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CATECHOLIC AND OTHER CONSTITUENTS OF THE LEAVES OF *TOXICODENDRON RADICANS* AND VARIATION OF URUSHIOL CONCENTRATIONS WITHIN ONE PLANT

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Abstract—Extracts of *T. radicans* were prepared for GC–MS by ethanol extraction of individual leaves followed by silylation. Analyses for the urushiol components were performed by monitoring ions at *m/e* 179 and the molecular ions of the various olefins. Higher concentrations of the urushiols were found in the younger leaves and, contrary to earlier analyses, the diene rather than triene was more abundant. Two isomers of the monoene were discovered by selected ion monitoring. Several sugars, polyols and related products were also identified.

INTRODUCTION

Extracts of poison ivy and poison oak (genus *Toxicodendron*, Anacardiaceae) contain pentadecylcatechols and are widely used in the diagnosis and immunotherapeutic treatment of sensitivity in humans [1, 2]. The skin reactivity of naturally allergic individuals varied when testing was carried out with partially purified pentadecylcatechol mixtures (urushiols) isolated from poison ivy leaves. The degree of sensitivity and the per cent of reactors seemed to be related to the natural abundance of each individual component. The diene is usually the most abundant, and also appeared to be the most reactive [3]. Although there is little direct evidence, it seems reasonable to assume that the immunotherapeutic efficacy of such extracts will also be dependent on their composition. Consequently, we have advocated analysis by gas chromatography–mass spectrometry (GC–MS) to define properly such mixtures [4].

Recently, Craig *et al.* [5] examined samples of various parts of *T. radicans* using a special gas chromatographic phase (OV-225) that provided separation of the unsaturated components of the urushiols as their trimethylsilyl ethers. Their technique involves separation of the urushiols as a class by chloroform extraction prior to silylation.

Because of our interest in the composition of leaf preparations, we sought a technique that would disclose leaf-to-leaf variations of the urushiols within one plant while minimizing any opportunity for their loss or degradation. A method was developed involving ethanol extraction of an individual leaf, silylation of the *total extract* and GC–MS. The resulting data, in addition to yielding results in some variance with the earlier work, provided identification of most of the ethanol-soluble components of the leaves, such as sugars and their cyclization products.

RESULTS AND DISCUSSION

The ion at *m/e* 179 was used as a crude measure of urushiol present [4] since its relative intensity was determined not to vary significantly in the homologues studied here. Also, silylated sugars overlapping the urushiols (Fig. 1) do not show significant intensity at this mass. The spectrometer was therefore set at *m/e* 179 and the oscillograph adjusted to a low speed so as to provide a trace of this mass at a fixed ion multiplier gain setting. The intensities of the GLC peaks eluting at the retention times of the urushiols were summed for quantitation of total urushiols. Known concentrations of pentadecylcatechol treated in the same manner were used for calibration of the spectrometer at the same gain settings.

The reconstructed gas chromatogram (*m/e* 462, Fig. 2) of the monoene shows two rather intense peaks, the smaller of which cannot be explained entirely by the $^{13}C_2$ and ^{30}Si satellites of the later-eluting diene (*m/e* 460). As determined from complete scans of the diene, approximately 11% of the intensity of *m/e* 460 must be subtracted from *m/e* 462 due to this cause. This still leaves appreciable intensity in the peak at this retention time and we conclude that 2 isomeric monoenes are present which we designate α and β, respectively. One of these is presumably 3-*n*-pentadec-8-enylcatechol identified earlier by Dawson [6]. We speculate the other is 3-*n*-pentadec-11-enylcatechol resulting from reduction of the other double bond of 3-*n*-pentadeca-8,11-dienylcatechol known to occur in the plant [6]. In contrast to several plants investigated earlier [4], heptadec(en)ylcatechols

