

Novel Method for Separation of GA₄/GA₇ Mixtures

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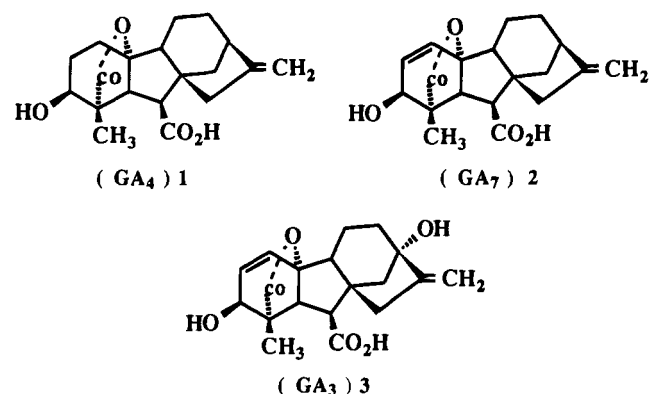
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Preparative separation of GA₄ **1** and GA₇ **2** from the commercially available mixture of GA₄ **1** and GA₇ **2** was achieved, which is predicated upon the discovery of the differential reactivities of GA₄ and GA₇ toward silyl ether formation and subsequent deprotection.

Keywords: Gibberellin acids (GA₄ and GA₇); separation; selective silylation and desilylation

INTRODUCTION

Gibberellins are powerful plant hormones that are responsible for flowering, root growth, stem elongation, fruit size, branching, etc. The mixture of GA₄ **1** and



GA₇ **2** and pure GA₃ **3** are the only gibberellins presently commercially produced in quantity from cultures of the fungus *Gibberella fujikuroi* (Takahashi et al., 1988; Jacobsen and Chandler, 1987). They are therefore convenient starting materials for the synthesis of less accessible gibberellins.

There has been a long-standing need for a method that effectively separates GA₄ **1** and GA₇ **2** from the mixture. In addition to being an important research tool for understanding the structure-activity relationships in plants via preparation and testing of less accessible gibberellins, it will provide the biologically desirable GA₇ **2** for commercial purposes.

Previously, tedious reversed-phase HPLC chromatography was used for preparative separation of the mixture of GA₄ **1** and GA₇ **2**, which was labor intensive and not feasible for the preparation of large quantities.

Laboratory chemical processes are used for the preparation of GA₄ **1** and GA₇ **2** in small quantities; however, they all involve multiple-step synthesis. For example, GA₇ **2** can be obtained from GA₃ **3** by a five-step reaction sequence (Beale and MacMillan, 1981) which involves selective protection of the 3- β -hydroxyl group of GA₃, preparation of the 13-methanesulfonyl derivative of the 3-acetate, hydrolysis of the acid chloride, and reduction of the bridgehead methanesulfonate followed by hydrolysis of the resulting acetate.

GA₄ can be obtained via Jones oxidation of a GA₄/GA₇ mixture followed by Selectride reduction (Bell and Turner, 1985). Another method for obtaining GA₄ is selective degradation of GA₇ from the mixture of GA₄

and GA₇, followed by isolation of GA₄. This method literally converts the biologically important GA₇ into degradation products (Crutcher, 1979).

