CHROM. 5449

Separation of gibberellin A_1 and dihydrogibberellin A_1 by argentation partition chromatography on a Sephadex column*

In samples of [3,4-^{3}H]gibberellin A_1 prepared by partial reduction of gibberellin A_3 with tritium the fully saturated dihydrogibberellin A_1 isomers are frequent contaminants[1]. [3,4-^{3}H]Gibberellin A_1 can be purified by chromatography on silicic acid[2] but separation from the dihydro derivatives is difficult. Impregnating the adsorbent with silver nitrate improved the resolution since gibberellin A_1 complexed with silver ions and was more strongly adsorbed, but neither preparative thin-layer chromatography (TLC) nor column chromatography on silver nitrate-treated silicic acid proved satisfactory for obtaining pure gibberellin A_1. Because of tailing the slower-moving gibberellin A_1 zone always contained some dihydro derivative. Severe loss of gibberellins also occurred during the TLC separation.

Silver nitrate is widely used as a complexing agent in adsorption chromatography to separate substances differing only in the number and geometry of their double bonds[3], but can also alter the partition coefficients of such compounds[4]. It may be incorporated in stationary phases to achieve specific separations by gas–liquid chromatography, and has been used in the fractionation of fatty acid esters by counter-current distribution[5,6]. Because many gibberellins are conveniently separated by partition chromatography on Sephadex columns[7] we examined the effect of silver ion complexing on the distribution of gibberellin A_1 and its dihydro derivatives. Whereas a mixture of these compounds was not resolved in the unsupplemented solvent systems tested, addition of silver nitrate altered the partition coefficient and permitted a separation of gibberellin A_1 from the fully saturated components.

Materials and methods

[^{3}H]Gibberellin mixture. The sample was obtained by catalytic reduction of gibberellin A_3 (K & K Laboratories, Jamaica, N.Y.) with tritium and 5% palladium/calcium carbonate[1,8]. Under the conditions used gibberellin A_1 and a small amount of the dihydrogibberellin A_1 were formed, and were separated from unreduced gibberellin A_3 and hydrogenolysis products by partition chromatography on Sephadex[1].

Sephadex partition chromatography. Silver nitrate (50 g) was dissolved in aqueous phase (500 ml) of the solvent mixture benzene–ethyl acetate–acetic acid–water (55:25:30:50) and the solution used to saturate Sephadex G-25 [fine bead form, 120 g, Pharmacia (Canada) Ltd., Montreal]. The swollen Sephadex was freed from excess aqueous phase, resuspended in organic phase, and packed in a 2.6 × 90 cm column. The column was equilibrated by irrigating with organic phase (approximately 200 ml) before the sample was applied. The sample in aqueous phase (2.5 ml) was adsorbed in dry Sephadex G-25 (1 g) and the impregnated gel packed on top of the column. Organic phase was passed through the column at approximately 120 ml/h and fractions (20 ml) were collected.

Gibberellins were identified by chromatographing a sample from each fraction on Silica Gel H plates (E. Merck & Co., Darmstadt, G.F.R.) previously dipped in

* Issued as N.R.C.C. No. 12032.

5% silver nitrate, dried, and activated at 100°. Zones were detected by spraying with sulfuric acid and heating at 110° (ref. 9), or by scanning for radioactivity. Elution of radioactivity was followed by counting a portion of each fraction with a liquid scintillation spectrometer.

Recovery of gibberellin A_1. Appropriate fractions were combined and evaporated to dryness at 40° *in vacuo*. The residue was redissolved in a mixture of ethyl acetate and very dilute (pH 2) sulfuric acid. Most of the gibberellin A_1 was removed from the aqueous phase by extracting with two additional portions of ethyl acetate, and the extracts were washed with water before being evaporated to dryness. The product was redissolved in ethanol, adsorbed on a small amount of silicic acid (Mallinckrodt CC-4, 200–320 mesh size) and, after removal of the solvent, packed on top of a column (2 × 15 cm) of the same adsorbent. The column was developed first with chloroform (100 ml), then 30% (750 ml), and finally 50% ethyl acetate (750 ml) while 10 ml fractions were collected. Gibberellin A_1 was eluted in fractions 85–140.

