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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF URUSHIOL CONGENERS IN POISON IVY AND POISON OAK

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SUMMARY

A high-performance liquid chromatographic procedure for the separation of urushiol congeners isolated from poison ivy and poison oak was developed. Each of the congeners was characterized by a retention index and the ratio of UV detector responses at 254 nm and 280 nm. The retention index of each congener was determined experimentally from a scale established by the relative retention of a series of 2-keto alkane standards (C_3–C_{23}). A simple semi-empirical equation using Hansch substituent constants (π values) was also used to estimate the retention index of each congener.

INTRODUCTION

Contact with certain members of the plant genus *Toxicodendron* causes irritation, inflammation and blistering of the skin. In the United States, the most widely encountered species of this genus are poison ivy (*T. radicans*), poison oak (*T. diversilobum*) and poison sumac (*T. vernix*). The composition of the allergenically active components, or so called "urushiol", has been reported to be a mixture of 3-*n*-alkenyl catechols with zero, one, two or three double bonds in the C_{15} side chain of poison ivy[1,2], and the C_{17} side chain of poison oak[3,4]. Because of the structural similarity of these congeners, their retention behaviors in gas–liquid chromatography (GLC) and liquid chromatography are also very similar[2–4]. In addition, because of the remarkable sensitivity of these alkenyl phenols to air oxidation and polymerization, the isolation of each individual congener in their underivatized forms by conventional gravity column chromatography is extremely difficult[2].

The primary objective of this study was to develop a high-performance liquid chromatographic (HPLC) system to separate and characterize the urushiol congeners of poison ivy and poison oak in their underivatized forms. Each of the congeners was

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characterized by a retention index scale and the ratio of the HPLC detector responses at 254 nm and 280 nm. The optimized HPLC conditions, absorbance ratios, and retention indices obtained with the analytical column were then used to separate each congener in 50–100-mg quantities using a semi-preparative HPLC column and the pure congeners were then characterized spectroscopically.

The second objective of the project was to determine whether the retention index of each congener could be predicted using a simple mathematical model. The concept of a retention index scale suitable for the use with the reversed-phase HPLC has been introduced recently to provide a general uniform way in which the HPLC properties of a given compound could be compared in a manner similar to the Kováts index in the GLC area[5]. It has been shown that the HPLC retention index of a given compound are fairly independent of the exact composition of the mobile phase[5] and can be estimated from the index of the structurally related parent compound and the Hansch substituent constant (or π value) for the compound[6]. The approach has been successfully applied to the estimation of retention indices for various types of drugs such as propranalol, anthranilic acid, barbiturate analogues and narcotic analgetics[6,7]. In this study, the retention index of three unsaturated congeners of poison ivy and four congeners of poison oak was estimated based on the observed value of the 3-pentadecyl-catechol (PDC) and π values for each of the substituents. In addition, diacetate derivatives of saturated C_{15} and C_{17} congeners were also characterized in the same manner and compared with the experimental values.

EXPERIMENTAL

Materials

A 30 cm × 3.9 mm I.D. reversed-phased column (μBondapak C_{18}, Waters Assoc., Milford, MA, U.S.A.) with a 10-μm particle size was used for this study. A 30 cm × 7.8 mm I.D. column with the same packing (μBondapak C_{18}, Waters Assoc.) was used for semi-preparative isolation. Methanol was of spectrograde (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) and chloroform was freshly distilled. The C_3–C_{23} 2-keto alkanes were obtained from Analabs (North Haven, CT, U.S.A.).

Analytical standards

PDC (m.p. 58–59°C; lit.[1] 58–59°C) and 3-heptadecyl-catechol (HDC) (m.p. 63–64°C; lit.[3] 63–65°C) was prepared by hydrogenation of poison ivy and poison oak urushiol respectively, using 10% Pd/C as catalyst. PDC acetate (m.p. 49–50°C) and HDC acetate (m.p. 57–58°C) were prepared by acetylation of PDC and HDC with acetic anhydride in pyridine.

