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OCCURRENCE AND GENOTYPIC DIFFERENCES OF CHLOROGENIC ACID IN DOUGLAS-FIR FOLIAGE

by

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ABSTRACT

Chlorogenic acid was identified in the foliage of Douglas-fir during the dormant season. Concentrations varied among genotypes and ranged between 75 and 390 p.p.m. in the fresh tissue.

Keywords: Plant physiology, Douglas-fir clones, chromatography, caffeoylquinic acid.

INTRODUCTION

Chlorogenic acid (3-O-caffeoylquinic acid) is one of the most widely distributed phenolic derivatives in plants (Hermann 1956, Buch 1960). Physiological studies have shown that the compound reacts synergistically with indoleacetic acid (Nitsch and Nitsch 1959) and undergoes changes in concentration with age (Koeppe et al. 1970) under the influence of various environmental factors (Zucker 1963, Koeppe et al. 1969) and during flower induction (Zucker et al. 1965). Further, other investigations have led to postulates of possible significant roles by chlorogenic acid in lignin synthesis (Stafford 1965), disease resistance (Farkas and Kiraly 1962), and allelopathic relations among plants (Koeppe et al. 1969). Because of these important effects and possible roles, lack of information on the occurrence of chlorogenic acid in conifers of the northern hemisphere, and our interest in

phenolic compounds which may affect animal preferences for forest trees, Douglas-fir (*Pseudotsuga menziesii*) was chosen for study.

The present paper, therefore, describes the isolation and identification of chlorogenic acid from Douglas-fir foliage, and compares its concentration in four different genotypes grown at the same location.

MATERIALS AND METHODS

Plant material.--Foliage was obtained from 10-year-old, 15-foot-tall Douglas-fir trees grown at the Dennie Ahl Seed Orchard in western Washington. Four clones, SD-10, -13, -19, and -22, were used. Composite samples of about 300 grams each were collected from each clone during the dormant season (February-March) in 2 consecutive years, 1968 and 1969. Each sample was taken from 10 to 15 trees selected at random each year and consisted of 3-inch tips of secondary laterals at a height of about 5 feet up the trees. Reproductive buds were removed if present, and samples were individually sealed in glass containers and brought to the laboratory in a portable cooler.

Separation and identification.--Fresh plant material was chopped and 10-gram samples were defatted in petroleum ether (30-60° C.) and then extracted with 80 percent methanol for 24 hours in Soxhlet. The alcohol extract containing the phenolic compounds was evaporated *in vacuo* and the residue was taken up in 5 milliliters of 80 percent methanol for chromatography on Whatman No. 1 paper. Two dimensional chromatograms were developed with n-butanol:acetic acid:water, BAW, 4:1:5, upper phase (A), or BAW, 4:1:2.2 (B) in the first direction and 2 percent aqueous acetic acid, HOAc (C), in the second direction (Cartwright and Roberts 1954, Hergert 1960). Separations were also accomplished by one-dimensional chromatography on paper freshly washed with 5 percent methanol with solvent methyl-isobutylketone:formic acid: water, KFW, 14:3:2 (D) (Koeppe et al. 1969).

Chlorogenic acid was located on the chromatograms by its fluorescence under an ultraviolet lamp before and after fuming with ammonia (Hulme 1953), and colors were developed with several specific reagents (Roberts and Wood 1951, Swain 1953).

Identification of chlorogenic acid was made through comparison with authentic material by co-chromatography in four solvents, and spectral analysis (ultraviolet on methanol solutions and infrared on KBr pellets). Additional evidence was obtained by hydrolysis of the compound with alkali (2N NaOH for 2 hours under N_2 at room temperature) and with acid (2N HCl for 1 hour at 100° C.) followed by chromatographic separation and identification of the products (co-chromatography with authentic material in three solvents, examination under ultraviolet light, and reactions with spray reagents specific for each product (Roberts and Wood 1951, Cartwright and Roberts 1955, Reio 1958)).

