechol C_{15} alkenyl side-chain congeners contained in the urushiol fraction of poison ivy (*Toxicodendron radicans*) and the C_{17} homologs of poison oak (*Toxicodendron diversilobum*). A number of liquid phases were investigated and demonstrated varying degrees of separation. The methods developed were applied to the analysis of the urushiol fractions obtained from different plant parts of poison ivy. The effects of extraction before and after drying demonstrated that a larger percentage of urushiol was obtained when the fresh plant material was extracted with ethanol.

Keyphrases □ Urushiol fractions—obtained from different plant parts of *Toxicodendron radicans*, GLC analysis □ GLC—analysis, urushiol fractions obtained from different plant parts of *Toxicodendron radicans* □ *Toxicodendron radicans*—urushiol fractions obtained from different plant parts, GLC analysis

Contact with certain members of the plant family Anacardiaceae causes irritation, inflammation, and blistering of the skin. In the United States, the most widely encountered members of the genus *Toxicodendron*, which produce these symptoms in sensitive individuals, are poison ivy (*T. radicans*), western poison oak (*T. diversilobum*), eastern poison oak (*T. quercifolium*), and poison sumac (*T. vernix*). The systematics and growth patterns of these plants were studied thoroughly (1).

Poison ivy urushiol is a mixture of 3-*n*-pentadec(en)ylcatechols containing zero, one, two, or three double bonds in the side chain (2–5). Recent work (6) showed the urushiol of poison oak (*T. diversilobum*) to be a mixture of heptadec(en)ylcatechols. Furthermore, small quantities of the C_{15} and C_{17} homologs were found in poison oak urushiol and poison ivy extracts, respectively (7). Structure–activity relationships relating the length of the side chain and the number of double bonds to the dermatological properties of urushiol were determined (8, 9). Poison ivy and poison oak extracts have been used in the detection and prophylactic treatment of sensitivity (10–12).

In spite of all these studies, there has been no published methodology designed to quantitate the individual congeners of the urushiol, and no analysis has been reported of the urushiol content of the various plant components of any member of this family. In light of several studies (9, 13), current analytical methods should be expanded to monitor formulation procedures that will enhance the production of reliable dosage forms. This report describes methods to analyze the total urushiol content and/or congener ratios of the C_{15} urushiol of poison ivy and the C_{17} urushiol of poison oak.

Table I—Column Study of Trimethylsilyl Derivatized C_{15} Urushiols of Poison Ivy

Liquid Phase	Column Temperature[a]	Type of Separation
8% OV-1	215°	No separation of congeners
2% OV-17	215°	Baseline separation of saturated congener, shoulder separation of monoene
5% OV-17	215°	Baseline separation of saturated congener, shoulder separation of monoene
5% OV-25	205°	Monoene from diene and triene
5% OV-225	210°	Complete congener separation

[a] Isothermal values for maximum resolution and minimum retention time.

EXPERIMENTAL[1]

Plant Material—Poison ivy[2] (*T. radicans*) was collected in October 1975 in Oxford, Miss., and used immediately after collection. Samples of the young leaves and stems were dried at room temperature to determine the effects of drying on the urushiol content. Poison oak was obtained as an extract[3].

Extraction and Partitioning—The different plant parts of poison ivy including young and old leaves, young and large stems, berries, and bark of large stems (10 g) were extracted separately with 95% ethanol at room temperature in small percolators until exhaustion. In each case, the ethanol extract was evaporated *in vacuo* (40°) to give a syrupy residue. The residue was partitioned between water (15 ml) and chloroform (3 × 30 ml). The chloroform extract was dried over anhydrous sodium sulfate and evaporated, and the residue was transferred quantitatively to a 10-ml volumetric flask with chloroform and diluted to volume.

Internal Standard—As reported previously[4], 4-androstene-3,17-dione was a satisfactory internal standard when the liquid phase was OV-1, OV-17, or OV-25. However, when a more polar liquid phase was used, *i.e.*, OV-225, elution of the internal standard presented a serious problem. Thus, for the OV-225 liquid phase, dotriacontane[5] was used. A stock solution of dotriacontane was prepared in *n*-heptane (5 mg/ml). The response factors were linear over a 10:1 concentration range, and the urushiols were calculated as pentadecylcatechol.

Preparation of Samples for GLC Analysis—Aliquots of 1 ml of the extract were transferred to 1.5-ml sealable vials and dried under a nitrogen stream. To each vial were added 0.50 ml of internal standard stock solution, 0.25 ml of pyridine, and 0.5 ml of bis(trimethylsilyl)trifluoroacetamide[6]. The vials were capped, slightly heated with an air gun, and allowed to stand 30 min prior to injection.

