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GIBBERELLIN A_9 GLUCOSYL ESTER IN NEEDLES OF *PICEA SITCHENSIS*

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Key Word Index—*Picea sitchensis*; Sitka spruce; Pinaceae; Gibberellin A_9 glucosyl ester; identification; preparation; biological activity.

Abstract—A polar gibberellin-like substance present in needles of *Picea sitchensis* was identified as GA_9-β-D-glucosyl ester on the basis of enzymatic hydrolysis and identification of the aglycone by GC–MS. The biological activity of the synthetic material was tested in two bioassays.

INTRODUCTION

In a previous publication [1] we reported on the occurrence of two GA-like fractions in needles of *Picea sitchensis* Carriere, and partially characterised the less polar of the two fractions as an isomer of GA_9. We report here on the identification of the more polar fraction as GA_9-β-D-glucosyl ester (GA_9GE) and on the activity of this substance in the lettuce hypocotyl and dwarf rice bioassays.

RESULTS AND DISCUSSION

15 kg of needles were extracted with 80% MeOH and purified by (1) cation exchange chromatography, (2) PVP column chromatography, (3) charcoal–celite chromatography, (4) silicic acid partition chromatography.

The distribution of biological activity from a silicic acid partition column eluted under the conditions previously described by Durley *et al.* [2] showed a major component at fractions 6–8 and another at fractions 45–46. TLC (solvent A) of a portion of the polar material in fractions 45 and 46 gave a single biologically active zone at R_f 0.3. Fractions 45 and 46 from the first column were purified by partition chromatography on a silicic acid column eluted with solvent A. The distribution of biological activity from this column showed a single component at fractions 22–25. The active fractions were combined and evaporated to give *ca* 240 μg of an amorphous solid with a GA activity in the lettuce hypocotyl test equivalent to 40 μg of GA_3. Silylation of this material followed by GLC analysis showed three major components. However, attempts to localise biological activity by means of preparative GLC were unsuccessful and resulted in a small portion of the biological activity appearing as a discrete peak immediately after the solvent peak whilst the remainder was spread over a large zone of the chromatogram indicating decomposition of the material on the GLC column.

Treatment of a part of the material with a purified β-glucosidase preparation, and analysis of the products by TLC (solvent A) gave a single zone of biological activity at R_f 0.8. These results indicated that the substance in question was a β-D-glucoside or glucose ester of a non-polar gibberellin.

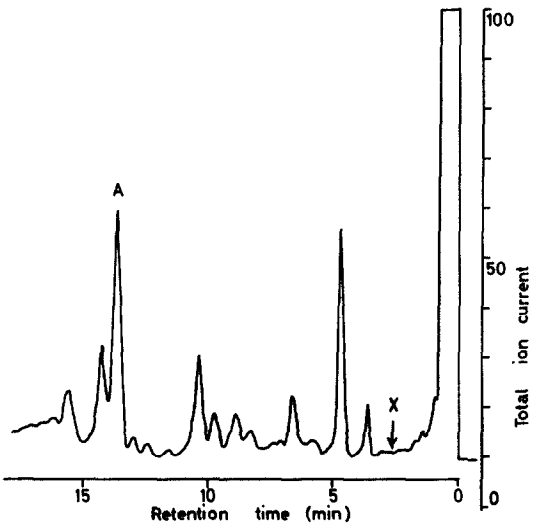

Fig. 1. Total ion current trace obtained during GC–MS of the hydrolysed sample; peak A represents TMSi GA_9.

The remaining material was treated with β-glucosidase and the reaction mixture evaporated and extracted several times with EtOAc. The extracted material was purified by partition chromatography on a silicic acid column (solvent A). The fraction containing the major proportion of the biological activity was silylated and analysed by GC–MS. The total ion current trace for this fraction is shown in Fig. 1. MS were taken at 7 sec intervals from point X to the end of the TIC trace. The MS taken at peak A were identical to those of GA_9 TMSi ester showing ions at *m/e*, 388(1.2)M^+, 373(16.3), 298(30.8), 281(4.5), 271(12.7), 270(57.8), 227(22.2), 226(32.2), 225(15.1), 183(9.4), 75(36.3), 73(100). The above evidence strongly indicated that the polar GA was GA_9-β-D-glucosyl ester. Since GA_9 has no free hydroxyl group, the carboxyl group is the only possible point of attachment of the glucose moiety. As further evidence for the identity of the compound, GA_9-β-D-glucosyl ester was