Quantitative determination.--Spots, located under ultraviolet light without exposure to ammonia, were cut from the chromatograms and eluted with methanol. Chlorogenic acid in the solutions was determined spectrophotometrically at a wave length of 328 nanometers (nm) using appropriate blanks. Average concentrations in the tissues were calculated based upon two replicates with three chromatograms per replicate. Data were subjected to analysis of variance and means were separated according to Tukey's test (Snedecor 1961).

RESULTS AND DISCUSSION

Identification.--Comparison of the compound isolated from Douglas-fir foliage with authentic chlorogenic acid (CGA) showed that the two chemicals were identical in fluorescence, chromatographic behavior, spectrophotometric characteristics, and hydrolysis products. Thus, under ultraviolet light, the compound exhibited blue fluorescence which changed to green upon exposure to ammonia (Hulme 1953). Reactions with several spray reagents were also typical of CGA (Roberts and Wood 1951, Hulme 1953, Swain 1953). Co-chromatography with authentic CGA gave one spot in three solvents and a pair of spots for the *cis* and *trans* forms, characteristic of cinnamic acid derivatives, in 2 percent HOAc (Williams 1955). Average R_f values (from three papers) were: 0.60 (A); 0.64 (B); 0.54, 0.72 (C); and 0.49 (D). Absorption spectra were similar to those in the literature (Barnes et al. 1950, Hulme 1953, Haslam et al. 1964)-- λ max. (70 percent methanol) = 328 nm with shoulders at 300, 233, and 216 nm; ν max. (KBr) = 1,260, 1,630, and 1,720 cm^{-1}. Further, both acid and alkaline hydrolyses yielded two spots as determined by chromatographic analysis of the products. The chromatographic behavior of these spots and their reactions with several spray reagents (Roberts and Wood 1951, Cartwright and Roberts 1955, Reio 1958) were identical with those of the known hydrolysis products of CGA--quinic and caffeic acids. R_f values of the acids were: 0.26 (A) and 0.89 (C) for quinic acid, and 0.79 (A) and 0.25, 0.61 (C) for caffeic acid.

Concentration.--Average concentration of CGA in the fresh foliage tissue (in p.p.m.) varied significantly ($P = 0.05$) among the four clones as follows: SD-10 = 170; SD-13 = 75; SD-19 = 390; and SD-22 = 113. On dry-weight basis, CGA concentrations (in milligrams per 100 grams of tissue) were: SD-10 = 39; SD-13 = 17; SD-19 = 91; and SD-22 = 26. These data indicate that Douglas-fir foliage contained low to moderate levels of CGA compared with other plant species (Sondheimer 1958).

Significance.--Chlorogenic acid is widely distributed in angiosperms. However, as far as is known, there are only two early reports of CGA in gymnosperms: *Podocarpus* and *Gnetum* (Hegnauer 1962). This study, therefore, reports, for the first time, the occurrence of CGA in the foliage of a northern-temperate conifer, Douglas-fir. Importance of this finding is not immediately apparent. However, the

genotypic differences in CGA found here, and the reports mentioned above on important effects and possible roles of CGA in plants, suggest that additional work to explore relationships between differences in important characteristics (e.g., seed-producing ability, susceptibility to injury by various biota, etc.) of various families of Douglas-fir and their CGA content may be useful in tree breeding programs.

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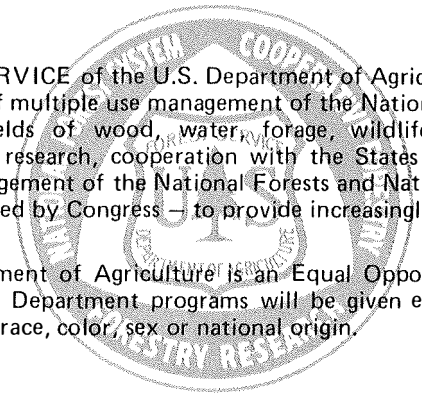

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