Results and discussion

Elution profiles for gibberellin A_1 and its dihydro derivative from Sephadex partition chromatography columns are shown in Fig. 1. Adding silver nitrate to the stationary phase retarded gibberellin A_1 which has an exocyclic methylene group at C-8 and can form a complex with silver ions. Recovery of gibberellin A_1 from frac-

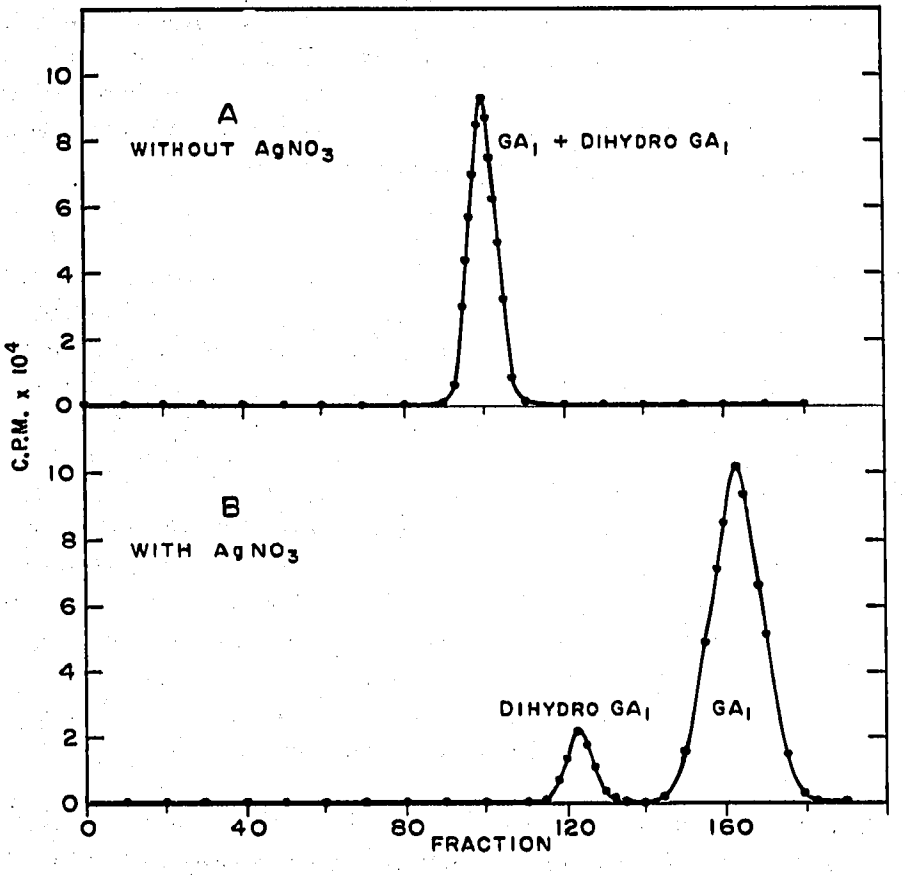

Fig. 1. Separation of ^{3}H-labeled gibberellin A_1 (GA_1) and dihydrogibberellin A_1 mixture on a 2.6 × 90 cm column of Sephadex with the solvent system benzene–ethyl acetate–acetic acid–water (55:25:30:50). Portions of the 20 ml fractions collected were counted for radioactivity.

tions 156–176 of the silver nitrate-treated column was not quantitative, but with the procedure used overall recoveries of 65–75% were obtained. Presumably the main cause of loss was hydrolysis of the lactone function under the acidic conditions used, but oxidation by free nitric acid may have been a factor. Silver ions partitioned into the organic phase and a small amount of silver acetate was present in each fraction collected. To remove this and impurities arising from degradation of the gibberellin chromatography on silicic acid was adopted as the final step in the procedure. Because of the slow loss of complexing agent from the stationary phase, columns could not be used more than three times without being either percolated with fresh aqueous phase or repacked.

Argentation partition chromatography provides a method for purifying [3,4-^{3}H]gibberellin A_1 and may be equally valuable for the separation of other unsaturated substances, especially acid-sensitive polar compounds which require solvent mixtures containing acetic acid.

I am grateful to Mr. J. C. BERRIGAN for his expert technical assistance.

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Received May 10th, 1971