Studies of the Selective O-Alkylation and Dealkylation of Flavonoids. 13.[1] An Improved Method for Synthesizing 5,6,7-Trihydroxyflavones from 6-Hydroxy-5,7-dimethoxyflavones

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The demethylation of five 6-hydroxy-5,7-dimethoxyflavones 1 and their acetates with 30% w/v anhydrous aluminum chloride in acetonitrile was studied, and the following results were found. In the demethylation of 6-hydroxy-4′,5,7-trimethoxyflavone (**1a**), 5,6-dihydroxy-4′,7-dimethoxyflavone (**2a**) and 5,6,7-trihydroxy-4′-methoxyflavone (**3a**) were produced. Although the ratio of the two products varied according to the amount of aluminum chloride used, it became constant after 12–24 h because the cleavage of the 7-methoxy group in **2a** was suppressed by iminoesterification of the 6-hydroxy group. In contrast, the demethylation of the 5- and 7-methoxy groups of acetate **4a** proceeded smoothly by the process shown in Scheme II. The amount of **3a** increased with increasing reaction time to give **3a** as the main product after 36–48 h. The same phenomena were observed in the demethylation of the other 6-hydroxyflavones **1b–1e** and their acetates **4b–4e**. The demethylation of the acetates is widely applicable as a general method for synthesizing 5,6,7-trihydroxyflavones because the protection of hydroxy groups also suppresses the further cleavage of the methoxy group adjacent to the acetoxy group on the B ring of compounds such as **4d** and **4e**.

Introduction

In a previous paper,[2] we reported that the demethylation of 6-hydroxy-5,7-dimethoxyflavones **1a–d** with anhydrous aluminum chloride in acetonitrile proceeds as shown in Scheme I and affords a mixture of the corresponding 5,6-dihydroxy-7-methoxyflavones **2a–d** and 5,6,7-trihydroxyflavones **3a–d**. Although the demethylation of 1 does afford 5,6,7-trihydroxyflavones **3a–d**, the yields of **3** are low (25–35%) because the 7-methoxy group is much more difficult to remove than the 5-methoxy group. Cleavage of the 7-methoxy group ceases after about 10 h. As we reported in a previous paper, the demethylation of 6-hydroxy-5,7,8-trimethoxyflavones also affords a mixture of the corresponding 5,6-dihydroxy- and 5,6,7-trihydroxyflavones. However, the mixture can be converted to the desired 5,6,7-trihydroxy-8-methoxyflavone in good yield by repeated demethylation[3] because the 7-methoxy group is much more easily cleaved than that of **1**. We hoped that the elucidation of the reason for the low yield of **3** would be useful for improving this method for synthesizing 5,6,7-trihydroxyflavones **3** from **1**. Thus, we reexamined the demethylation of 1 with anhydrous aluminum chloride in acetonitrile and established a convenient method for synthesizing **3**.

Results and Discussion

Demethylation of 6-Hydroxy-4′,5,7-trimethoxyflavone (1a) and Its Derivatives 2a, 4a, and 5a. The products of the demethylation of **1a** with aluminum chloride in acetonitrile were examined by HPLC using porous polymer.[4] After the reaction mixture was treated with dilute hydrochloric acid, the chromatogram showed the presence of a significant amount of monoacetate **5a** in addition to **2a** and **3a**. The corresponding monoacetate was also produced in the demethylation of 6-hydroxy-5,7,8-trimethoxyflavones. The presence of acetate suggests that an imino ester is formed in the reaction and that its formation suppresses further demethylation of **2a**. The detection of the imino ester, however, was difficult because the reaction mixture had been treated with dilute hydrochloric acid. Therefore, in the subsequent experiments, the demethylated products were hydrolyzed with methanolic hydrochloric acid and then analyzed by HPLC.

The influence of the amount of reagent on the demethylation of **1a** was examined first, and the results are shown in Figure 1. The ratios of **3a** to **2a** became constant after 12–24 h but increased when the amount of aluminum chloride was increased. In these reactions, the viscosity of the mixture increased as the reaction time increased, and consequently, the mixture formed a gel. Decreasing the amount of reagent increased gel formation.

The 5-methoxy group of the acetates 4 of 1 is selectively cleaved with 5% w/v anhydrous aluminum chloride in acetonitrile to give quantitatively the monoacetates **5** of **2**. However, the cleavage of the 7-methoxy group proceeds simultaneously at a very slow rate, and at longer reaction times **3** is observed among the reaction products.[5] Thus, **3a** is obtained from acetates **4a** and **5a** as well as from **1**.