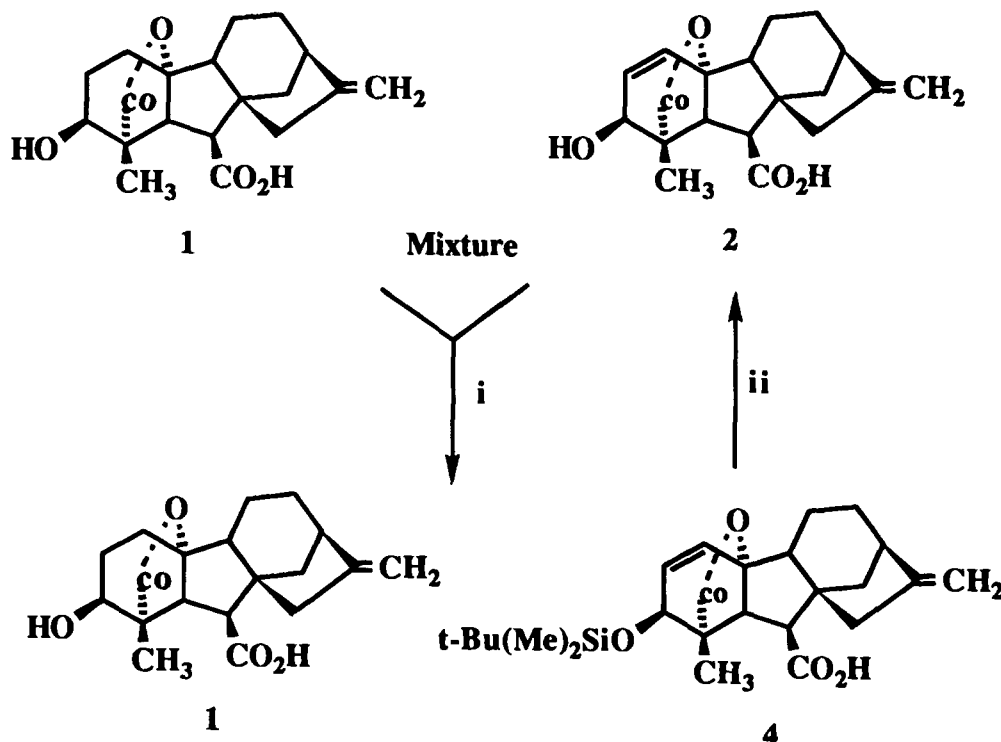
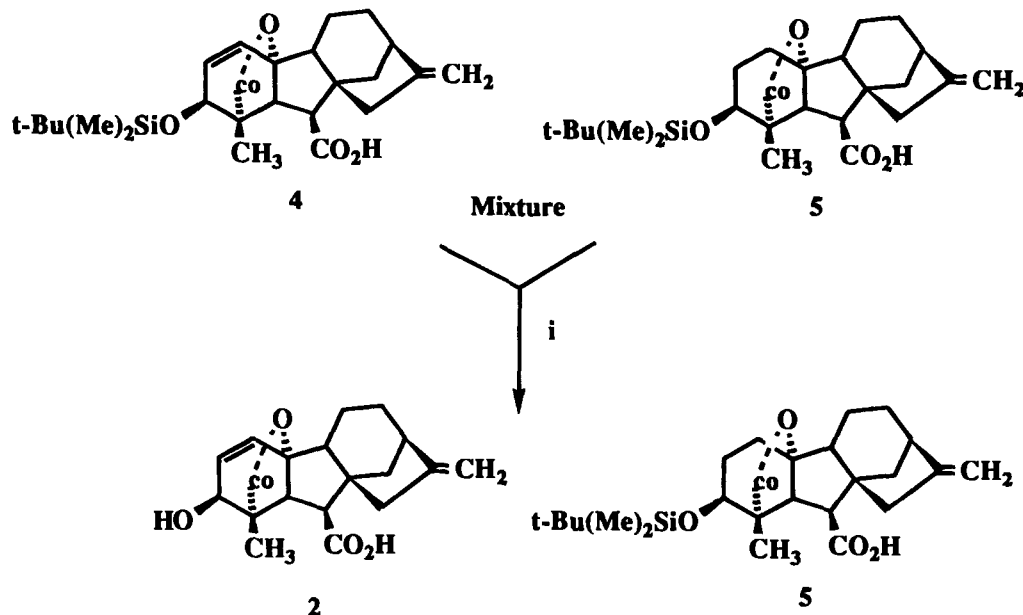
None of these methods can provide GA₄ and GA₇ in large quantities efficiently. In this paper we report a novel method for separation of GA₄ and GA₇, which is predicated upon the discovery of the differential reactivities of GA₄ and GA₇ toward silyl ether formation and subsequent deprotection.

