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Short Communication

A Rapid, Simple Synthesis and Purification of Abscisic Acid Glucose Ester[1]

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ABSTRACT

A simple and rapid technique was developed to synthesize abscisic acid glucose ester. The free acid of abscisic acid (ABA) was combined with $CsHCO_3$ to form the Cs salt of ABA. The Cs salt of ABA was then combined with acetobromo-α-D-glucose tetraacetate, and the tetraacetate derivative of abscisic acid glucose ester was formed. Acetate groups were selectively removed from the glucose moiety with a crude enzyme preparation derived from *Helianthus annuus* seeds. Abscisic acid glucose ester was purified via silica gel column chromatography and identified by micro NMR.

ABA-GE[4] is a metabolite of the free acid form of the plant growth regulator ABA (6, 8). ABA-GE has been identified in immature fruits of yellow lupin (3), pseudocarp of field rose (6), and *Xanthium* and spinach leaves (1). The amount of ABA-GE found in plant tissues is generally about one-tenth of the free ABA level (7); however, ABA-GE increased in *Xanthium* leaves to a level several times greater than free ABA after water stress wilting-recovery cycles (1). The physiological relevance of ABA-GE in plants is at present unknown (8).

With the development of antibodies specifically directed against ABA for use in radio- and enzyme-linked immunoassay (9, 11, 12), reasonable quantities of potential cross-reacting compounds, such as ABA-GE, are required to determine antibody specificity. ABA-GE can potentially cross-react with antibodies produced against ABA which had been conjugated to a carrier protein (BSA) through its C-1 carboxy function (9, 11, 12) and may cross-react with antibodies produced against ABA-BSA conjugates coupled through C-4 of ABA (12). A synthetic procedure previously used to produce ABA-GE reacted the free acid of ABA with *O*-substituted halocarbohydrates in the presence of amines (*e.g.* triethylamine) as catalyst (5). In this report we describe an alternative synthetic approach to the synthesis of ABA-GE and simple purification procedure.

MATERIALS AND METHODS

±ABA (Fluka; 264.32 mg, 1.0 mmol) was gradually added and stirred into 2.0 ml of dioxane in a 25 ml round bottom flask at room temperature. After the ABA had completely dissolved, 1.25 ml of absolute ethanol and 3.25 ml of distilled H_2O were gradually added which resulted in a clear yellowish solution. Cesium bicarbonate (194.0 mg, 1.0 mmol) was added and the pH of the reaction mixture was lowered to 7.0 by adding several mg more ABA, after which it was gently stirred for 30 min. The volume was reduced by one-half via rotary evaporation at 40°C. Absolute ethanol (5 ml) and then benzene (5 ml) were added, and the mixture was reduced to dryness. The resulting yellow crystals were redissolved in ethanol (10 ml) and then benzene (10 ml) was added, and the solution was evaporated to one-third. Benzene (15 ml) was added and the solution was reduced to one-third as before. Benzene was added twice more to the capacity of the 25 ml round bottom flask and evaporated as described above and the final solution was reduced to dryness. The yellow-white ABA-Cs salt crystals were dried *in vacuo* over P_2O_5 for 3 h.

One ml dry DMF was added to the ABA-Cs salt at room temperature while gently stirring. Acetobromo-α-D-glucose tetraacetate (Sigma; 415.32 mg, 1.01 mmol) in 0.75 ml dry DMF was added dropwise to the solution. Another 0.25 ml DMF was used to rinse the acetobromo-α-D-glucose tetraacetate from its weighing container and also added to the solution. The resulting solution was stirred overnight at room temperature. The mixture was centrifuged at 7000 rpm for 15 min and the supernatant loaded onto a silica gel G-60 (EM Scientific 230–400 mesh) 1.2 × 100 cm column equilibrated in chloroform:methanol (96:4, v/v). The ABA-GE tAc was eluted with 300 ml of the above solvent mixture into 4 ml fractions number 17 to 30. Those fractions which showed one spot, R_F 0.824 on TLC (Whatman reverse-phase MKFG silica gel 1 × 3 cm, 200 μm thickness) plates developed in chloroform:methanol (96:4, v/v and visualized by UV) were pooled. Other fractions containing ABA-GE tAc and minor contaminants were rechromatographed on a new silica gel G-60 column as described previously after solvent evaporation, and those fractions showing a single spot on TLC were collected again. All fractions containing purified ABA-GE tAc were pooled, and the solvent was evaporated. The white ABA-GE tAc crystals were dried *in vacuo* over P_2O_5 and stored at −18°C (73–75 m.p.; 247 mg).