Although the 7-methoxy group in **4a** was cleaved slowly with increasing reaction time, the cleavage was accelerated by the addition of a small amount of water. This acceleration was also observed in the demethylation of 4′,5,6,8-tetramethoxyflavones to 5,6-dihydroxy-4′,8-dimethoxyflavones.[6] Therefore, in the following demethylations, acetonitrile containing a small amount of water (0.1–0.2%, v/v) was used as a solvent. The demethylation of hydroxyflavones **1a** and **2a** and their acetates (**4a** and **5a**) was examined under the same conditions in order to compare their reactivities. The results are shown in Figure 2. Time vs conversion curves for the reaction of **1a** and its acetate **4a** were superimposable with those of **2a** and its monoacetate **5a**, respectively. The ratios of **3a** to **2a** in the reactions of acetates **4a** and **5a**, however, increased when the reaction time was increased, and the yields of **3a** reached 95% at 48 h. The product ratios were hardly

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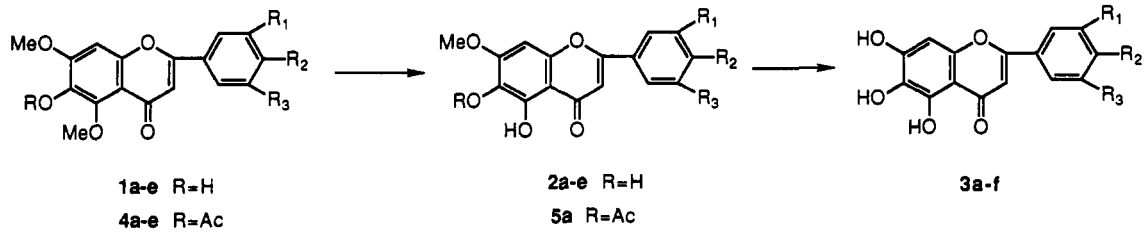
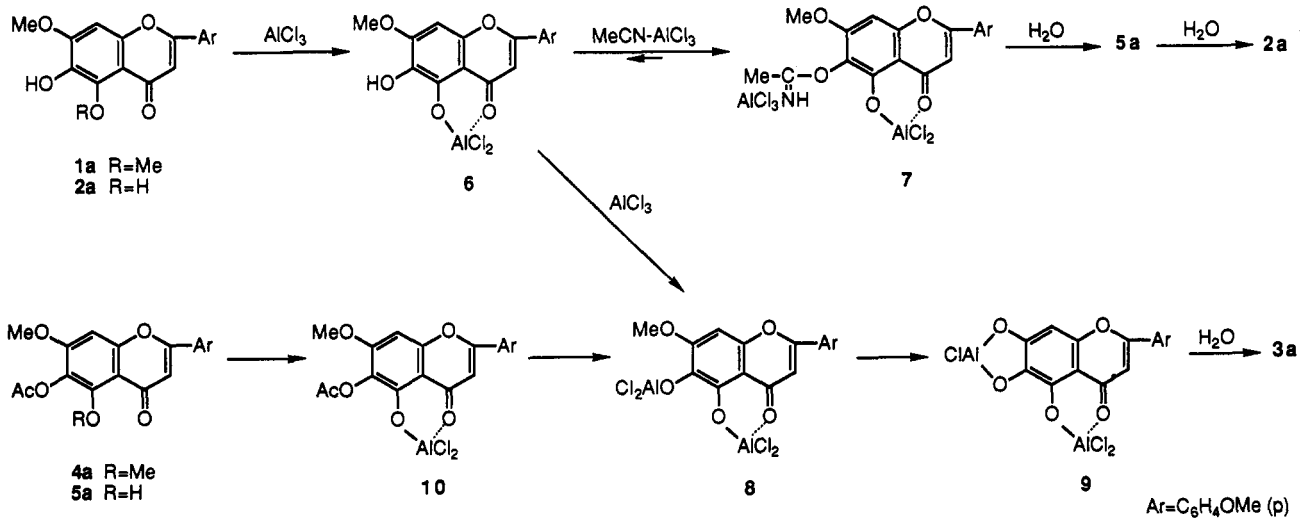
Scheme I



a: R_1=R_3=H, R_2=OMe b: R_1=R_2=OMe, R_3=H c: R_1=R_2=R_3=OMe d: R_1=OMe, R_2=OH, R_3=H
e: R_1=OH, R_2=OMe, R_3=H f: R_1=R_2=OH, R_3=H