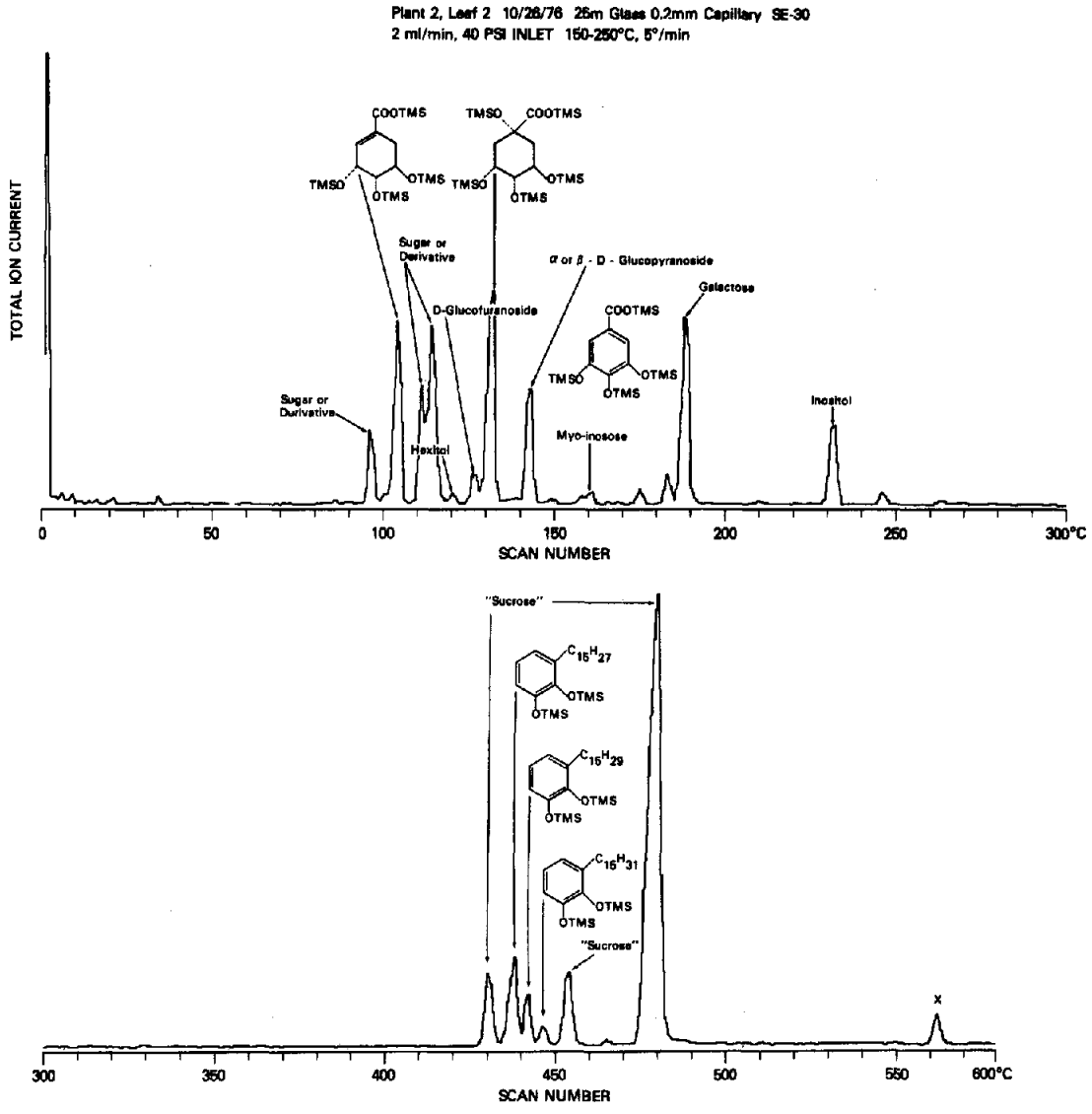

Fig. 1. Reconstructed total ion gas chromatogram of silylated extract of leaf from *T. radicans*. Conditions as on figure.

or other homologues were not detected here. In agreement with the results of Craig *et al.* [5], Table 1 shows that the urushiol content of the smaller, more immature leaves growing at the top of the plant is 5–10 times greater than the older leaves. One might speculate that such leaves are more in need of the protection afforded by these toxic substances. This may also account in part for the fact that the danger of being poisoned is greater in the spring and summer than late fall or winter [7].

Table 1 also shows the relative proportions of the various pentadec(en)ylcatechols in these leaves. In every case (except leaf 5 of plant 2) the diene (MW 460) is the most abundant component and the triene (MW 458), the least abundant. The exception here (Table 1) was considerably more damaged and perhaps much older than the other leaves. Its high proportion of saturated pentadecylcatechol may reflect either the greater stability of this substance (as in the case of saturated vs unsaturated fatty acids) or an increase in its biosynthesis with age. Other than this, we cannot relate the degree of saturation to the age or weight of the leaf. These results are similar to our earlier findings where *T. radicans* collected in the New York area and in Bethesda, Maryland yielded urushiols whose major component was the diene. The exception found at that time was an urushiol prepared from plants collected in Mississippi where the major component was the triene. The recent work of Craig [5] on urushiol prepared from plants collected in Mississippi also had triene as the major component. It thus appears that plants collected in Mississippi are a different strain of *T. radicans* or yield an urushiol of somewhat different composition due to climatic or soil differences. It is clear, however, that there is considerable variation in unsaturation from leaf to leaf in a single plant and it would be fortuitous indeed if a collection of such leaves would be a constant character of one species. Again it is clear that such extracts must be individually analysed prior to pharmacological use.