Plant extracts

Two samples containing crude extract from poison ivy and poison oak plants were prepared following the procedures described elsewhere[3]. Prior to HPLC separation, both samples were further purified by gravity column chromatography. General procedures were as follows: 10–20 g of crude extract was loaded on 250 g silica gel 60 (Brinkman, Westbury, NY, U.S.A., 70–230 mesh, suspended as chloroform slurry and packed in a 35 cm × 4.8 cm glass column) and eluted with chloroform and 50 ml fractions were collected. The fractions were monitored using thin-layer chroma-

tographic (TLC) analysis and a $FeCl_3$ spray. Those fractions that showed a positive reaction (blue to purple color) were combined and analyzed by GLC for their total urushiol content[8]. The results showed that the poison ivy fraction contains 78% (w/w) of urushiols and the poison oak fraction contained 61% urushiols.

Chromatographic conditions

A Waters Assoc. Model 202 chromatograph equipped with a U6K injector and a Model 6000 pump was used for the study. A Model 440 dual-wavelength UV detector was used in series with the column and were operated at 254 nm and 280 nm. The chromatograms were obtained on a dual-pen recorder. The mobile phase was methanol–water (85:15) and the flow-rate was set at 2.0 ml/min for the analytical column and 3.0–3.5 ml/min for the semi-preparative column

Retention index measurement

The basic concept and method of the construction of the retention index scale for a given reversed-phase system has been reported previously[5] The retention index (I) of a given 2-keto alkane standard was given a value equal to 100 times the number of carbons in the standard The retention index of a compound (I_x) was calculated from the capacity factor observed for the compound (k'_x), the capacity factor for a 2-keto alkane standard eluting just before the compound (k'_N) and the capacity factor of the next higher homolog (k'_{N+1}) using the following equation:

$$I_x = 100 \frac{\log k'_x - \log k'_N}{\log k'_{N+1} - \log k'_N} + 100N \qquad (1)$$

The capacity factors were determined from the observed retention time of a given compound and the retention time of the solvent front via injection of methanol.

RESULTS AND DISCUSSION

Figs 1 and 2 illustrate the chromatograms obtained with the analytical HPLC column from the poison ivy and poison oak extracts. The notations on the graphs were adapted from that of fatty acids[10], thus (15:0) indicates poison ivy congener with a saturated C_{15} side chain on the catechol moiety and (17:3) indicates the poison oak congener with a C_{17}-triene side chain. Except for the compounds of (15:0) and (17:1), all components of poison ivy and poison oak urushiol congeners gave a baseline separation using the system as described above.

In a previous study it was demonstrated that the use of the absorbance ratio at 254 nm and 280 nm for chromatographic peaks greatly facilitated the identification of constituents in the complex mixtures[9]. For example, in the separation of poison ivy urushiol, some of the minor components in the plant extracts can easily be distinguished from the urushiol peaks by their characteristic absorbance ratios. The values for the A_{254}/A_{280} ratio for poison ivy and poison oak urushiol congeners as well as the ratio for PDC and HDC diacetates were listed in Table I. All of the urushiol congeners were found to have similar absorbance ratios ranging from 0.22 to 0.35 which would be consistent with their common chromophore (catechol, $\lambda_{max,\ ethanol}$ 278 nm, $\log \varepsilon = 3.42$)[12]. Upon acetylation, the chromophore exhibited a marked

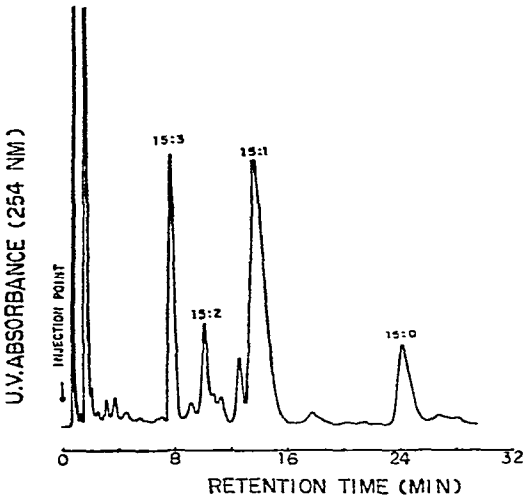

Fig. 1. HPLC chromatogram of poison ivy urushiol on μBondapak C_{18} column eluting with 85% methanol in water at a flow-rate of 2 ml/min. Though a dual-wavelength detector was used, only the response of the 254 nm detector is shown.