MATERIALS AND METHODS

Selective Silylation of GA₇ 2 from a Mixture of GA₄ 1/GA₇ 2. To a solution of a mixture of GA₄ **1** and GA₇ **2** (99.3 g, 0.3 mmol) in DMF (480 mL) was added imidazole (61.3 g, 0.9 mol); after the imidazole was completely dissolved, *tert*-butyldimethylsilyl chloride (72.4 g, 0.48 mol) was added. The reaction mixture was stirred for 2 days at room temperature under nitrogen. To the mixture was added 400 mL of acetic acid and 500 mL of water; a white solid (GA₇-silyl ether) was precipitated and filtered to give 26 g of GA₇-silyl ether **4**: ¹H NMR (DMSO-*d*₆) δ 0.10 (s, -SiCH₃), 0.88 (s, -Si-*t*-Bu), 1.08 (s, 18-H₃), 2.78 (d, 10 Hz, H-5), 3.11 (d, 10 Hz, H-6), 4.09 (d, 4 Hz, H-3), 4.85 and 4.97 (each br, 17-H₂), 5.77 (d, d, 10, 4 Hz, H-2), 6.40 (d, 10 Hz, H-1); MS (FAB) 445 (M + H). To the filtrate was added an excess of water; a white solid precipitated to give 38.28 g of crude GA₄, which was further purified by suspending the crude GA₄ with a solution of Et₂O/Hex (1:1) (4 mL/g) to remove the remaining GA₇-silyl ether. GA₄ (31.50 g) was obtained, which had physical characteristics consistent with those of an authentic sample: ¹H NMR (DMSO-*d*₆) δ 0.99 (s, 18-H₃), 2.39 (d, 12 Hz, H-5), 3.02 (d, 11 Hz, H-6), 3.55 (m, H-3), 4.84 and 4.96 (each br, 17-H₂), 5.34 (d, 4.5 Hz, OH), 12.46 (s, -CO₂H); MS (FAB) 333 (M + H).

Desilylation of GA₇-Silyl Ether 4. To a solution of silyl ether of GA₇ (26 g, 58.6 mmole) in THF (5 mL) was added a solution of tetrabutylammonium fluoride in THF (11 mL, 1.0 M solution). The solution was stirred for 8 h at room temperature under nitrogen. To the reaction mixture was added 1.0 M citric acid solution (50 mL). THF was removed under vacuum. To the residue was added an excess of 1.0 M citric acid; a white solid was precipitated to give 18.36 g of GA₇, which was crystallized from acetone/H₂O to give 15.20 g of GA₇. The GA₇ had physical characteristics consistent with those of an authentic sample: ¹H NMR (DMSO-*d*₆) δ 1.07 (s, 18-H₃), 2.50 (d, 12 Hz, H-5), 3.07 (d, 11 Hz, H-6), 3.88 (m, H-3), 4.86 and 4.97 (each br, 17-H₂), 5.57 (br d, -OH), 5.81 (dd, 10, 4 Hz, H-2), 6.34 (d, 10 Hz, H-1), 12.56 (br s, -CO₂H); MS (FAB) 331 (M + 1).

Silylation of GA₄ 1/GA₇ 2 from a Mixture of GA₄ 1/GA₇ 2. To a solution of a mixture of GA₄ and GA₇ (44 g, 0.13 mmol) in DMF (155 mL) was added imidazole (90 g, 1.33 mol). After imidazole was completely dissolved, *tert*-butyldimethylsilyl chloride (100 g, 0.66 mol) was added. The reaction mixture was stirred for 2 days at 45 °C under nitrogen. To the mixture

Scheme 1. Reagents: i, (a) Imidazole/DMF, RT, (b) *t*-Bu(Me)₂SiCl; ii, (*t*-Bu)₄NF/THFScheme 2. Reagents: i, (*t*-Bu)₄NF/THF, RT

was added 700 mL of acetic acid, 500 mL of THF, and 500 mL of water. A white solid (silyl ethers of GA₄/GA₇) was precipitated and filtered to give 49 g of the silyl ethers of GA₄/GA₇.