RESULTS AND DISCUSSION

GLC methods for the identification of urushiol have been reported (14, 15), and the use of GLC–mass spectrometry for the quantitation of the

[1] A Beckman GC-45 gas chromatograph equipped with dual hydrogen flame-ionization detectors and interfaced to a digital computer (PDP-8) was used. Glass columns, 0.64 cm o.d. and 2 mm i.d. × 2.43 m, were packed with 80–100-mesh Gas Chrom Q, coated with the desired liquid phase. The operating conditions for the OV-225 column were: column, 210°; detector, 260°; and injection port, 230°. Nitrogen was the carrier gas with a flow rate of 15 ml/min. For GLC–mass spectrometry, a Varian 2740 instrument was used with the same column packing material and helical 2-mm i.d. × 1.2-m glass columns. This unit was interfaced to a DuPont 21-492 mass spectrometer.

[2] The plant material was 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.

[3] Supplied by Hollister-Stier Laboratories, Spokane, Wash.

[4] Final Report to Food and Drug Administration, "Poison Oak (*Rhus toxicodendron*) and Poison Ivy (*Rhus radicans*) Preparation and Analysis of Dosage Forms," Contract FDA 73-155 (223-73-1155), Mar. 1, 1973, to Nov. 30, 1974 (submitted Mar. 1975).

[5] Aldrich Chemical Co., Milwaukee, Wis.

[6] Pierce Chemical Co., Rockford, Ill.

Table II—Total Urushiol and Congener Analysis of Poison Ivy Plant Parts[a]

Plant Part	Total Urushiol, %	Composition of Total Urushiol, % Saturated	Monoene	Diene	Triene
Fresh young leaves	0.38	7.2	17.8	9.1	65.9
Dry young leaves[b]	0.08	23.2	23.2	11.7	41.9
Large leaves	0.03	t[c]	19.0	12.2	68.0
Large stems	0.17	4.6	7.8	35.0	52.6
Dry young stems[b]	0.20	6.4	15.2	28.0	50.4
Fresh young stems	0.39	3.8	13.1	27.0	56.1
Bark	0.47	t[c]	8.1	31.3	59.9
Fruits	0.37	5.8	7.6	13.1	73.5

[a] Analysis performed on 5% OV-225, which separated all four congeners found in C_{15} urushiol. [b] Calculated as percent wet material. [c] Trace amount (<1% of total urushiol).

different congeners was discussed previously (16). The described techniques offer significant improvement over previous methods. Derivatization *via* silylation of the catechol nucleus is quantitative and rapid and does not require any workup when compared to the preparation of acetates. Separation of the saturated, mono-, di-, and triolefinic components is sufficient for the quantification of each. Furthermore, the necessity of a mass spectrometric analysis and such techniques as mass fragmentography are avoided.

The GLC analysis of the urushiol fraction is straightforward when trimethylsilyl derivatives are used. These derivatives were stable for at least 2 days and had good GLC characteristics. The degree of silylation and stability of these derivatives were confirmed using GLC–mass spectrometric techniques.

The choice of column used in the analysis is determined by the degree of separation and type of information desired. For example, with an OV-1 column, no separation of the congeners in the urushiol fraction is observed. Thus, if the object is to determine the total urushiol content under a single GLC peak, a nonpolar liquid phase should be used. But if it is desired to quantitate each congener in the urushiol fraction, a more polar liquid phase is needed. The best success was achieved with an OV-225 column (Fig. 1). As might be expected, liquid phases of intermediate polarity, such as OV-25, produced partial separation of these congeners.

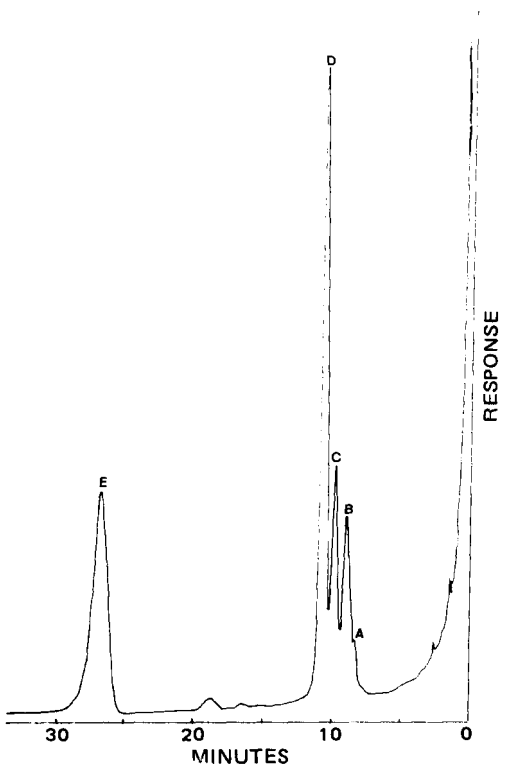

Figure 1—*Gas chromatogram of the trimethylsilyl derivatives of poison ivy urushiol (C_{15}) using OV-225 column. Key: A, saturated congener (relative retention time 0.31); B, monoene (relative retention time 0.33); C, diene (relative retention time 0.37); D, triene (relative retention time 0.40); and E, dotriacontane.*

