Novel Method for Separation of GA_4/GA_7 Mixtures

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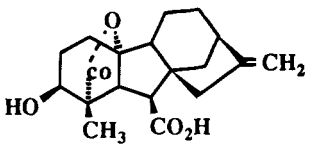
Preparative separation of GA_4 **1** and GA_7 **2** from the commercially available mixture of GA_4 **1** and GA_7 **2** was achieved, which is predicated upon the discovery of the differential reactivities of GA_4 and GA_7 toward silyl ether formation and subsequent deprotection.

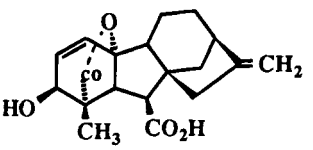
Keywords: *Gibberellin acids (GA_4 and GA_7); separation; selective silylation and desilylation*

INTRODUCTION

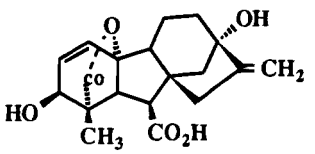
Gibberellins are powerful plant hormones that are responsible for flowering, root growth, stem elongation, fruit size, branching, etc. The mixture of GA_4 **1** and



(GA_4) **1**



(GA_7) **2**



(GA_3) **3**

GA_7 **2** and pure GA_3 **3** are the only gibberellins presently commercially produced in quantity from cultures of the fungus *Gibberella fujikoroi* (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 GA_4 **1** and GA_7 **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 GA_7 **2** for commercial purposes.

Previously, tedious reversed-phase HPLC chromatography was used for preparative separation of the mixture of GA_4 **1** and GA_7 **2**, which was labor intensive and not feasible for the preparation of large quantities.

Laboratory chemical processes are used for the preparation of GA_4 **1** and GA_7 **2** in small quantities; however, they all involve multiple-step synthesis. For example, GA_7 **1** can be obtained from GA_3 **3** by a five-step reaction sequence (Beale and MacMillan, 1981) which involves selective protection of the 3-β-hydroxyl group of GA_3, 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.

GA_4 can be obtained via Jones oxidation of a GA_4/GA_7 mixture followed by Selectride reduction (Bell and Turner, 1985). Another method for obtaining GA_4 is selective degradation of GA_7 from the mixture of GA_4 and GA_7, followed by isolation of GA_4. This method literally converts the biologically important GA_7 into degradation products (Crutcher, 1979).

None of these methods can provide GA_4 and GA_7 in large quantities efficiently. In this paper we report a novel method for separation of GA_4 and GA_7, which is predicated upon the discovery of the differential reactivities of GA_4 and GA_7 toward silyl ether formation and subsequent deprotection.

MATERIALS AND METHODS

Selective Silylation of GA_7 2 from a Mixture of GA_4 1/GA_7 2. To a solution of a mixture of GA_4 **1** and GA_7 **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 (GA_7–silyl ether) was precipitated and filtered to give 26 g of GA_7–silyl ether **4**: ^{1}H NMR (DMSO-d_6) δ 0.10 (s, $-SiCH_3$), 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 GA_4, which was further purified by suspending the crude GA_4 with a solution of Et_2O/Hex (1:1) (4 mL/g) to remove the remaining GA_7–silyl ether. GA_4 (31.50 g) was obtained, which had physical characteristics consistent with those of an authentic sample: ^{1}H 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, $-CO_2H$); MS (FAB) 333 (M + H).

Desilylation of GA_7–Silyl Ether 4. To a solution of silyl ether of GA_7 (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 GA_7, which was crystallized from acetone/H_2O to give 15.20 g of GA_7. The GA_7 had physical characteristics consistent with those of an authentic sample: ^{1}H 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-Hz), 5.57 (br d, $-OH$), 5.81 (dd, 10, 4 Hz, H-2), 6.34 (d, 10 Hz, H-1), 12.56 (br s, $-CO_2H$); MS (FAB) 331 (M + 1).