4d: R_1=OMe, R_2=OAc, R_3=H 4e: R_1=OAc, R_2=OMe, R_3=H

Scheme II



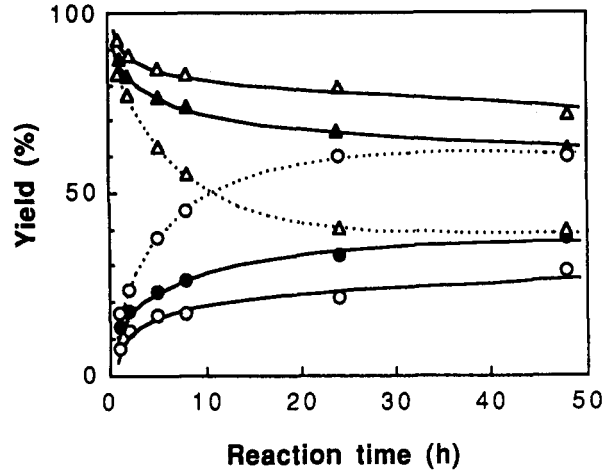


Figure 1. Time vs conversion of the demethylation of **1a** (100 mg) with 30% w/v anhydrous aluminum chloride in acetonitrile (1.5 mL, -△-, -○-; 3 mL, -▲-, -●-; 6 mL, ---△---, ---○---) at 70 °C: **2a**, △ and ▲; **3a**, ○ and ●.

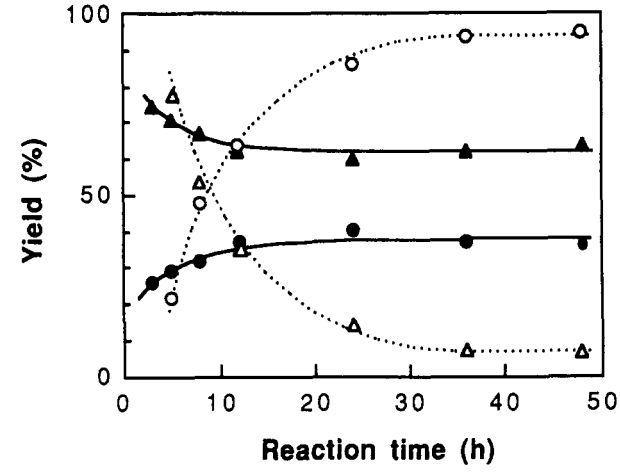


Figure 2. Time vs conversion of the demethylation of **1a** (—) and its acetate **4a** (---) (each 80 mg) with anhydrous aluminum chloride (0.6 g) in acetonitrile (2 mL) containing water (4 μL) at 70 °C: **2a**, △ and ▲; **3a**, ○ and ●.

affected by the amount of reagent, which is in contrast to the reactions of the 6-hydroxyflavones. In these demethylations, the protection of the 6-hydroxy group in **1a** or **2a** seems to suppress the cleavage of the 7-methoxy group, since the cleavage of a methoxy group adjacent to a hydroxy group proceeds via a cyclic aluminum complex.[7-9]

On the basis of these observations, we believe the reaction mechanism to be the one shown in Scheme II. The 5-methoxy group of **1a** is rapidly cleaved, and aluminum complex **6** is formed. Complex **6** is converted to imino ester **7** and aluminum complex **8**. Further demethylation of **8** via cyclic aluminum complex **9** leads to **3a**. Because imino ester **7** is in equilibrium with **6**, it also is demethylated to **9**. However, the demethylation of **7** is very slow because the equilibrium between **6** and **7** is suppressed as the viscosity of the reaction mixture increases at longer reaction times. Consequently, the reaction stabilizes as a mixture of **7** and **9**, and the ratio of **3a** to **2a** after

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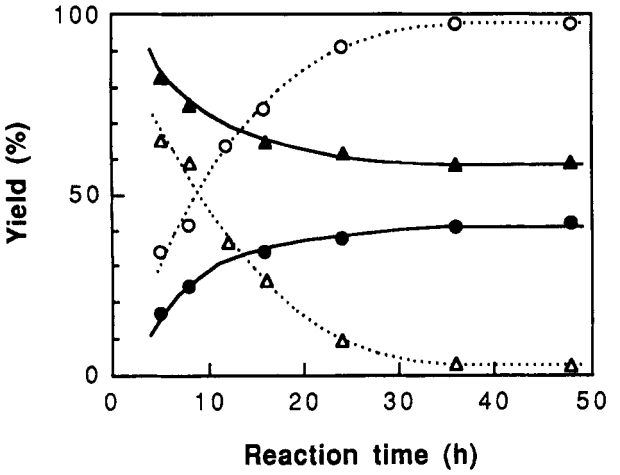

Figure 3. Time vs conversion of the demethylation of **1b** (—) and its acetate **4b** (---) (each 80 mg) with anhydrous aluminum chloride (0.6 g) in acetonitrile (2 mL) containing water (4 μL) at 70 °C: **2b**, △ and ▲; **3b**, ○ and ●.

