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PHYTOCHEMISTRY OF THE *SALICACEAE*

V. THE USE OF A GAS–LIQUID CHROMATOGRAPHIC SCREENING TEST TO DETECT PHYTOCHEMICAL VARIATIONS IN *POPULUS DELTOIDES* MARSH.

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SUMMARY

A detailed description is given of a gas–liquid chromatographic screening test for the detection of phenolic glycosides in 10-mg samples of plant material. The test was applied to a large number of *P. deltoides* Marsh. bark samples in order to examine and compare glycoside content patterns in the "western" (var. *occidentalis*) and "eastern" (var. *deltoides*) types.

INTRODUCTION

Complex hybridization, accompanied by morphological differences within widely distributed species, has contributed to difficulties in the taxonomic treatment of *Populus*. The North American cottonwood and its forms (*P. deltoides*) has remained one of the difficult groups in the genus. Dode[1] was the first worker to recognize "western" North American cottonwood as *P. sargentii* and "eastern" specimens as *P. deltoides*. This classification was generally accepted by taxonomists for a number of years, even though the two taxa were difficult to define on the basis of morphological characteristics or geographical distribution. Most of the recent treatments have recognized the two respective taxa as var. *occidentalis* and var. *deltoides* within *P. deltoides*[2–4].

Regardless of taxonomic treatment, it is generally accepted that the "western" and "eastern" forms intergrade in central North America. This area of intergradation has been recognized by some workers as occurring in South Dakota, Nebraska, Kansas, Oklahoma and Texas[3], while others have placed it to the north in Manitoba and Saskatchewan[5]. Specimens from Manitoba and Saskatchewan have thus been

variously treated as either the western form or as intermediates between the two varieties.

A number of small morphological differences have been reported between western and eastern Canadian cottonwoods[4], but many western Canadian specimens in the Morden Research Station show minor leaf and twig differences in addition. Morphological differences between cottonwood stands in regions adjacent to the Mississippi River indicate that variation also exists in the southern range of the species[3].

Analysis of chemical variation in plants has been a widely used technique in recent years, especially when plant classification problems existed. For example, in studies of the *Picea glauca–engelmanii* complex, chemical characteristics proved useful in indicating areas where hybridization had occurred[6–8]. No comparable studies have been reported for the intergrading varieties of *P. deltoides*, although only minor chemical variations have been found[9] within collections from Manitoba.

The objectives of the present study were to determine the pattern of chemical variation between overlapping geographical ranges of *P. deltoides* and to relate any differences in chemical content to reported intraspecific variations.

EXPERIMENTAL AND RESULTS

Plants collected from various parts of Canada and the U.S.A. were grown under uniform field conditions at Morden, Manitoba. The original geographical sources of the specimens are given in Table I.

The gas chromatographs used to analyse the samples were a Beckman GC 4 and a Varian 1840, both being equipped with flame ionization detectors and a temperature programmer. The columns used were 0.3% OV-1 and 0.3% OV-17 as described by Bolan and Steele[10]. Helium was used as the carrier gas on one instrument and nitrogen on the other, both at a flow-rate of 100 ml/min. Both gases performed efficiently and their different characteristics did not affect the analyses in any significant way, as shown by regular tests with the same sample on both chromatographs. The temperature programme used for both columns was 10 min of isothermal operation at 190° followed by a rise of 4°/min to 245°. For all analyses, the detector temperature was 300° and the injector temperature 250°.

About 10 mg of coarsely powdered, dried bark were placed in a 5-ml flask and refluxed for 2 h with 2 ml of reagent grade acetone. The extract was filtered and the marc and flask were washed with acetone (1 ml). The combined filtrates were evaporated to dryness under vacuum at room temperature and the residue was shaken manually with successive portions of cold distilled water (0.5–1.0 ml each). The aqueous extracts were filtered through a very small cotton plug and combined in a specially designed 15-ml standard taper test-tube with a capillary base having a capacity of 100 μl (ref. 11). The lower half of the tube was immersed in water at approximately 50° and the solvent was removed *in vacuo*. When the tube was completely dry, 100 μl of Tri-Sil (Pierce Chemical Co.) were added to the residue and allowed to react for 10 min. A 25-μl Hamilton syringe was then used to inject 10 μl of the resulting solution into the gas chromatograph. The chromatograms were recorded on 1 mV full scale deflection recorders.

All retention values were recorded relative to trimethylsilylated arbutin. When necessary, the entire experiment was repeated in order to confirm the presence or