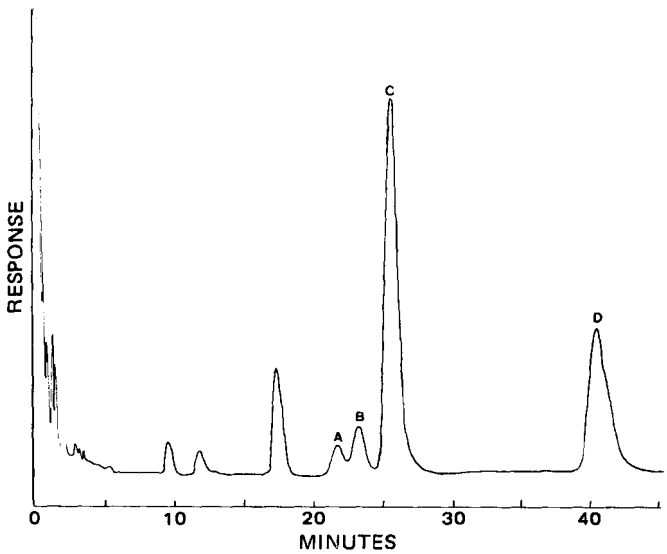

Figure 2—*Gas chromatogram of the trimethylsilyl derivatives of poison oak urushiol (C_{17}) using OV-25 column. Key: A, monoene; B, diene; C, triene; and D, androst-4-ene-3,17-dione.*

However, the OV-25 column, like the OV-225 column, provided complete separation of the C_{17} components of poison oak urushiol (Fig. 2).

Table I summarizes the GLC column analysis; this survey is not meant to be exhaustive but does represent the full range of separation required in any analysis of poison ivy urushiol by this technique. Application of this method to the analysis of poison ivy plant parts provides information on the distribution of urushiol throughout the plant.

Table II shows the total urushiol content and also provides a breakdown of the congeners found in the different plant parts. The highest concentration of total urushiol (0.47%) was found in the bark. This material was obtained by scraping the bark from the larger stems (2–4 cm in diameter). The fresh young leaves, fresh young stems, and fruits contained ~0.40% urushiol. In all cases, the large plant parts contained significantly less urushiol than those from younger plants. In all plant parts, the triene was the predominant congener, comprising more than 50% of the total urushiol when the plants were wet extracted; the fruits contained the highest triene concentration (73.5%). The leaves were the only plant parts to contain more monoene than diene. The highest diene concentration (35.0%) of the total urushiol was observed in the large stems.

Air drying of the stems and leaves reduced the total urushiol content. However, the most dramatic effect occurred with the young leaves, where a significant change in the congener ratios as well as in the total urushiol content was observed.

Chloroform, hexane, and ethanol were evaluated to determine the best solvent for extraction. Hexane was selected for the extraction of urushiol from dried material since the extracts were comparatively clean. However, if, as with the leaves, a significant amount of urushiol is lost upon drying, ethanol becomes the solvent of choice for wet extraction.

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GLC–Mass Spectral Analysis of Fungal Metabolites

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Received July 23, 1976, from **P.O. Box 899, Los Altos, CA 94022*, ‡*104 Whitney Avenue, Los Gatos, CA 95030*, and §*Ultrachem Corporation, Walnut Creek, CA 94596.* Accepted for publication July 11, 1977.

Abstract □ Four metabolites, hispidin, bisnoryangonin, muscimole, and ibotenic acid, from potentially psychoactive mushrooms were analyzed by GLC–mass spectrometry as their trimethylsilyl derivatives. This method was applied to the first two compounds in *Gymnopilus punctifolius* (Peck) Singer and to the last two compounds in *Amanita pantherina* (Fr.) Secr.

Keyphrases □ Hispidin—GLC–mass spectral analysis in mushrooms □ Bisnoryangonin—GLC–mass spectral analysis in mushrooms □ Muscimole—GLC–mass spectral analysis in mushrooms □ Ibotenic acid—GLC–mass spectral analysis in mushrooms □ GLC–mass spectrometry—analyses, hispidin, bisnoryangonin, muscimole, and ibotenic acid in mushrooms □ *Gymnopilus punctifolius*—GLC–mass spectral analysis of various components □ *Amanita pantherina*—GLC–mass spectral analysis of various components

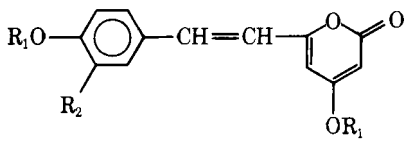
A previous report described the GLC–mass spectral analysis of the trimethylsilyl derivatives of psilocin and psilocybin (1). As part of continuing studies of the chemical constituents of higher fungi, this report describes the GLC analysis of the trimethylsilyl derivatives of four such compounds and their mass spectral features. Bisnoryangonin (Ia), hispidin (IIa), muscimole (IIIa), and ibotenic acid (IVa) occur in various genera of the Hymenomycetes.

