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DIHYDROCONIFERYL ALCOHOL IN DEVELOPING XYLEM OF *PINUS CONTORTA*

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Key Word Index *Pinus contorta* spp. *latifolia*; Pinaceae; lodgepole pine; tracheid differentiation; phenylpropanoids; dihydroconiferyl alcohol; 3-(3-methoxy-4-hydroxyphenyl)-propan-1-ol.

Abstract Dihydroconiferyl alcohol was identified by GC/MS as an endogenous constituent of developing xylem in *Pinus contorta* ssp. *latifolia*. Its role in regulating wood formation in *P. contorta* is discussed.

INTRODUCTION

Exogenous 3-(3-methoxy-4-hydroxyphenyl)-propan-1-ol (dihydroconiferyl alcohol, DCA) enhances gibberellin promotion of lettuce hypocotyl elongation [1], auxin promotion of cucumber hypocotyl elongation [2] and auxin-induced cell expansion [3]. DCA acts synergistically with kinetin to promote soybean callus growth and is active in tobacco callus and radish leaf senescence cytokinin bioassays [4]. DCA also promotes wilting of pine seedlings [5].

Before its role as a growth regulator was known, it was isolated and identified from bark of *Pinus sylvestris* [6], from maple syrup (xylem sap of *Acer saccharum* concentrated by extended boiling) [7], and in water prehydrolysates (175°) of pine wood [8]. Subsequently, Higuchi *et al.* [9] reported two glucosides, *viz.* DCA γ-*O*-β-D-glucopyranoside and DCA 4-*O*-β-D-glucopyranoside, in air-dried leaves of *Pinus contorta*. As a plant growth regulatory molecule, DCA was first identified in cotyledons of lettuce [1]. Investigations by Purse *et al.* [10] yielded an unidentified non-polar fraction from the spring sap of sycamore (*Acer pseudoplatanus* L.) that promoted soybean callus growth, and GC/MS evidence was presented for DCA in a similarly bioactive fraction derived from maple syrup [4]. The first report that DCA is in developing xylem is provided here together with bioassay findings that suggest DCA may have a regulatory role in vascular development.

RESULTS

A purified, derivatized (TMSi) fraction from developing xylem analysed by EI GC/MS (24 eV) yielded *m/z* (rel. int.): 326 $[M]^+$ (96), 311 $[M - CH_3]^+$ (30), 236 $[M - 90]^+$ (36), 209 $[M - 117]^+$ (40), 206 $[M - 120]^+$ (100), 179 $[M - 147]^+$ (30). The mass spectrum was in full agreement with that of synthetic TMSi-DCA and with previous reports [4, 11]. This result was readily confirmed using tissues obtained from two additional trees in midsummer. The similarity between these mass spectra and that of authentic TMSi-DCA indicates that DCA is endogenous to developing xylem of *P. contorta*. Due to a lack of suitable internal standard, tissue levels of endogenous DCA were not quantified, but GC peak areas of extracted TMSi-DCA suggested levels similar to those of TMSi-IAA-OMe in mid-summer [12].

Eluting from the GC as a small shoulder on the late side of extract-purified TMSi-DCA was a substance, unidentified, that yielded the spectrum *m/z* (rel. int.): 326 $[M]^+$ (100), 324 (59), 311 (28), 305 (37), 293 (17), 236 (25), 209 (28), 206 (63), 179 (18). The *m/z* 326 base peak and molecular ion suggests this compound is an isomer of DCA.

Applications of exogenous DCA (100 ppm wt/wt) in anhydrous lanolin were made to transverse apical surfaces of 15-cm-long, 1-year-old, disbudded-defoliated cuttings of *P. contorta*, using six cuttings per treatment. Cuttings were stood upright in tap water and maintained at 20–25° under 18 hr photoperiod for 3 weeks, renewing applications weekly. Subsequent microscopy of transverse sections showed both cambial cell division and differentiation of thick-walled earlywood tracheids to have occurred in DCA-treated cuttings. Control cuttings that received anhydrous lanolin exhibited no evidence for either cell division or tracheid differentiation. This experiment was done with branch cuttings harvested in late winter and then stored in darkness at 4° with leaves and buds intact for 2 months. In two subsequent experiments that employed freshly collected, disbudded-defoliated, branch cuttings, exogenous DCA had no observable promotory effect on either cambial activity or xylem development. DCA has been found to inhibit IAA-degrading enzymes [2] and in view of the high IAA concentrations in the cambial region [12], it could well be that DCA antagonizes IAA degradation in the regulation of stem growth and development.

The presence of DCA glucosides in *P. contorta* leaves [9] could, but need not necessarily, be physiologically related to DCA levels of developing xylem. Evidence for DCA glucosides in developing xylem was not sought in my investigations; however, coniferin, the glucoside of coniferyl alcohol, is well established to be present in the cambial region [13] and absent from leaves [14] suggesting that glucosylation of DCA could also occur in the stem. Moreover, it has been stated that glucosides do not move in the phloem in appreciable amounts (Ziegler, p. 217 in [15]).