TABLE I

SOURCES OF *P. DELTOIDES* COLLECTIONS

Locality	*River system*	*Latitude (N)*	*Longitude (W)*	*Accession No.*
Alberta*	Red Deer River–Saskatchewan River	50°45′	111°30′	1778-70
	Milk River–Missouri River	49°05′	111°40′	1784-70
Montana*	Marias River–Missouri River	48°30′	111°50′	1787-70
	Yellowstone River–Missouri River	46°30′	105°50′	1788-70
North Dakota*	Little Missouri River–Missouri River	46°45′	103°50′	1791-70
	Missouri River	46°50′	100°25′	1792-70
Saskatchewan*	Saskatchewan River	50°40′	107°57′	1795-70
Manitoba*	Saskatchewan River	54°00′	101°80′	1793-70
	Assiniboine River	50°12′	98°95′	1798-70
		50°12′	98°95′	1799-70
Ontario*	St. Lawrence River	43°10′	80°50′	1797-70
		43°50′	80°50′	2533-71
	Ottawa River–St. Lawrence River	45°40′	76°10′	2491-71
Minnesota**	Mississippi River	45°50′	91°25′	2526-71
		44°15′	92°00′	2527-71
		44°18′	92°00′	2528-71
		44°25′	92°00′	2529-71
		44°25′	92°10′	2530-71
		44°30′	92°20′	2532-71
Illinois (Northern)**	Mississippi River	41°45′	90°15′	2505-71
		41°45′	90°15′	2506-71
		41°45′	90°15′	2507-71
		41°50′	90°10′	2508-71
		41°55′	90°10′	2525-71
Illinois (Southern)**	Mississippi River	41°35′	90°20′	2509-71
	Ohio River	37°25′	89°25′	2500-71
		37°20′	88°35′	2501-71
		37°20′	88°35′	2502-71
		37°20′	88°40′	2503-71
		37°20′	88°40′	2504-71
Missouri**	Missouri River	38°55′	92°29′	2521-71
		38°55′	92°28′	2522-71
		38°55′	92°25′	2523-71
		38°38′	92°11′	2524-71
Mississippi**	Mississippi River	33°55′	91°05′	2516-71
		33°45′	91°00′	2517-71
		33°40′	91°00′	2518-71
		33°40′	91°00′	2519-71
		33°30′	91°00′	2520-71
Louisiana**,***	—	—	—	2510-71
				2511-71
				2513-71
				2515-71

* Normally regarded as western variety (var. *occidentalis*).
** Normally regarded as eastern variety (var. *deltoides*).
*** The exact location of these specimens is not available.

absence of minor peaks, the exact shapes of shoulders, etc., and the heights of peaks in one chromatogram relative to another. From one to six specimens from each locality division (Table I) were used. The samples within each division were found to be very similar to each other and differences within the divisions were much less significant than differences between the "eastern" and "western" types. Representative chromatograms from each locality division are shown in Fig. 1.