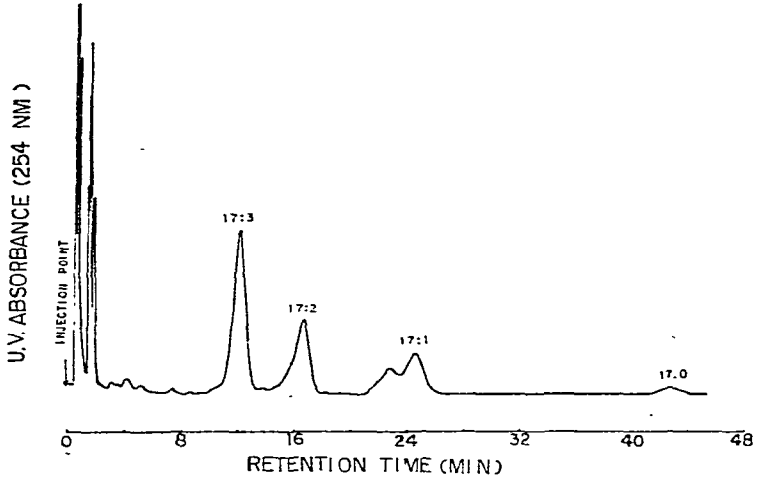

Fig. 2. HPLC chromatogram of poison oak urushiol on μBondapak C_{18} column eluting with 85% methanol in water at a flow-rate of 2 ml/min.

hypsochromic shift (PDC-diacetate, $\lambda_{max,\ methanol}$ 258 nm, log $\varepsilon = 2.25$; HDC-diacetate, $\lambda_{max,\ methanol}$ 258 nm, log $\varepsilon = 2.44$). The absorbance ratios determined with the HPLC system for the two ester derivatives (Table I) were consistent with the decrease in absorbance at 280 nm and the increase in absorbance at 254 nm that accompanied the hypsochromic shift.

In the initial phase of the project, pure reference standards were only available for PDC, HDC, and their diacetate derivatives. In order to assign the remainder of the HPLC peaks, the fractions from the HPLC column were collected and examined

TABLE I

HPLC SEPARATION OF POISON IVY AND POISON OAK CONGENERS

Compound	*Retention time (min)*	A_{254}/A_{280}	*Retention index*	
			Observed	*Calculated*
PDC (15:0)	23.84	0.274	1771	ref.
PDC-monoene (15:1)	13.96	0.356	1620	1645
PDC-diene (15:2)	10.20	0.293	1506	1518
PDC-triene (15:3)	7.84	0.347	1416	1393
PDC-diacetate	27.56	39.7	1814	1823
HDC (17:0)	42.98	0.222	1969	1971
HDC-monoene (17:1)	24.63	0.241	1795	1845
HDC-diene (17:2)	16.63	0.272	1668	1718
HDC-triene (17:3)	12.24	0.235	1573	1593
HDC-diacetate	47.50	49.3	2026	2023

by TLC, GLC and mass spectroscopy. TLC analysis using $FeCl_3$ spray showed that each of the peaks was a catechol with an R_F value that corresponded to one in the original sample. GLC analysis using a trimethylsilyl derivatization procedure[8] indicated the presence of a single congener at a retention time consistent with the assignments shown in Figs. 1 and 2. Direct probe mass spectral analysis of the fractions showed the molecular ion peaks at *m/e* 314, 316, 318 and 320 representing the tri-, di-, mono- and saturated PDC. Each spectrum also showed the presence of a common fragment at *m/e* 123 which would correspond to the dihydroxytropylium ion that was common to all of the congeners[3]. Using the semi-preparative system, 50–100 mg of each congener was collected and examined by ^1H and ^{13}C-nuclear magnetic resonance (NMR) spectral analysis. The ^1H-NMR spectra showed peaks at 6.7 ppm for aromatic protons and at 5.4 ppm for the vinyl protons. The ratios of the integrals of the vinyl/aromatic signals were consistent with the number of vinyl bonds in the assigned structure. The ^{13}C-NMR spectra also showed each of the six aromatic carbon signals and the corresponding number of vinyl carbon signals. The details of the ^{13}C-NMR spectral assignments will be presented in a subsequent publication.