Silylation of GA_4 1/GA_7 2 from a Mixture of GA_4 1/GA_7 2. To a solution of a mixture of GA_4 and GA_7 (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

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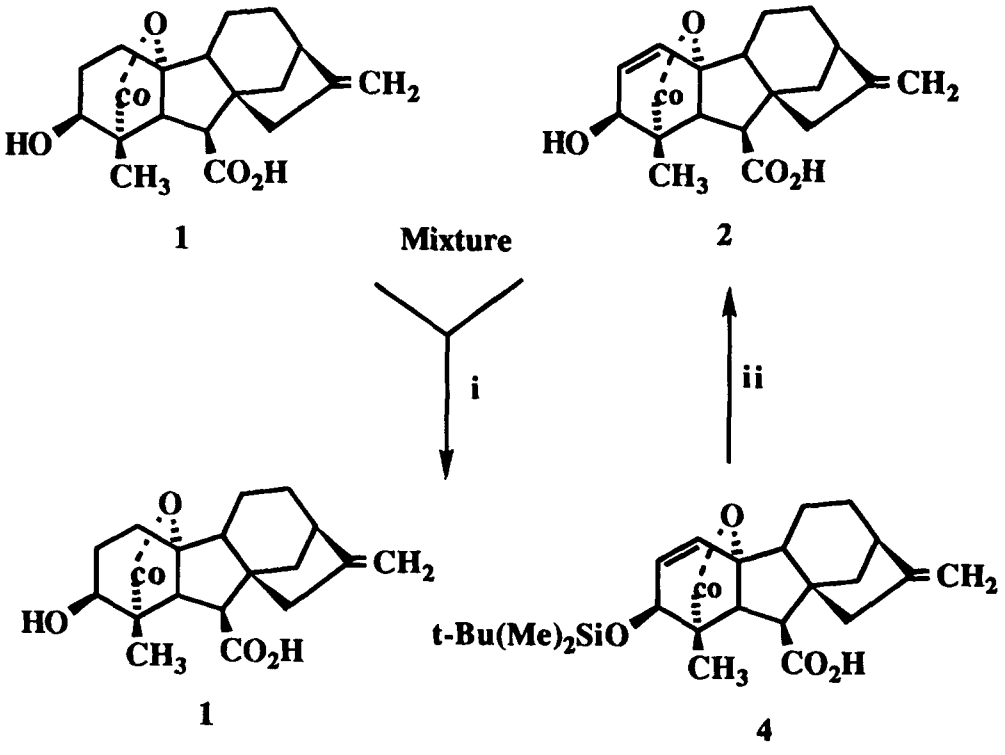
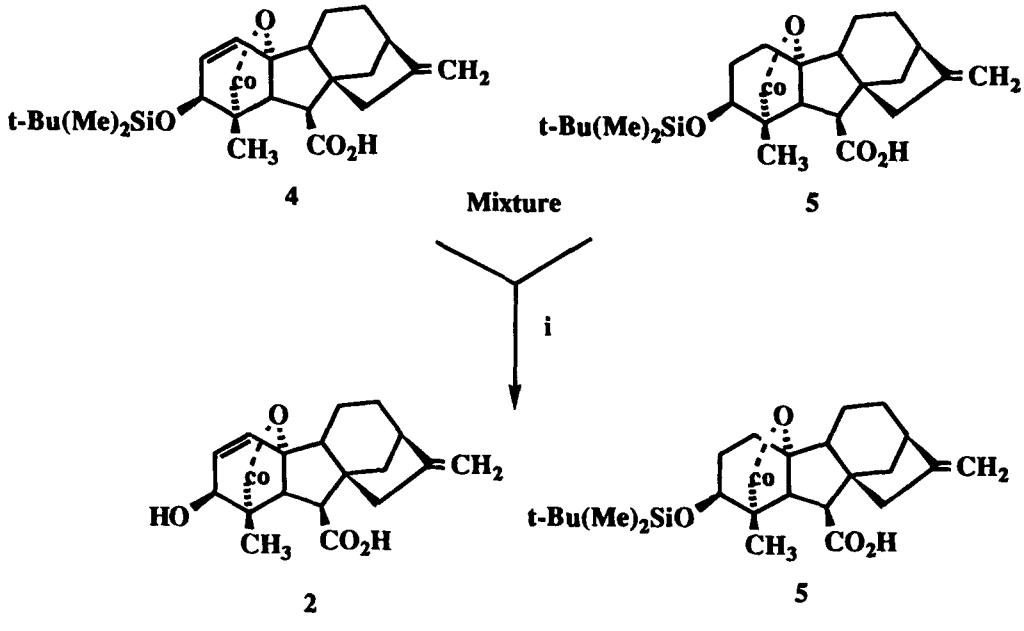
Scheme 1. Reagents: i, (a) Imidazole/DMF, RT, (b) *t*-Bu(Me)$_2$SiCl; ii, (*t*-Bu)$_4$NF/THF