The preparation of ABA-GE from ABA-GE tAc was adapted from the procedure of Lehmann *et al.* (4) and was followed with slight modification in purification. Briefly, 3 gm of dehusked, ripe, mature sunflower *Helianthus annus* seeds were ground in an ice-cold mortar and pestle with 25 ml, 100 mM Na-phosphate buffer (pH 7.0) and sand until homogeneity. The homogenate was centrifuged for 20 min at 20,000 rpm which resulted in the

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[4]Abbreviations: ABA-GE, β-D-glucopyranosyl abscisate; ABA, ±abscisic acid; ABA-GE tAc, tetraacetate derivative of ABE-GE; DMF, dimethylformamide.

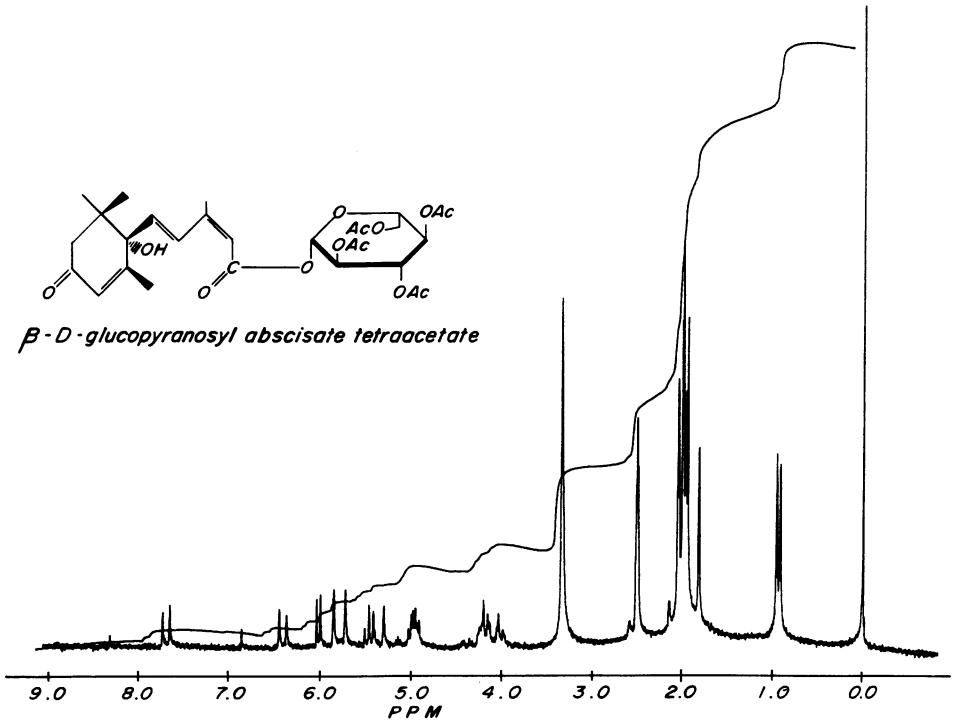

FIG. 1. NMR spectra of β-D-glucopyranosyl abscisate tetracetate in deuterated DMSO.

