PREPARATION OF TRITIUM-LABELLED GIBBERELLIN GA_3 AT HIGH SPECIFIC ACTIVITY

G. Ayrey* and J.M. Chapman[Δ]
Queen Elizabeth College (University of London), Campden Hill,
London W8 7AH, U.K.

SUMMARY

Gibberellin GA_3 was prepared in low yield but at high specific activity (6.68 Ci/mmol) by platinum catalysed exchange with tritiated water at 80°C for 16 hours.

Key words: Gibberellin GA_3, Tritium, Catalysed-exchange

INTRODUCTION

Gibberellins labelled with tritium have been extensively used to investigate the localisation and metabolic fate of these growth substances within various plant tissues.[1-3] Previously radiolabelled GA_3 has been prepared using the Wilzbach method[4-6] which is well known for the disadvantage of producing compounds of moderate specific activity with the possibility of additional small quantitites of very high specific activity impurities. For circumstances where a high specific activity is required, heterogeneous catalysis in a tritiated solvent offers a greater chance of success[7] with the added advantage of easier purification since the impurities are produced with similar specific activity. We required a source of gibberellin GA_3 with very high specific activity for use as an internal standard for a series of extractions of gibberellins from plant material. The high specific activity was essential as plant material contains only small amounts of gibberellin and it was important that the added radioactive

*Isotope Unit
[Δ]Department of Biology

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standard should not contribute significantly to the natural pool of growth substances present in the tissue.

Platinum catalysed exchange with tritiated water is normally carried out at temperatures of 120-140°C for extended periods of time. Unfortunately the lactone ring of the gibberellins is fairly susceptible to hydrolysis under these conditions. Nevertheless, sufficiently mild conditions were found which yielded adequate material for our purposes, though after extensive purification by thin layer chromatography the yield was less than 3%.

EXPERIMENTAL AND RESULTS

Preparation of tritiated GA_3

Platinum oxide (0.05g) was washed into a 5 cm^3 'break-seal' reaction tube using ethanol/acetic acid (3:1) (4 cm^3) and was reduced with H_2 gas for 45 minutes. The solvent was reduced in volume and gibberellic acid (0.005g) in ethanol (2 cm^3) was added. The tube was attached to a high vacuum line and all the ethanol/acetic acid mixture pumped off. Tritiated water (1 cm^3, ∿ 50 Ci) was then distilled in, the tube was sealed under vacuum and heated in an oil bath at 80°C for 16 hr. The tritiated water was then removed in vacuo via the break-seal, and portions of ethanol (3 x 3 cm^3) distilled into the tube and off again to remove exchangeable tritium. Finally the product was dissolved in acetone (3 cm^3) and removed from the vacuum line. The acetone solution and washings (total of 8 cm^3) were transferred by syringe to a small filter beaker and the solution filtered to remove the catalyst. The resulting clear solution was evaporated to ∿ 0.5 cm^3 at room temperature with a gentle stream of nitrogen.

Purificataion

Up to 200 µl of the acetone solution was loaded in a streak onto thin layer silica gel G plates (20 x 5 cm Eastman No.6061) which had been previously activated for 1 hr at 80°C. A separate marker spot of 0.025 µCi [^{14}C]-GA_3 (1.7 mCi/mmol : The Radiochemical Centre, Amersham) was also included and the plate developed in a mixture of ethyl acetate : chloroform : acetic acid (15 : 5 : 1).

Radioactivity on the plates was located using a Packard chromatograph scanner and the area corresponding to the position of the marker GA_3 (R_f 0.5) scraped off and eluted with acetone (3 cm^3). After filtration the acetone solution was evaporated to about 0.5 cm^3 and the procedure repeated using a mixture of benzene : n-butanol : acetic acid (80 : 15 : 5; R_f 0.2). Finally the whole procedure was repeated again using a mixture of benzene : acetic acid : water (8 : 3 : 5; R_f 0.0). After extraction of the purified GA_3 from the TLC plate with acetone (3 cm^3) the acetone was removed at room temperature with a stream of nitrogen. At this stage the product co-chromatographed with pure authentic GA_3 and gave only one peak on the radiochromatogram. As the quantity was too small to be weighed accurately, it was dissolved in water (4.3 cm^3) and assayed as described below.

Assay of GA_3 amount

The amount of GA_3 present in the aqueous solution was determined using the barley endosperm assay[8] which is specific for gibberellins and sensitive to extremely low levels of the compound. This produced a value of 0.25 ng of GA_3 in 8 µl of aqueous solution (an overall yield of 134.4 ng : 2.7%). The radioactivity in the same volume was determined by liquid scintillation counting to be 10,940 dpm (radiochemical yield 2.65 µCi). The specific activity of the GA_3 obtained was thus 6.88 Ci/mmol.

CONCLUSIONS

The method described was found to be useful for producing small amounts of high specific GA_3. The overall yield was low (2.7%) most probably because of the ease of hydrolysis of the lactone ring of GA_3. However, since only small quantities of the labelled compound are usually needed this should present no particular problem.

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