BACKGROUND

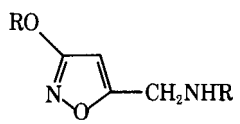
The styrylpyrone derivatives Ia and IIa were analyzed by TLC and IR, UV, and mass spectrometry (2, 3). Compound IIa was first reported (4–6) in certain species of Polyporaceous fungi. One or both of these compounds have been detected in various species of *Gymnopilus* Karsten (Cortinariaceae), *Pholiota* (Fr.) Kummer [including *Flammula* (Fr.) Kummer] (Strophariaceae or Cortinariaceae), and *Naematoloma* Karsten [= *Hypholoma* (Fr.) Kummer] (Strophariaceae) (2, 7–10). These and related compounds may be of considerable use in biochemical systematics (10–13).

The historical literature of Japan suggests the ethnomycological importance of *G. spectabilis* (Fr.) Smith[1] [= *Pholiota spectabilis* (Fr.) Quel.] (15, 16), but only occasional incidents of intoxication due to this species have been reported recently (17, 18). The presence of the related styrylpyrone yangonin (V) in the rhizomes of kava-kava (*Piper methysticum* Forst.) (19), the narcotic pepper used in the South Pacific, lends support to the possibility that this class of compounds might be psychoactive. However, limited pharmacological studies of the pure kava pyrones have not accounted for the activity of the whole plant (20).

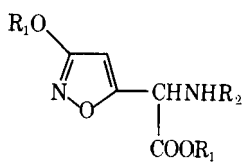
Compounds IIIa and IVa were analyzed previously by paper chromatography (21–23) and paper electrophoresis (21, 24). Instrumental analysis of the pure compounds was performed by IR (21) and mass spectrometry (25). These compounds appear to be confined to a few species of the genus *Amanita* Pers. ex Hook., principally *A. muscaria* (L. ex Fr.) Hooker (21–23, 26), *A. pantherina* (Fr.) Secr. (23, 26), and *A. cothurnata* Atk. (26). The ethnobotanical importance of *A. muscaria* has been established (27). Although these mushrooms are the cause of numerous intoxications, both accidental and intentional (28), pure compounds IIIa and IVa have received limited pharmacological study (29–31).



Ia: $R_1 = R_2 = H$
Ib: $R_1 = (CH_3)_3Si$, $R_2 = H$
IIa: $R_1 = H$, $R_2 = OH$
IIb: $R_1 = (CH_3)_3Si$, $R_2 = (CH_3)_3SiO$
V: $R_1 = CH_3$, $R_2 = H$



IIIa: R = H
IIIb: R = $(CH_3)_3Si$



IVa: $R_1 = R_2 = H$
IVb: $R_1 = R_2$ $(CH_3)_3Si$
IVc: $R_1 = (CH_3)_3Si$, $R_2 = H$

The previously described methods have been adequate for the qualitative analysis of these compounds. However, quantitative analysis by these methods suffers from several disadvantages, especially for the highly polar, zwitterionic IIIa and IVa.

The facile decarboxylation of IVa to IIIa during extraction, analytical, and isolation procedures (23, 26) leads to an inaccurate estimation of individual components present in the mushroom. Occasionally, enrichment of fungal extracts by ion-exchange chromatography is necessary before analysis can proceed (26). Use of colorimetric reagents to visualize spots on a chromatogram can often be misleading, especially in quanti-

Table I—Chromatography Conditions, Retention Times [a], and Percent of Trimethylsilyl Derivatives

	Ib	IIb	IIIb	IVb	IVc
SE-30	250°, R_t 8.5 min	250°, R_t 15 min	150–250°, R_t 2.1 min	150–250°, R_t 5.4 min	150–250°, R_t 3.7 min
OV-101	—	—	100–200°, R_t 6.0 min	100–200°, R_t 13.1 min	100–200°, R_t 10.8 min
Percent [b] present in fungal extracts	0.44 (0.42) [c]	0.25 (0.21) [c]	0.046	0.002	—

[a] Relative to the solvent front. [b] Based on dry weight. [c] By isolation.

[1] This species might be identical with *G. junonious* (Fr.) Orton (14).