Scheme 2. Reagents: i, (*t*-Bu)$_4$NF/THF, RT



was added 700 mL of acetic acid, 500 mL of THF, and 500 mL of water. A white solid (silyl ethers of GA_4/GA_7) was precipitated and filtered to give 49 g of the silyl ethers of GA_4/GA_7.

Selective Desilylation of GA_7–Silyl Ether 4 from a Mixture of GA_4–Silyl Ether 5 and GA_7–Silyl Ether 4. To a solution of a mixture of GA_4–silyl ether and GA_7–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 GA_4–silyl ether: ^{1}H NMR (DMSO-d_6) δ 0.07 (s, $-SiCH_3$), 0.08 (s, $-SiCH_3$), 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 GA_7, which has ^{1}H NMR data and physical characteristics consistent with those of an authentic sample.

RESULTS AND DISCUSSION

We have discovered that GA_4 and GA_7 react differently with trialkylsilyl chloride in the presence of imidazole in DMF. For example, GA_7 reacts with *tert*-butyldimethylsilyl chloride in the presence of imidazole in DMF at room temperature to form the GA_7–silyl ether 4, while GA_4 is inactive under this condition (Scheme 1).

A greater kinetic selectivity for silylation of GA_7 versus GA_4 may be attributed to the more accessible steric environment of ring A of GA_7 (more planar) than ring A of GA_4 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 GA_4 and GA_7, and 0.6 equiv of the reagent selectively reacted with GA_7 from a 50%/50% mixture of GA_4 and GA_7) 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 GA_4. Under forcing conditions such as higher temperature (45 °C) or excess silylating agent (5 equiv), both GA_4 and GA_7 can be completely converted to their silyl ethers.

The differentiation in the reactivity toward *tert*-butyldimethylsilyl chloride made it possible to separate GA_4 and GA_7 from a readily available mixture of GA_4 and GA_7.

It was found that the silyl ether of GA_7 formed from the above reaction has completely different physical properties from those of GA_4, such as solubility in organic solvent and in water. On the basis of those differences, the silyl ether of GA_7 can be easily separated from GA_4 by simple selective precipitation. The separated silyl ether of GA_7 can then be desilylated by simply treating it with tetrabutylammonium fluoride to afford GA_7 (Scheme 1).

We have also discovered that the silyl ethers of GA_4 and GA_7 have shown different reactivities toward desilylation. Once again this may be attributed to a kinetic selectivity (steric of ring A) favoring GA_7 reaction. For example, a mixture of *tert*-butyldimethylsilyl ethers of GA_4 and GA_7 was treated with tetrabutylammonium fluoride (2 equiv) in THF at room temperature. It was found that the silyl ether of GA_7 was desilylated first, while the GA_4–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 GA_4– and GA_7–silyl ether were completely desilylated.

The combination of the selectivities in silylation and desilylation makes the separation of the GA_4 and GA_7 doubly efficient; i.e., the difficulty in control of the contamination of the GA_7–silyl ether by GA_4–silyl ether can be circumvented in the desilylation stage.

In summary, pure GA_4 **1** and GA_7 **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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