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Table 1. Lettuce (Arctic King) hypocotyl bioassay. Data expressed as hypocotyl length in mm ± standard error ($n = 10$)

Gibberellin	μg Gibberellin/ml				
	10^{-2}	5×10^{-2}	10^{-1}	5×10^{-1}	10^{0}
GA_9	3.2 ± 0.2	4.7 ± 0.6	6.0 ± 0.4	9.0 ± 0.5	10.3 ± 0.3
GA_9-β-D-glucosyl ester	2.3 ± 0.4	3.2 ± 0.2	3.8 ± 0.3	6.6 ± 0.4	8.6 ± 0.6
Controls 2.1 ± 0.2					

Table 2. Dwarf Rice (Tan-gimbuzo) bioassay. Data expressed as the length (mm) of the 2nd leaf sheath ± standard error ($n = 4$)

Gibberellin	ng Gibberellin/plant			
	12.5	25	125	250
GA_9	14.7 ± 1.1	18.5 ± 2.0	28.5 ± 2.9	33.7 ± 2.5
GA_9-β-D-glucosyl ester	11.2 ± 1.3	13.7 ± 0.9	21.8 ± 2.1	25.8 ± 1.8
Controls 9 ± 0.9				

synthesised by the method of Hiraga *et al.* [3] and was shown to have identical chromatographic properties to those of the naturally occurring material. In addition it was also hydrolysed by β-glucosidase at a similar rate to that of the naturally occurring substance, and showed substantial decomposition under the GLC conditions used. Although injection of large quantities (>5 μg) of the synthetic material produced a GLC peak which was shown by GC–MS to be due to the intact tetra-TMSi GA_9GE, TMSi GA_9 was also identified as a breakdown product on these chromatograms. The observed behaviour of small amounts (< 2 μg) of the synthetic material on GLC clearly paralleled that of the naturally occurring material. From the above data we concluded that it is most probable that the more polar GA-like substance from needles of *Picea sitchensis* is GA_9-β-D-glucosyl ester.

This finding confirms the previous report [4] on the occurrence of gibberellin glucosyl esters in higher plants and furnishes the first evidence for the presence of this type of GA conjugate in leaves.

The biological activity of GA_9-β-D-glucosyl ester was compared with GA_9 in the lettuce hypocotyl bioassay and in the dwarf rice bioassay. (Table 1) and showed that the GA_9 ester was almost as active as the free GA_9, suggesting facile hydrolysis of the glucosyl ester under the condition used in the bioassays. This is in agreement with the work of Hirage *et al.* [3] which demonstrated that the glucosyl esters of GA_1 and GA_4 are readily hydrolysed in rice seedlings. Liebisch [5] recently reported that GA_3 glucosyl ester is readily hydrolysed in germinating maize seedlings but if this compound is applied 14 days after germination no hydrolysis can be detected.

The rapid hydrolysis of GA_9 glucosyl ester in actively growing tissues and changes in the amount of this compound present in buds at bud burst if *Picea sitchensis* [1] indicates that the endogenous compound may represent a storage form of GA that can be released as free GA during periods of fast growth.

EXPERIMENTAL

Chromatography. Si gel partition chromatography with solvent A (EtOAc–$CHCl_3$–HOAc, 15:5:1) was carried out as follows: (i) a column 20 × 1 cm was packed with Si gel (Woelm) suspended in the solvent; (ii) the sample was placed on the top of the column dissolved in 300 μl of the running solvent; (iii) 20 × 3 ml fractions followed by 10 × 10 ml fractions were collected at a flow rate of 3 ml/min. TLC was performed on 0.5 mm thick layers of Si gel GF, pre-washed with EtOAc and chromatograms were developed with EtOAc–$CHCl_3$–HOAc (15:5:1). Derivatisation procedures, GLC and GC–MS conditions were those described previously[1].

Enzymatic hydrolysis. Enzymatic hydrolysis was carried out using β-glucosidase from sweet almonds (Boeringer) at 37° in 0.1 M acetate buffer at pH 5.4 for 48 hr.

Bioassay. Biological activity was monitored throughout by the lettuce hypocotyl bioassay.

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