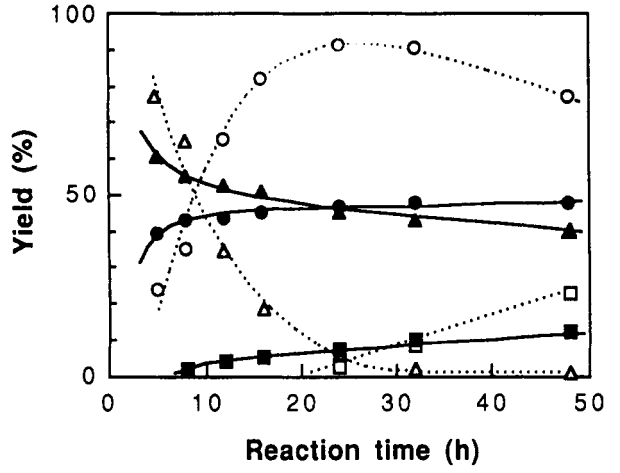

Figure 4. Time vs conversion of the demethylation of **1c** (—) and its acetate **4c** (---) (each 80 mg) with anhydrous aluminum chloride (0.6 g) in acetonitrile (2 mL) containing water (4 μL) at 70 °C: **2c**, △ and ▲; **3c**, ○ and ●; further demethylation products, □ and ■.

treatment of the reaction mixture with dilute hydrochloric acid becomes constant. Increasing the amount of aluminum chloride decreases gel formation, thus encouraging the equilibration of **7** and **6** and increasing the ratio of **3a** to **2a**. The reaction process for **2a** can also be explained by the same mechanism.

In the demethylation of acetates **4a** and **5a**, aluminum complex **10**, which forms rapidly, is slowly converted into aluminum complex **8** by an exchange reaction between the acetyl group and aluminum chloride. Because the acetyl group suppresses iminoesterification, further demethylation gives **3a**, via **9**, in high yield. The demethylation of the acetates is a general method for synthesizing 5,6,7-trihydroxyflavones **3** as long as the reaction proceeds without the cleavage of the methoxy groups on the B ring. Therefore, the demethylation of some 6-hydroxy-5,7-dimethoxyflavones with B-ring methoxy groups was also examined.

Demethylation of 6-Hydroxy-5,7-dimethoxyflavones 1b–1e and Their Acetates 4b–4e. The analysis of the products of demethylation of **1b**, **1c**, **4b**, and **4c** was carried out by HPLC, but the products from **1d**, **1e**, **4d**, and **4e** were analyzed by their ^{1}H NMR spectra because it was not possible to separate the products by HPLC. The time vs conversion curves of these reactions are shown in Figures 3–8.

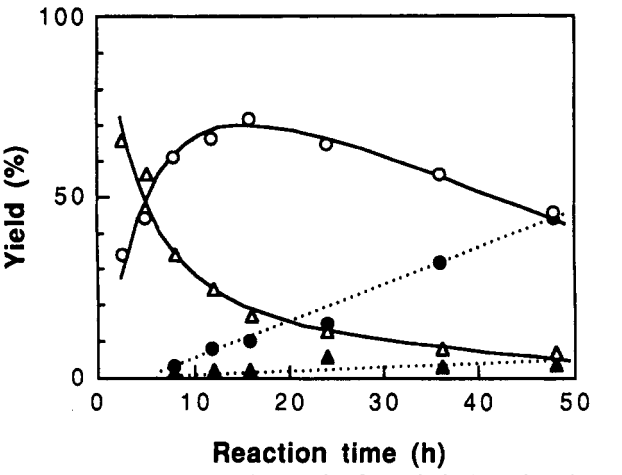

Figure 5. Time vs conversion of the demethylation of **1d** (100 mg) with anhydrous aluminum chloride (1.2 g) in acetonitrile (4 mL) containing water (4 μL) at 70 °C: **2d**, △; **3d**, ○; **2f**, ▲; **3f**, ●.