In the course of this work we also identified several of the other ethanol-extractable components. It can be

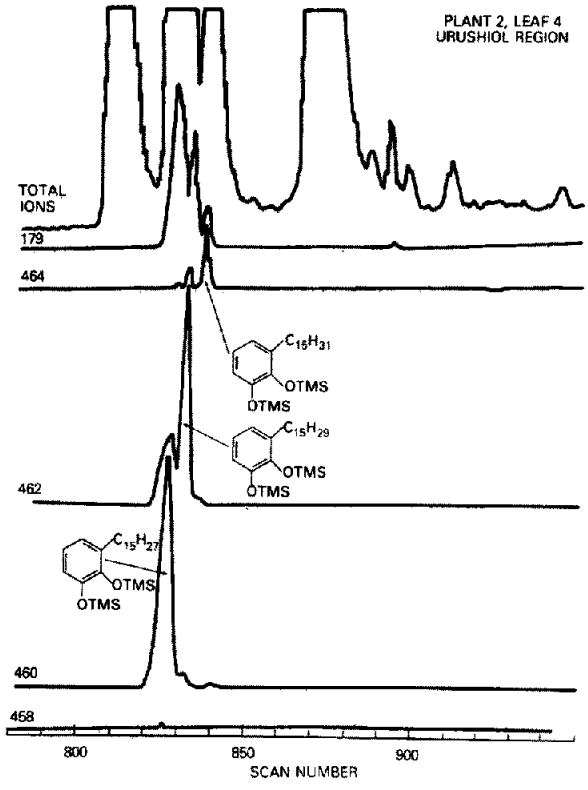

Fig. 2. Reconstructed gas chromatograms of urushiol region using molecular ions of saturated (464), monounsaturated (462), diunsaturated (460) and triunsaturated pentadecylcatechols. The monounsaturated (462) trace shows two components.

seen from Fig. 1 that the gas chromatogram is divided into two main groups of peaks. The first (scan numbers 90–250) is due mainly to monosaccharides and their cyclization products, shikimic acid and quinic acid; the latter (scan number 420–500) to disaccharides and the urushiols.

Mono- and disaccharides and their derivatives were identified as a group by virtue of abundant ions at *m/e* 204, 205, and 217. Except in the case of quinic acid, the individual compounds were identified by reference to compilations of mass spectra data [8]. The structure of quinic acid penta-TMS was deduced from deuteration of the ions at *m/e* 537 (M−15, 2-2/3 TMS), 435 (M−COOTMS, 4 TMS), 345 (M−435−90, 3 TMS), and 255 (M−345−90, 2 TMS), i.e. it is a tetrahydroxy acid-TMS of molecular weight 552. Myo-inosose at scan number 159 is tentatively identified by the abundant ions at *m/e* 73 (100), 305 (65, 3 TMS), and 147 (3).

The 'hexitols' at scan numbers 120, 175, and 183 show mass spectra very similar to, but distinct from, inositol (scan number 232). They do not resemble spectra of mannitol or galactitol. The 'sugar or derivative' at scan number 97 shows ions at *m/e* 217 (73), 257 (11), 303 (6), and 393 (11). The last ion (*m/e* 393) contains three TMS groups (from deuteration) and presumably represents loss either of OTMS, CH_2OTMS, or COOTMS from sugar derivatives whose molecular weights would be either 194, 208 or 222, respectively, each containing four hydroxyls. It could not be further identified. The sugar derivatives at scan numbers 112 and 113 showed very similar spectra; in each case the last abundant ion appeared at *m/e* 437 and contained 4 TMS groups (deuteration). Again one can logically add the elements of OTMS, CH_2OTMS, or COOTMS to give penta-TMS derivatives of compounds whose original molecular weights are 166, 180 and 194. Hexoses have molecular weights of 180 but the spectra did not agree with literature spectra [8] of any of the anomers of lactose, galactose, glucose, talose, mannose, gulose or altrose.

The mass spectra of the disaccharides at scan numbers 430, 454, and 478 were all nearly identical with that of sucrose and quite different from those of lactose, maltose, trehalose, turanose, palatinose, cellobiose, isomaltose, melibiose and gentiobiose.