EXPERIMENTAL

Collection of developing xylem. Specimens of *Pinus contorta* var. *latifolia* (Engelm.) growing at the side previously described [16] were felled in mid-summer. Developing xylem (7-year-old main stem region of 20-year-old-trees) was collected as previously described [12], wrapped in aluminium foil and immediately submerged in liquid N_2. The frozen tissue was pulverized at liquid N_2 temperatures in the laboratory in a hand-operated meat grinder. The frozen powdered tissue was lyophilized and *ca* 5 g (dry wt) of tissue were used for each analysis.

Extraction of DCA. Tissues were extracted with pre-chilled (4°) 80% MeOH (15:1, vol.:dry wt) in darkness at 4° for 4 hr, filtered (Whatman No. 1) under red. pres., and the residue re-extracted and filtered twice more. The three combined extracts were reduced to aq. *in vacuo* (30°) by thin film rotary evaporation, frozen, thawed and centrifuged (22×10^3 *g*) for 1 hr at 4°.

Purification procedures. The supernatant at pH 3.0 (1 N HCl) was partitioned 3 × against an equal volume of Et_2O. The combined Et_2O extracts were dried in N_2 and CH_2N was added in order to methylate the carboxyl groups of substances for another investigation (Savidge, R. A., unpublished).

EtOH-soluble material was injected onto a steel column (150 × 10 mm i.d.) packed with ODS Hypersil (Shandon, Cheshire, England). Reversed phase HPLC was done with two Constametric III pumps (Laboratory Data Control (LDC), Riviera Beach, FL) controlled by a Gradient Master (LDC). A 5–100% methanol gradient (water buffered at pH 7.0 with triethylammonium hydrogen carbonate) was eluted at 5 ml min^{-1}, monitoring the eluate with a Spectromonitor III UV detector (LDC) at 282 nm. The fraction eluting between 41–43% MeOH, corresponding to authentic DCA, was collected and reduced to dryness at 30° *in vacuo* by rotary evaporation.

Derivatization and analysis by GC/MS. The residue in BSA was derivatized at 60° for 1 hr prior to injecting onto a 0.4 × 150 cm glass column (3% OV-1 on Gas Chrom Q) with He flowing at 35 ml min^{-1}, temp. programming from 170–300° at 8° min^{-1}. The jet separator was set at 260°, the Kratos MS 30 ion source at 180°. Scanning was done at 3 sec dec^{-1} at a resolution of 1000 over *m/z* 28-600, using DS-50S software.

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REFERENCES

1. Shibata, K., Kubota, K. and Kamisaka, S. (1974) *Plant Cell Physiol.* **15**, 191.
2. Sakurai, N., Shibata, K. and Kamisaka, S. (1975) *Plant Cell Physiol.* **16**, 845.
3. Kamisaka, S., Sakurai, N. and Shibata, K. (1983) *Plant Cell Physiol.* **24**, 369.
4. Lee, T. S., Purse, J. G., Pryce, R. J., Horgan, R. and Wareing, P. F. (1981) *Planta* **152**, 571.
5. Ueda, T., Oku, H., Tomita, K., Sato, K. and Shiraishi, T. (1984) *Nippon Shokubutsu Byori Gakkaiho* **50**, 166.
6. Oksanen, H. (1965) *Suomen Kemistilehti* **B38**, 257.
7. Underwood, J. C., Filipic, V. J. and Bell, R. A. (1969) *J. Ass. Off. Analyt. Chem.* **52**, 717.
8. Sears, K. D., Beelik, A., Casebier, R. L., Engen, R. J., Hamilton, J. K. and Hergert, H. L. (1971) *J. Polymer Sci. C* **36**, 425.
9. Higuchi, R., Aritomi, M. and Donnelly, D. M. X. (1977) *Phytochemistry* **16**, 1007.
10. Purse, J. G., Horgan, R., Horgan, J. M. and Wareing, P. F. (1976) *Planta* **132**, 1.
11. Riley, R. G. and Kolattukudy, P. E. (1975) *Plant Physiol.* **56**, 650.
12. Savidge, R. A., Heald, J. K. and Wareing, P. F. (1982) *Planta* **155**, 89.
13. Freudenberg, K. and Harkin, J. M. (1963) *Phytochemistry* **2**, 189.
14. Marcinowski, S. and Grisebach, H. (1977) *Phytochemistry* **16**, 1665.
15. Zimmermann, M. H. (1964) *The Formation of Wood in Forest Trees.* Academic Press, New York.
16. Savidge, R. A. and Wareing, P. F. (1984) *Can. J. For. Res.* **14**, 676.