Table I summarizes the retention times and retention indices of all the components being studied. The experimentally observed retention indices were obtained using eqn. 1 in the experimental section, while the calculated values were estimated by the following equation[6,7]:

$$I_x = 200\,\pi_x + I \tag{2}$$

where π_x was the sum of the Hansch substituent constants[11] for the tested compound, and I was the value experimentally observed for the reference compound (PDC, $I = 1771$). Though the standard π_x values were used for most of the substituents in the series, it was found that a value of -0.63 for each double bond gave a better agreement with the experimental values of the retention index. Using this method, the index calculated for the PDC-monoene (15:1) would be $200(-0.63) + 1771 = 1645$.

The observed and calculated retention indices of all of the components were also plotted on Fig. 3 for comparison, and the solid diagonal line in the figure repre-

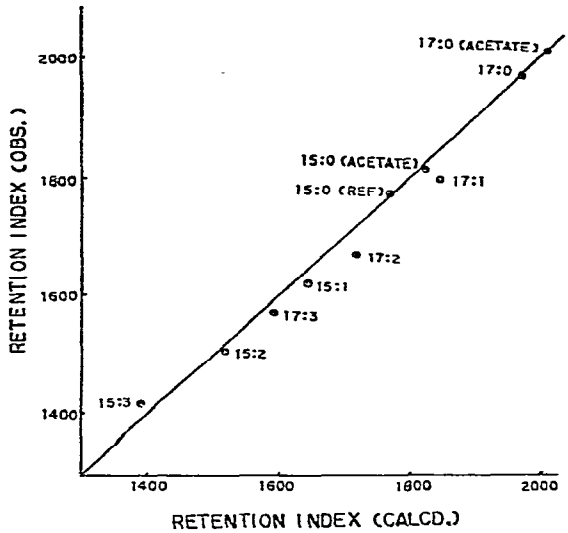

Fig. 3. Comparison of retention indices determined experimentally with the values estimated using eqn. 2 for congeners of poison ivy urushiol, poison oak urushiol and diacetate of PDC and HDC.

sents those points where the estimated and observed values are equal. It was found that there was a very good agreement between the predicted and the observed indices. Since the PDC series and the HDC series differ by two methylene groups, their retention indices should differ by 200 units and this was generally observed ($I_{17:0} - I_{15:0} =$ 198 units). It is also interesting to note that unsaturation decreases the retention of the solute by allowing interaction to occur between the mobile phase and the π electrons as reported elsewhere[10]. This increases the solubility of the unsaturated congeners in the mobile phase while decreasing the solubility in the stationary phase, resulting in a net decrease in retention time. Using a value of -0.63 for the π value for the double bond and eqn. 2, it would be expected that the monoene would have a retention index 125 units lower than the fully saturated congener. The retention indices observed for the monoenes of the poison ivy and poison oak congeners were in agreement with the predicted values and the trend continued for the diene and triene series (Table I). Though the simple model would predict equal incremental retention index shifts, it was observed that there was trend for the addition of each double bond to have a slightly smaller shift than that produced by the addition of the previous double bond.

The retention index observed for the diacetate derivatives were also found to be in good agreement with the calculated values. In the calculation of the indices for these two compounds, it was assumed that the acidic catechol groups were not ionized and that these groups were being replaced by simple aromatic acetate esters. Using the standard π values for these groups[11], the value estimated for the PDC-diacetate was nine units too high and the estimate for the HDC-diacetate was three units too low.

In conclusion, the HPLC method was found to offer a simple method not requiring derivatization for the characterization of the components of both poison ivy and poison oak. The retention index and A_{254}/A_{280} ratio for each component was

found to be useful in assignments of peaks in the chromatograms obtained from the extracts of the natural products. The retention index of the natural constituents and their derivatives can be accurately estimated using a simple additivity relationship with Hansch substituent constants.

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