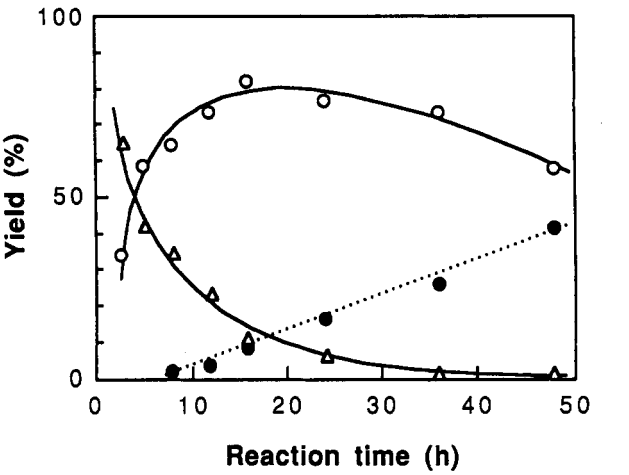

Figure 6. Time vs conversion of the demethylation of **4d** (100 mg) with anhydrous aluminum chloride (1.2 g) in acetonitrile (4 mL) containing water (4 μL) at 70 °C: **2d**, △; **3d**, ○; **3f**, ●.

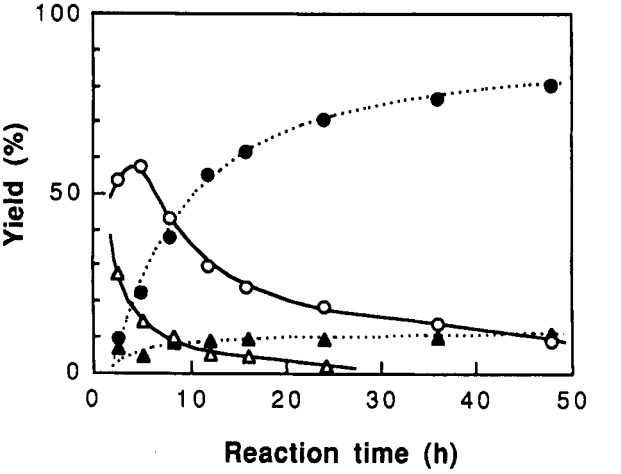

Figure 7. Time vs conversion of the demethylation of **1e** (100 mg) with anhydrous aluminum chloride (1.2 g) in acetonitrile (4 mL) containing water (4 μL) at 70 °C: **2e**, △; **3e**, ○; **2f**, ▲; **3f**, ●.

Table I. Molar Extinction Coefficients at 338 nm for the 5,6-Dihydroxy-7-methoxyflavones 2 and 5,6,7-Trihydroxyflavones 3 in Methanol

compd	a	b	c	g	h
2	26 300	26 200	22 900		
3	16 500	16 500	13 000	16 500	16 200

Table II. ^{1}H NMR Spectral Data for 5,6,7-Trioxygenated Flavones (2d, e, f and 3d, e, f) in DMSO-d_6[a]

compd	C_3-H	C_8-H	$C_{2'}$-H	$C_{6'}$-H	$C_{5'}$-H	OMe	5-OH
3d	6.86s	6.61s[b]	7.55d′	7.55dd	6.94d	3.90s	12.81s
2d	6.93s	6.94s	7.59d′[b]	7.60dd[b]	6.94d	3.91s, 3.93s	12.67s
3f	6.63s[c]	6.54s[b]	7.39d′	7.41dd	6.89d		12.81s
2f	6.70s[b,c]	6.86s	7.43d′	7.44dd	6.90d	3.92s	12.67s
3e	6.72s[c]	6.57s	7.42d′	7.53dd	7.08d	3.87s	12.77s
2e	6.78s[c]	6.90s	7.47d′	7.57dd	7.10d	3.86s, 3.93s	12.63s

[a] δ-values (400 MHz): d, doublet (J = 8.5 Hz); d′, doublet (J = 2.0 Hz); dd, (J = 8.5, 2.0 Hz). [b] These signals were employed for the product analysis of **1d** and **4d**. [c] These signals were employed for the product analysis of **1e** and **4e**.