Finally, the peak at scan number 562 shows ions at *m/e* 147 (7), 267 (8, 1-2/3 TMS), 281 (3), 355 (26, 3 TMS) and 456 (65, 4 TMS). From these data it does not appear to be a sugar. Since the ion at *m/e* 267 contains 1-2/3 rather than two TMS groups, it cannot be a catechol related to the urushiols [4].

Table 1.

Leaf #	Leaf wt (wet) mg	Wt % urushiols	Relative amounts of urushiols				
			Triene (458)	Diene (460)	Monoene (462) α	Monoene (462) β	Saturated (464)
Plant 1							
1	208	2.0	0.002	0.48	0.19	0.20	0.13
2	209	2.1	0.004	0.49	0.15	0.21	0.14
3	226	2.4	0.002	0.52	0.31	0.09	0.07
4	502	0.7	0.004	0.55	0.16	0.18	0.10
5	515	0.3	0.006	0.59	0.16	0.14	0.10
Plant 2							
1	151	2.8	0.010	0.61	0.18	0.12	0.08
2	465	2.6	0.005	0.53	0.11	0.29	0.07
3	456	1.5	—	0.53	0.09	0.29	0.09
4	601	0.35	0.019	0.48	0.05	0.41	0.05
5	738	0.25	—	0.26	0.06	0.17	0.51

EXPERIMENTAL

On 9 July 1976, 5 leaves were taken from each of 2 plants of *Toxicodendron radicans* (L.) Kuntze growing as vines on trees in Frederick County, MD. Leaves were numbered 1–5 as removed from the top to the bottom of the vine and immediately brought to the laboratory. One leaf from each location was weighed, cut into small pieces and placed in 5 ml 95% EtOH. The tubes were then flushed with N_2, sealed and allowed to stand overnight. For qualitative analysis, 2 aliquots (0.5 ml each) were withdrawn from each sample and evapd under N_2. Bis (trimethylsilyl) trifluoroacetamide (100 μl) or its D-9 derivative were added to each, the vials stoppered tightly and heated for 1 hr at 65°. After standing 5 days at room temp. (the silylated solutions were found to be stable in the presence of excess BSTFA for at least several weeks when carefully stoppered), 1–2 μl samples were analysed with an LKB 2091 GC–MS computer system, using a 1/10 splitter and a 25 m × 0.2 mm id glass capillary column coated with OV-1 liquid phase. Spectra were taken every 3 sec, the data being acquired by an on-line PDP-11 disk system using the LKB programs. The scans were inspected using reconstructed gas chromatograms for qualitative identification of the components and to determine relative amounts of the various urushiols as described below.

To determine absolute amounts of urushiols present, aliquots of EtOH extracts representing 20 mg (wet wt) of plant material were withdrawn, the EtOH evapd under a stream of N_2, 0.2 ml of *bis* (trimethylsilyl)trifluoroacetamide (or its D-9 analogue) added and the stoppered vials heated at 65°. After *ca* 2 hr, the samples had completely dissolved leaving a slightly yellow soln. After remaining at this temp. overnight, 1.8 ml $CHCl_3$ was added and 2 μl samples (equivalent to 0.02 mg of plant material) injected on the GC–MS system using a 2 m × 1.5 mm column packed with 3% OV-1 Gaschrom Q (Applied Sciences Lab., PA)

REFERENCES

1. Epstein, W. L., Baer, H., Dawson, C. R. and Khurana, R. G. (1974) *Arch Dermatol* **109,** 355.
2. Kligman, A. M. (1958) *Arch. Dermatol.* **74,** 47.
3. Johnson, R. A., Baer, H., Kirkpatrick, C. H., Dawson, C. R. and Khurana, R. G. (1972) *J. Allergy Clin. Immunol.* **49,** 27.
4. Gross, M., Baer, H. and Fales, H. M. (1975) *Phytochemistry* **14,** 2263.
5. Craig, J. C., Waller, C. W., Billets, S. and Elsohly, M. A. (1978) *J. Pharm. Sci.* **67,** 483.
6. Symes, W. R. and Dawson, C. R. (1954) *J. Am. Chem. Soc.* **76,** 2959.
7. Crooks, D. M. and Klingman, D. L. (1976) *Farmers' Bull.* No. 1972. U.S. Department of Agriculture, Jan. 1976, p. 11, U.S. Government Printing Office, Stock 001-000-03554-1, Washington, D.C.
8. Markey, S. P., Urgan, W. G. and Levine, S. P. (eds.) *Mass Spectra of Compounds of Biological Interest.* Publication #TID 26553-Pl. National Technical Information Service, Springfield, VA 22161, U.S.A.