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IDENTIFICATION OF ABSCISIC ACID IN *ABIES BALSAMEA*

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Abstract—Abscisic acid was positively identified in dormant buds of the conifer *Abies balsamea* on the basis of chromatographic behavior, inhibition in a bioassay, and mass spectrometry. Sublimation was a valuable adjunct to chromatographic separation techniques.

INTRODUCTION

ALTHOUGH the growth regulator abscisic acid (ABA)[1-3] has been detected in a wide variety of plants, including many deciduous trees,[4,5] there are few reports of its isolation from conifers.[4,6-8] There is circumstantial evidence for the occurrence of ABA in balsam fir (*Abies balsamea* L.),[9-13] and, before commencing investigations of its physiological role, it was expedient to demonstrate that ABA is indeed present in the buds of this species.

RESULTS AND DISCUSSION

The presence of ABA in dormant buds of balsam fir was clearly demonstrated by the following observations. Bioassay revealed strong inhibitory activity associated with material from the appropriate R_f zone at each stage in successive chromatographic purifications of the acidic extract. This activity was retained on sublimation. Material, corresponding in retention time with MeABA and displaying the expected inhibitory activity, was isolated by preparative GLC of the esterified sublimate.

GLC analysis, after UV irradiation of the isolated material, revealed, in addition to the presumptive MeABA, the emergence of a new peak whose (longer) retention time corresponded to 2-*trans* MeABA. Cochromatography of the irradiation products with the mixture of 2-*cis* and *trans* isomers obtained by irradiating a sample of authentic MeABA[6] confirmed their identity. Finally, the MS of the natural and 2-*trans* isomers of MeABA

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derived from balsam fir showed essentially the same characteristic fragmentation pattern as authentic MeABA.[14,15] Sublimation was found to be a very useful adjunct to chromatography in the separation of ABA from the complex mixture of acids extracted from balsam fir.

EXPERIMENTAL

Plant Material. The whorl of dormant buds (plus a few needles and a short length of the proximal internode) was collected from the apex of terminal and lateral shoots in the uppermost one or two whorls of wild balsam fir trees on 2–3 September 1971 and immediately immersed in 80% MeOH.

Extraction. The plant material (8·6 kg fr. wt) was homogenized in 80% MeOH, then left to soak for 4–5 days at 2° in the dark. The extract was filtered through glass wool and the solids re-extracted with fresh 80% MeOH overnight. Most of the MeOH was removed from the combined extracts by evaporation under reduced pressure at 40°. The resulting aqueous suspension was acidified to pH 3·0 with 5% H_2SO_4 and extracted 5× with *ca.* 0·14 vol. Et_2O. The combined ether fractions were then extracted 4× with *ca.* 0·14 vol. sat. $NaHCO_3$ solution. The $NaHCO_3$ layer was adjusted to pH 7·5, washed with *ca.* 0·2 vol. Et_2O, then acidified to pH 3·0 and extracted 3× with *ca.* 0·2 vol. Et_2O. The combined ether fractions from the acidic solution were dried over $MgSO_4$ and evaporated to dryness under vacuum.

PC and TLC. The residue was dissolved in Et_2O, applied to Whatman No. 3MM paper and developed in *iso*PrOH–NH_4OH–H_2O (8:1:1). The material in the β-inhibitor region, 0·5–0·8 R_f, was eluted with MeOH and, with a marker of authentic ABA chromatographed on silica gel GF_{254} first in benzene–EtOAc–HOAc (14:6:1), then in benzene–$CHCl_3$–HCO_2H (2:10:1; plates developed 3×).

Sublimation. After the TLC, the amorphous material separated was sublimed in a tube at 0·1 mm Hg pressure while raising the temperature slowly to 220°. Bioassay revealed that the sublimate retained inhibitory activity, while the residue was inactive.

GC, Irradiation and MS. Before GLC experiments, the sublimate was esterified by treatment with diazomethane in ether. Analytical and preparative GLC was carried out on a Varian Aerograph Model 705 gas chromatograph equipped with flame ionization detector. With the exception of the final preparative separation of the *cis* and *trans* MeABA isomers, all separations were effected using a 305 × 0·64 cm (o.d.) stainless steel column, packed with 5% QF 1 on 50/60 mesh chromosorb W AW DMCS. Operation was isothermal at column temp. 200°; He was the carrier gas with column flow-rate about 120 ml/min. Under these conditions, the R_t of MeABA was around 9 min. The column employed for the preparative separation of *cis* and *trans* MeABA for MS studies was stainless steel (305 × 0·64 cm), packed with 3% SE 30 on 60/80 mesh chromosorb W AW DMCS. The isomerization of MeABA was effected by exposure to direct sunlight for 1–2 days in flint glass sample vials. The MS were obtained using a Hitachi–Perkin–Elmer RMU-6D mass spectrometer with direct inlet system, temp. 170°, chamber voltage, 70V. The molecular ion of authentic MeABA at m/e 278 is of low relative abundance[14] and was barely discernible in the spectra of the *cis* and *trans* isomers from balsam fir. The peak of m/e 260 (M^+–H_2O) was present, however, and all of the most significant ions of MeABA, including those at *m/e* 190 (base peak), 162, 134, 125 and 91, were readily apparent in the spectra of the two isomers from the fir extract.

Bioassay. Inhibitory activity was determined with the oat, first internode test.[16] It was established that both ABA and its methyl ester are active in this bioassay.

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