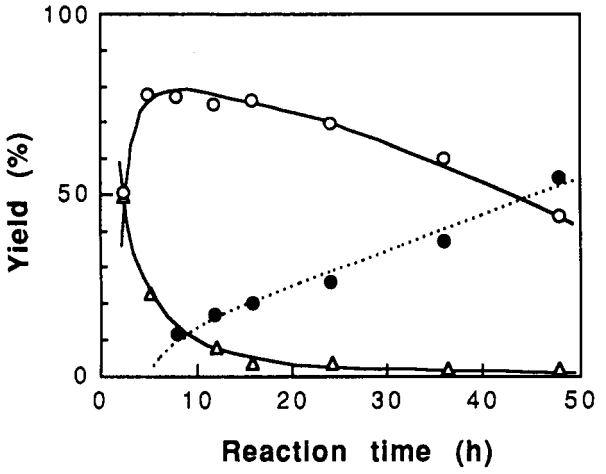

Figure 8. Time vs conversion of the demethylation of **4e** (100 mg) with anhydrous aluminum chloride (1.2 g) in acetonitrile (4 mL) containing water (4 μL) at 70 °C: **2e**, △; **3e**, ○; **3f**, ●.

The demethylation of flavones **1b** and **1c**, which have no free hydroxy groups on the B ring, and their acetates **4b** and **4c** proceeds as in the cases of the reactions of **1a** and **4a**. This result shows that 5,6,7-trihydroxyflavones **3b** and **3c** can be synthesized in high yield by the protection of the hydroxy group with an acetyl group (Figures 3 and 4). However, in the demethylation of **1c** and **4c**, the adjacent 3′-, 4′-, and 5′-methoxy groups on the B ring of **1b** and **4b**, were more easily cleaved than 3′- and 4′-methoxy groups of **1b** and **4b**, and a small amount of further demethylation products was obtained. The cleavage of the methoxy groups proceeded in the order 4′, 3′, and 5′, which was also the case for the demethylation of 8-hydroxy-3′,4′,5,5′,7-pentamethoxyflavone,[8] an isomer of **1c**. 4′,5,6,7-Tetrahydroxy-3′,5′-dimethoxy- (**3g**), 3′,4′,5,6,7-pentahydroxy-5′-methoxy- (**3h**), and 3′,4′,5,5′,6,7-hexahydroxyflavones (**3i**)[10] were isolated from the demethylation of **4c**.

The demethylation of 6-hydroxyflavones **1d** and **1e** also seems to proceed by the process shown in Scheme II, but the cleavage of the methoxy group on the B ring is accelerated by the adjacent hydroxy group. The quantity of 3′,4′,5,6,7-pentahydroxyflavone (**3f**) increases with increasing reaction time (Figures 5 and 7). More **3f** is formed from **1e** than from **1d**. One of the reasons that the 4′-methoxy group is easier to cleave than the 3′-methoxy group may be that the cleavage of the 4′-methoxy group is accelerated by the resonance between the 4′-methoxyl oxygen atom and the 4-carbonyl group. This possibility suggests that the cleavage of the 7-methoxy group may also be accelerated by the same type of resonance as well as the participation of the adjacent 6-hydroxy group.

The formation of 3′,4′,5,6-tetrahydroxy-7-methoxyflavone (**2f**) is not observed in the demethylations of the acetates **4d** and **4e** (Figures 6 and 8). This fact shows that the cleavage of the acetoxy group at the 6-position with aluminum chloride is faster than that at the 3′- or 4′-position in the B ring. The protection of the hydroxy groups by the acetyl group prevents not only the formation of the imino ester but also the cleavage of the methoxy group on the B ring, since the methoxy group is cleaved with participation of the adjacent hydroxy group after the cleavage of the 3′- or 4′-acetoxy group. That is, the demethylation of the acetylated 6-hydroxy-5,7-dimethoxyflavones is widely applicable as a general method for synthesizing 5,6,7-trihydroxyflavones **3**.

Experimental Section

The high-performance liquid chromatography (HPLC) was carried out by a method described in a previous paper[4] with a UV monitor at 338 nm and a column (2.1 i.d. × 500 mm) packed with Hitachi gel no. 3011. A mixed solvent of methanol, ethyl methyl ketone, and water was used as an eluent. For the separation of demethylated products, a column (20 i.d. × 600 mm) packed with Hitachi gel no. 3019 was used. Methanol was used as an eluent. ^{1}H NMR spectra were recorded in DMSO-d_6 at 400 MHz with tetramethylsilane as an internal standard, and the chemical shifts are given in δ values. The flavones used here were prepared by the method described in previous papers.[2,11] The values of elemental analyses were within 0.35% of theoretical values.