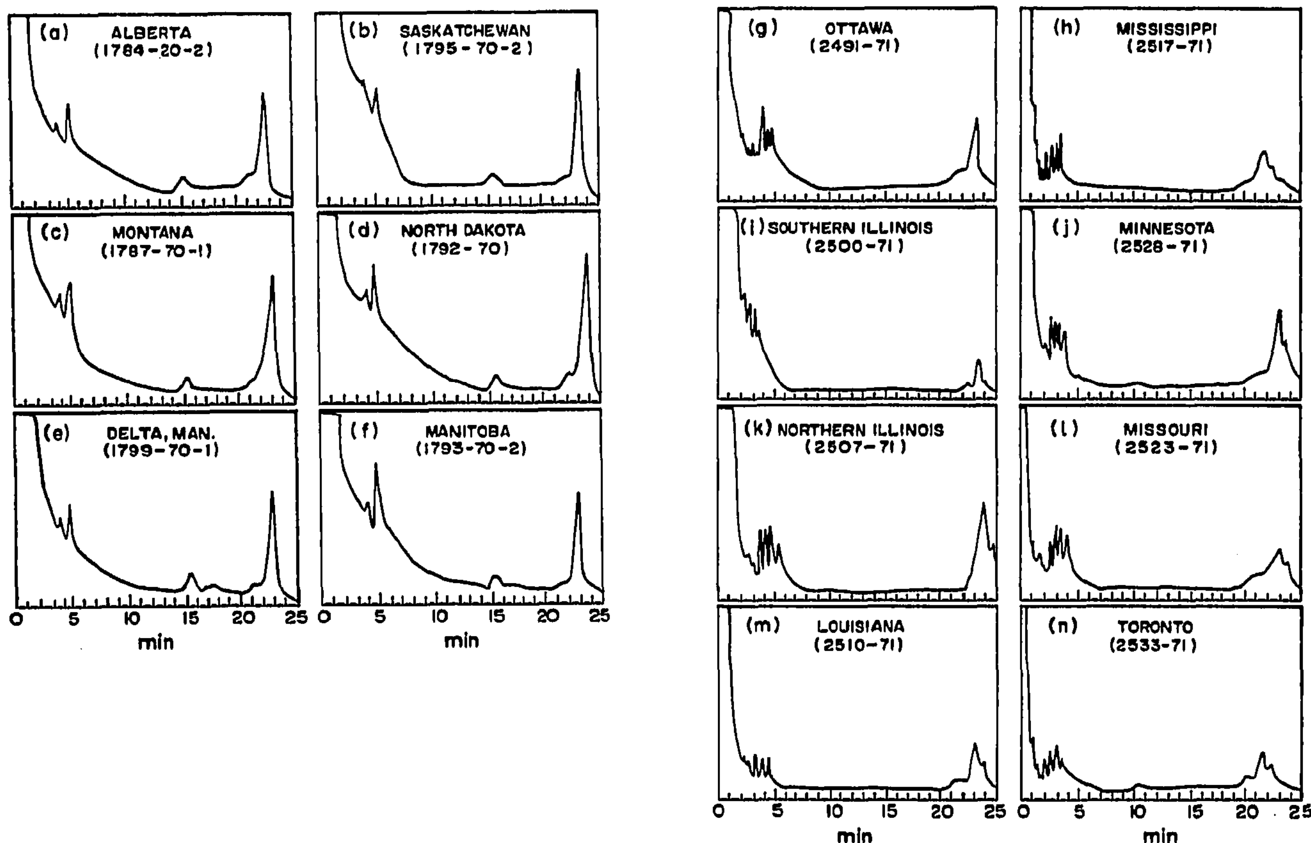

Fig. 1. Representative chromatograms of *P. deltoides* on OV-17.

DISCUSSION

In preparing and analysing the extracts, every precaution was taken to ensure reproducibility and as little variation as possible. The Manitoba samples (1798-70 and 1799-70) were collected in both 1970 and 1971 and were run repeatedly as checks; trimethylsilylated arbutin was used several times each day to ensure further the reproducibility of the retention times. The chromatograms showed that within each locality division, the samples had a remarkably similar glycoside pattern, both quantitatively and qualitatively.

Acetone was used as the extraction solvent because, unlike other solvents, it

does not decompose pure glycosides under the conditions of the test[12]. The bark samples were refluxed with solvent, rather than extracted in a Soxhlet apparatus, as less time was required for complete extraction. The marc remaining after 2 h of extraction was tested by further boiling with fresh solvent, but no glycoside was then detectable.

The weight of plant material used may be varied within wide limits, but 10 mg was found to be about the smallest weight which was still representative of the powdered samples under test. As approximately one tenth of the total silylated extract is injected, this test is effectively carried out on 1 mg of plant material. When larger weights of plant sample are to be used, the same solvent/sample ratio is recommended.

The water extraction step was used routinely so as to avoid carrying non-glycosidic substances into the final solution. If this step is omitted, undesirable contaminants may show on the chromatogram, including fats, hydrocarbons, terpenes and fatty alcohols. When preliminary tests show that no interfering compounds are present, the water extraction step can be omitted and the Tri-Sil added directly to the residue after evaporation of the acetone. The contact between water and extracts should always be minimized in any event[12], and the procedure described requires about 30 min from filtration of the crude extract to addition of Tri-Sil.

The special tube[11] used for the final reaction with Tri-Sil ensures complete contact of the residue with the reagent and permits the withdrawal of a representative sample for injection. The capillary portion helps to prevent evaporation of the reagent and the stoppered tube can be stored in a refrigerator at 5° for several days without deterioration of the silylated extract.

An examination of Fig. 1 shows very clearly that the specimens can be divided into two major groups on the basis of the phenolic glycoside chromatograms. These two groups appear to correspond closely to the geographical ranges of the "eastern" and "western" varieties of *P. deltoides*. The chromatograms consist of three sections: (*i*) at 3 to 6 min; (*ii*) 6 to about 20 min; and (*iii*) after 20 min.

The "eastern" samples (Mississippi, Northern and Southern Illinois, Louisiana, Missouri, Minnesota and Ottawa, Ontario) show multiple peaks between 2 and 4.5 min, no peaks between 5 and 20 min and a triple peak after 20 min. The latter triple peak has a prominent shoulder on the upward slope of the main peak and a smaller second peak or shoulder on the downward slope of the main peak. The Ottawa, Ontario sample (2491-71) was the only exception in that it lacked the smaller second peak on the downward slope of the main peak. Somewhat more variation (mainly quantitative) was noted in the glycoside patterns within the "eastern" samples than within "western" samples.

The "western" samples (Alberta, Saskatchewan, Manitoba, Montana and North Dakota) show a double peak at 4.5–5.5 min, a small but definite peak at *ca.* 15.5 min and a large peak at *ca.* 22–23 min. This large peak is invariably larger than the largest peak in the "eastern" samples and shows a definite shoulder on the upward slope. There is no peak on the downward slope comparable with that in "eastern" samples. As only a small chemical variation is seen within the large "western" sample area, it appears as if one or two samples are properly representative of this whole region. Chromatographic evidence does not support placing of Manitoba and Saskatchewan specimens in the "eastern" var. *deltoides* or even as intermediates between the "eastern" var. *deltoides* and "western" var. *occidentalis*. Samples from

further east and south, including Minnesota, Missouri and Illinois, appear to be typical of the "eastern" var. *deltoides*.

Chromatographic results only partially support the reported morphological distinctions in the southern range of "eastern" var. *deltoides*. Chromatograms from the northern section of the Mississippi River (Northern Illinois and Minnesota) were slightly different from those of Mississippi and Louisiana. These two southern samples were, however, very similar to a sample from the northerly Toronto source which is far removed from the Mississippi River.

The results obtained in this study confirm the value of the method used to correlate glycoside pattern variations with other factors in the *Salicaceae*.

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