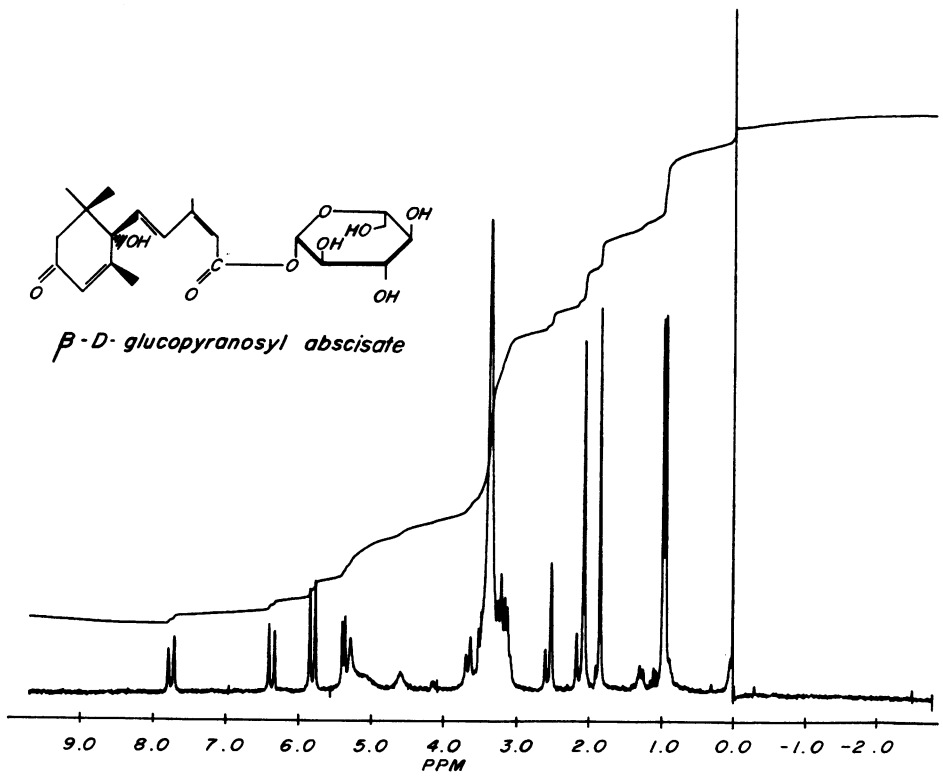

FIG. 2. NMR spectra of β-D-glucopyranosyl abscisate in deuterated DMSO.

formation of three layers. The middle layer (17.0 ml) was collected. To a solution of ABA-GE tAc (60 mg) in 3.5 ml ethanol, 11.0 ml of Na-phosphate buffer was slowly added while gently stirring at room temperature. The 17.0 ml of crude sunflower seed enzyme preparation was added and allowed to react for 24 h. The reaction was stopped with 20 ml of ethanol and the mixture was centrifuged for 20 min at 20,000 rpm. The supernatant was collected and reduced in volume to 1 to 2 ml via rotary evaporation, and the concentrated solution was filtered through glass wool and loaded onto a silica gel G-60 column (1.2 × 100 cm) equilibrated in chloroform:methanol:water (75:22:3, v/v). The putative ABA-GE was eluted in 4 ml (fractions 52–65) in the above solvent system. The putative ABA-GE was identified by TLC on the aforementioned Whatman reverse phase silica gel (R_F 0.45; ABA-GE tAc, R_F 0.95) with the above solvent system. The fractions containing ABA-GE were pooled, and the solvent was removed by evaporation. The resultant ABA-GE was dissolved in .5 ml ethanol and then 10 ml benzene and these solvents were reduced via rotary evaporation. Approximately 10 ml benzene was added and reduced two more times. Anhydrous methanol was added dropwise to the dry ABA-GE, and the solution was filtered through glass wool to remove any traces of

silica gel. Methanol was removed by evaporation under a stream of dry N_2 gas. The residue dried *in vacuo* over P_2O_5 (24.81 mg; 55.5%). The identities of ABA-GE tAc and ABA-GE were verified by analysis of micro NMR spectra (Bruker AM-200 spectrometer). The spectra were obtained at 200 mHz.

RESULTS AND DISCUSSION

The preparation of esters of plant growth regulators has been previously accomplished by reaction of the free acids with *O*-substituted halocarbohydrates in the presence of triethylamine catalysts (5). However, the use of amines in reaction with alkyl halides can result in racemization of asymmetric carbons. Moreover, the tertiary amine, triethylamine, reacting with an alkyl halide can undergo alkylation to form an amine salt. This reaction side product can reduce the formation of new product and thus reaction yield, thereby requiring more extensive purification. A need exists for a versatile and easy procedure to prepare plant growth regulator esters under mild conditions. Cesium carbonate or cesium bicarbonate have been used to form cesium salts of amino acid and peptides (10) which can be reacted with alkyl halides to form esters (2, 10).

The ABA-Cs salt is formed easily by adjusting the reaction mixture to pH 7.0 as had been described for amino acids and peptides (10). The purified ABA-Cs salt reacts upon the addition of acetobromo-α-D-glucose in DMF. TLC after 5, 15, and 30 min showed progressively increasing amounts of ABA-GE tAc formed. Yields of ABA-GE tAc (Fig. 1) and ABA-GE (Fig. 2) reported were lower than those obtainable via workup of a larger number of fractions because only those fractions showing no evidence of cross-contaminating compounds were selected after column chromatography.

In conclusion, the free acid of ABA can be reacted with $CsHCO_3$ to form ABA-Cs salt, which can then be reacted with halocarbohydrates to form esters of ABA. The preparation of ABA-GE by this procedure and purification by silica gel column chromatography is simple, easily scaled. The synthesis of other plant growth regulator esters should be achievable by utilizing this procedure.

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