General Method for Analysis of the Demethylated Products. In a test tube (18 i.d. × 150 mm) fitted with a calcium chloride tube was dissolved the flavone **1**, **4**, or **5a** in 30% w/v solution of anhydrous aluminum chloride–acetonitrile, and the solution was heated at 70 °C in a thermostated oil bath. A small amount of the reaction mixture (0.1–0.2 mL) was removed at intervals, diluted with 1–2% hydrochloric acid (1–2 mL), and heated at 70–80 °C for 20–30 min. The mixture was allowed to stand in a refrigerator until the aqueous layer became colorless. The separated crystals were collected, washed with water, dissolved in methanol (ca. 3 mL) containing 10% hydrochloric acid (0.5 mL), and refluxed for 2–3 h. In the demethylations of **1a–1c** and their acetates, the solution (or the concentrated solution) was directly analyzed by HPLC, and the yields of the products were calculated from the chromatogram and molar extinction coefficients of the flavones as shown in Table I.

In the demethylations of **1d** and **1e** and their acetates (**4d** and **4e**) the solution obtained from the hydrolysis of the demethylated product was concentrated, diluted with water, and allowed to stand in a refrigerator until the aqueous layer became colorless. The separated crystals were collected, washed with water, and dried. The crystals were analyzed by ^{1}H NMR, and the amount of each component was determined by integration of the signals as shown in Table II.

3′,5,6,7-Tetrahydroxy-4′-methoxyflavone (3e). Acetate **4e** (190 mg) was dissolved in 30% w/v anhydrous aluminum chloride in acetonitrile (5 mL) containing water (5 μL), heated at 70 °C for 12 h, and then diluted with 5% hydrochloric acid (5 mL). The mixture was dissolved in methanol and refluxed for 2–3 h. The solution was concentrated under reduced pressure, diluted with water, and allowed to stand overnight. The separated crystals were collected and recrystallized from methanol to give **3e** (70

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mg, 50%): mp 252-253 °C; UV λ_{max} nm (log ϵ) (EtOH) 347 (4.31), 283 (4.21); (EtOH-$AlCl_3$) 370 (4.39), 295 (4.24); (EtOH-NaOAc) 366 (4.22), 324 (4.07), 277 (4.25). The mother liquor was separated by preparative HPLC: **3e** (40 mg, 29%); **2e** (9 mg, 6%); **3f** (6 mg, 4%). Tetraacetate: mp 250-251 °C (from $CHCl_3$-MeOH); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 × OAc), 2.41 (s, OAc), 3.86 (s, OMe), 6.58 (s, C_3-H), 7.00 (d, $C_{5'}$-H, J = 9 Hz), 7.41 (s, C_8-H), 7.49 (d, $C_{2'}$-H, J = 2.5 Hz), 7.66 (dd, $C_{6'}$-H, J = 2.5, 9 Hz). The isolation of **3e** from *Arnica viscosa* has been reported by Wolf et al.,[12] but its physical properties have not been reported.

Identification of the Demethylated Product from 4c. Acetate **4c** (1 g) was dissolved in 30% w/v anhydrous aluminum chloride in acetonitrile (20 mL) and heated at 70 °C for 48 h. The mixture was treated with dilute hydrochloric acid, and the product obtained was recrystallized from methanol to give **3c**.[2] The mother liquor was separated by preparative HPLC, and the following three demethylation products were obtained: 3',4',5,5',6,7-hexahydroxyflavone (**3i**)[10] (15 mg), 3',4',5,6,7-pentahydroxy-5'-methoxyflavone (**3h**) (34 mg) [mp 234-235 °C (from aqueous methanol); UV λ_{max} nm (log ϵ) (EtOH) 360 (4.31), 282 (4.13); (EtOH-$AlCl_3$) 383 (4.40), 301 (4.12); (EtOH-NaOAc) 400 sh (4.18), 375 (4.22); ^{1}H NMR (DMSO-d_6) δ 3.90 (s, OMe), 6.58 (s, C_8-H), 6.77 (s, C_3-H), 7.16 (2 H, s, $C_{2',6'}$-H), 12.82 (s, C_5-OH). Pentaacetate of **3h**: mp 284-286 °C (from $CHCl_3$-MeOH); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 4 × OAc), 2.41 (s, OAc), 6.55 (s, C_3-H), 7.24 (2 H, s, $C_{2',6'}$-H), 7.45 (s, C_8-H)], and 4',5,6,7-tetrahydroxy-3',5'-dimethoxyflavone (**3g**) (65 mg) [mp 290-292 °C (from aqueous methanol); UV λ_{max} nm (log ϵ) (EtOH) 360 (4.31), 284 (4.16); (EtOH-$AlCl_3$) 379 (4.45), 303 (4.15); (EtOH-NaOAc) 403 (4.22), 377 (4.23), 321 (4.06); ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 2 × OMe), 6.66 (s, C_8-H), 6.85 (s, C_3-H), 7.27 (2 H, s, $C_{2',6'}$-H), 12.81 (s, C_5-OH)]. Tetraacetate of **3g**: mp 259-261 °C (from $CHCl_3$-MeOH); ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3 × OAc), 2.42 (s, OAc), 3.87 (s, 2 × OMe), 6.55 (s, C_3-H), 7.02 (2 H, s, $C_{2',6'}$-H), 7.46 (s, C_8-H).