Selective Desilylation of GA₇-Silyl Ether 4 from a Mixture of GA₄-Silyl Ether 5 and GA₇-Silyl Ether 4. To a solution of a mixture of GA₄-silyl ether and GA₇-silyl ether (4.45 g, 10 mmol) in THF (20 mL) was added tetrabutylammonium fluoride trihydrate (6.31 g, 20 mmol). The mixture was stirred at room temperature for 8 h; 20 mL of acetic acid and 25 mL of water were added to the mixture, and a white solid was precipitated to give 1.3 g of GA₄-silyl ether. ¹H NMR (DMSO-*d*₆) δ 0.07 (s, -SiCH₃), 0.08 (s, -SiCH₃), 0.90 (-Si-*t*-Bu), 0.95 (s, 18-H₃), 2.40 (d, 10 Hz, H-5), 3.10 (d, 10 Hz, H-6), 4.844 and 4.950 (each br, 17-H₂); MS (FAB) 447 (M + H). To the filtrate was added an excess of water. A white solid was precipitated to give 1.1 g of GA₇, which has ¹H NMR data and

physical characteristics consistent with those of an authentic sample.

RESULTS AND DISCUSSION

We have discovered that GA₄ and GA₇ react differently with trialkylsilyl chloride in the presence of imidazole in DMF. For example, GA₇ reacts with *tert*-butyldimethylsilyl chloride in the presence of imidazole in DMF at room temperature to form the GA₇-silyl ether 4, while GA₄ is inactive under this condition (Scheme 1).

A greater kinetic selectivity for silylation of GA₇ versus GA₄ may be attributed to the more accessible steric environment of ring A of GA₇ (more planar) than ring A of GA₄ and was achieved by using slightly more

than 1 equiv of *tert*-butyldimethylsilyl chloride (1.6 equiv, 1 equiv of the silylating reagent reacted with the carboxyl groups of GA₄ and GA₇, and 0.6 equiv of the reagent selectively reacted with GA₇ from a 50%/50% mixture of GA₄ and GA₇) at room temperature. Increasing the amount of silylating agent in excess of this amount or raising the temperature above room temperature resulted in the formation of silyl ether of GA₄. Under forcing conditions such as higher temperature (45 °C) or excess silylating agent (5 equiv), both GA₄ and GA₇ can be completely converted to their silyl ethers.

The differentiation in the reactivity toward *tert*-butyldimethylsilyl chloride made it possible to separate GA₄ and GA₇ from a readily available mixture of GA₄ and GA₇.

It was found that the silyl ether of GA₇ formed from the above reaction has completely different physical properties from those of GA₄, such as solubility in organic solvent and in water. On the basis of those differences, the silyl ether of GA₇ can be easily separated from GA₄ by simple selective precipitation. The separated silyl ether of GA₇ can then be desilylated by simply treating it with tetrabutylammonium fluoride to afford GA₇ (Scheme 1).

We have also discovered that the silyl ethers of GA₄ and GA₇ have shown different reactivities toward desilylation. Once again this may be attributed to a kinetic selectivity (steric of ring A) favoring GA₇ reaction. For example, a mixture of *tert*-butyldimethylsilyl ethers of GA₄ and GA₇ was treated with tetrabutylammonium fluoride (2 equiv) in THF at room temperature. It was found that the silyl ether of GA₇ was desilylated first, while the GA₄-silyl ether **5** was left intact (Scheme 2). Increasing the amount of desilylating agent in excess

of 2 equiv and raising the temperature to above room temperature resulted in the loss of selectivity; both GA₄- and GA₇-silyl ether were completely desilylated.

The combination of the selectivities in silylation and desilylation makes the separation of the GA₄ and GA₇ doubly efficient; i.e., the difficulty in control of the contamination of the GA₇-silyl ether by GA₄-silyl ether can be circumvented in the desilylation stage.

In summary, pure GA₄ **1** and GA₇ **2** can be obtained efficiently by using this novel process which can be readily accomplished by a two-step reaction sequence, selective silylation followed by selective desilylation.

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