Supplementary Material Available: Table III with analytical data for the new demethylated products **3e**, **3g**, and **3h** and their acetates (1 page). Ordering information is given on any current masthead page.

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Regioselective Synthesis of Δ^6-, Δ^7-, and Δ^8-14α-Cyanosterol Derivatives: Versatile Precursors to 14α-Demethylase Inhibitors

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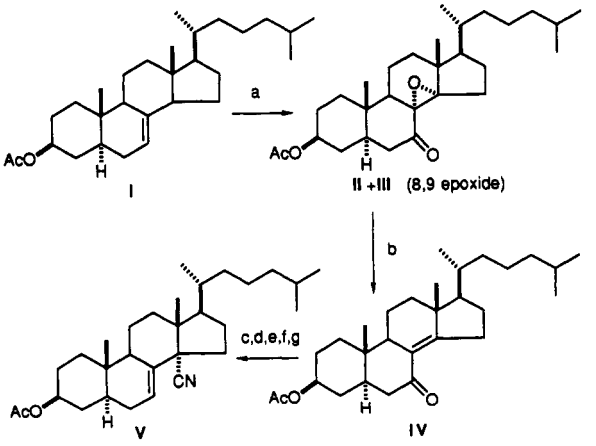
The efficient preparation of 3β-(benzoyloxy)-4,4-dimethyl-5α-cholest-8(14)-en-7-one (**4a**) and 3β-(benzyloxy)-5α-ergost-8(14)-en-7-one (**4b**) and their conversion to Δ^6-, Δ^7- or Δ^8-14α-functionalized sterols is reported. The alkylaluminum-mediated 1,4-addition of HCN (Nagata reaction) to enones **4a,b** is used to introduce the 14α substituent. Reduction of these conjugate addition products (**5a,b**) affords the 7α-hydroxysterols (**6a,b**). The dehydration of **6a** with Martin sulfurane reagent regioselectively produces the Δ^6-14α-cyanosterol **8**. Alternatively, mesylation of **6a** and elimination affords a mixture of Δ^7:Δ^6-sterols (3:1) from which the Δ^6-sterol is removed by selective ozonolytic degradation. The ozonolytic stability of the Δ^7-14α-cyanosterols is also exploited in the preparation of 14α-cyanosterols possessing modified side chains. Ozonolysis of 3β-(benzoyloxy)-5α-ergost-7,22-diene-14α-carbonitrile (**9b**) gave **18**, which is used to prepare compounds containing the "24 methenyl" (**21**) and "lanosterol" (**23**) side chains. The trapping of the intermediate aluminum enolate formed in the hydrocyanation of **4a** gives an 8β-bromosterol **13**. Dehydrobromination of **13** provides a novel regioselective synthesis of the difficult to prepare Δ^8-14α-functionalized sterol **14**.

Introduction

Lanosterol 14α-demethylase is the rate-limiting enzyme in the conversion of lanosterol to cholesterol in mammals and to ergosterol in fungi. Inhibitors of the fungal enzyme have proven utility in the treatment of both human and plant fungal disease.[1] Additionally, the inhibition of the mammalian enzyme has been suggested as a potential target for treatment of hypercholesterolemia.[2] As part of a program to prepare selective inhibitors of sterol 14α-demethylase, we required efficient and regiospecific syntheses of 14α-functionalized sterols which could serve as precursors to rationally designed substrate analogs. Because the side-chain structure and the position of nuclear unsaturation (Δ^7 and Δ^8 preferred) were known to be important variables for 14α-demethylase substrates,[3] we wanted a method which would accommodate the synthesis of Δ^6-, Δ^7-, and Δ^8-14α-functionalized sterols with various side chains. On the basis of the known biosynthetic pathways, the side chains which we considered of primary

Scheme I[a]



[a] (a) CrO_3; (b) chromatography, then treatment of **II** with Zn, HOAc; (c) Et_2AlCN; (d) $NaBH_4$; (e) MsCl, pyridine; (f) 2,4,6-collidine, Δ; (g) chromatography, $AgNO_3$ on silica.

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