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Regioselective Acetylation of 7-Deacetylforskolin with ^{11}C-Acetyl chloride

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SUMMARY

Reaction conditions were studied to control the acetylating position on 7-deacetylforskolin using [^{11}C]acetyl chloride. In a preliminary study using non-labeled acetyl chloride, pyridine, lutidine, triethylamine, N,N-diisopropylethylamine, dimethylaminopyridine (DMAP) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were tested as the base in the acetylation. Pyridine was effective in selective acetylation to yield forskolin in any solvent, and DBU was effective for 1-acetyl-7-deacetylforskolin. Among toluene, dichloromethane and dichloroethane, toluene was the most suitable as the solvent for the selective acetylation of the 7-OH group to give forskolin with any base.

In the selectivity of acetylation with [^{11}C]acetyl chloride, more [^{11}C]forskolin was obtained than [^{11}C]1-acetyl-7-deacetylforskolin (70:30) in the presence of pyridine in toluene. [^{11}C]1-Acetyl-7-deacetylforskolin was preferentially synthesized with DBU in dichloromethane, and the ratio of [^{11}C]1-acetyl-7-deacetylforskolin to [^{11}C]forskolin was 98:2.

For the yield of the [^{11}C]acetylated product, DBU in dichloromethane was also suitable to obtain [^{11}C]1-acetyl-7-deacetylforskolin. However, that of pyridine in

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toluene did not confer any advantages upon the yield of [^{11}C]forskolin compared with DMAP in toluene.

INTRODUCTION

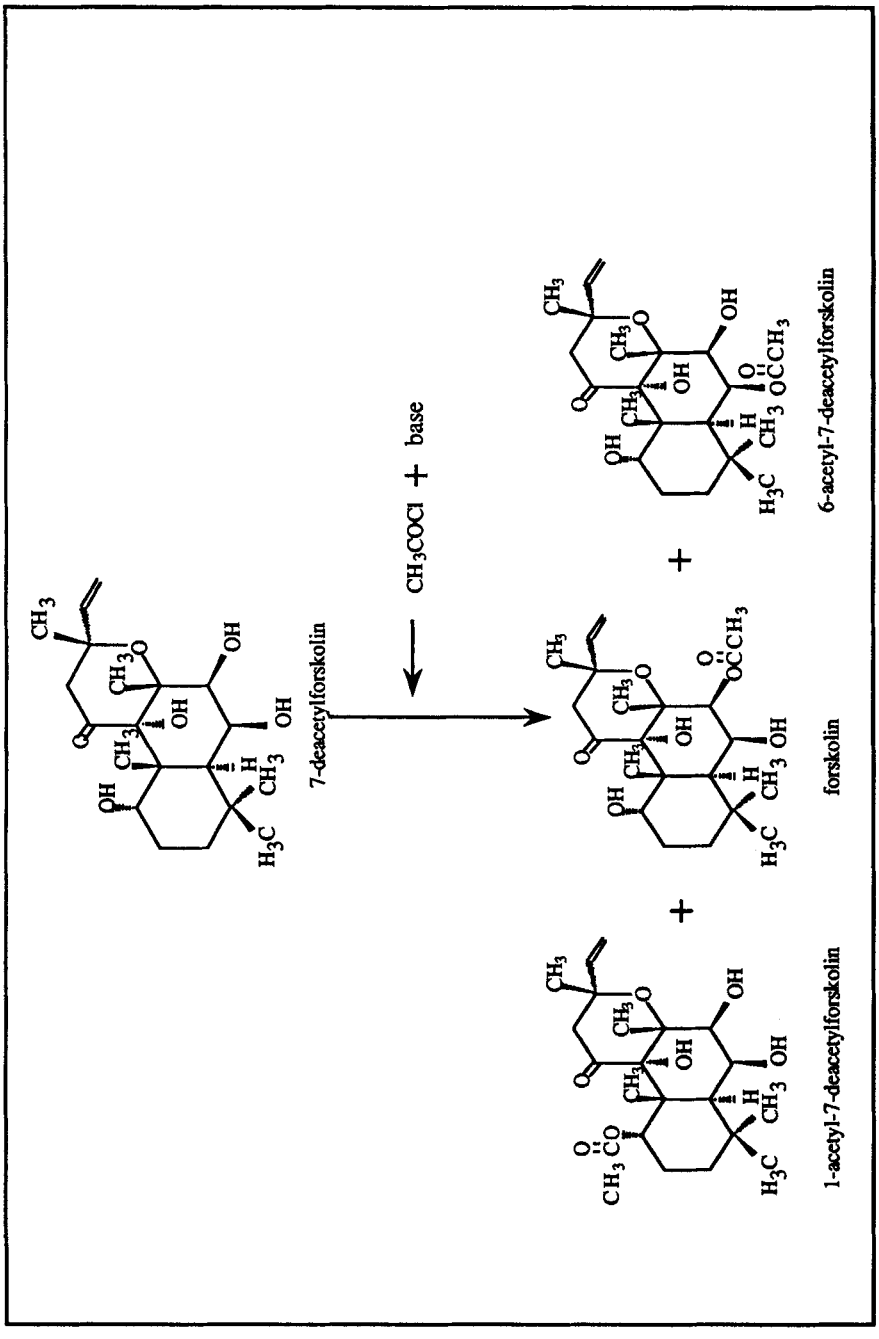
To visualize the adenylate cyclase-related second messenger system using a tracer, we reported the synthesis of [^{11}C]forskolin from 7-deacetylforskolin and [^{11}C]acetyl chloride, and its specific accumulation in the heart (1). We found that [^{11}C]1-acetyl-7-deacetylforskolin was synthesized as a by-product with [^{11}C]forskolin. Since the 1- and 9-OH groups on forskolin are critical for its ability to activate adenylate cyclase (2,3), we suggested that the [^{11}C]1-acetyl-7-deacetylforskolin could be used as a non-specific forskolin analog to estimate the specific uptake of [^{11}C]forskolin (1).

The purpose of this study was to find the optimum conditions under which [^{11}C]forskolin or [^{11}C]1-acetyl-7-deacetylforskolin could be selectively synthesized, by acetylating 7-deacetylforskolin with [^{11}C]acetyl chloride. First, a preliminary experiment was performed using unlabeled acetyl chloride, with various bases and solvents. Then experiments with [^{11}C]acetyl chloride were performed under the reaction conditions that demonstrated selectivity in the unlabeled experiment. The likelihood of controlling the selectivity of [^{11}C]acetylation with the bases and solvents in the reaction was studied.

MATERIALS AND METHODS

Unlabeled experiment

The precursor for forskolin and its analogs, 7-deacetylforskolin, was synthesized from forskolin (Wako Pure Chemical Co. Ltd.) according to the method of Kosley et al. (4). Five milligrams (14 μmol) of 7-deacetylforskolin were dissolved in 0.5 mL of freshly distilled toluene, dichloromethane or dichloroethane. Thereafter, 140 μmol of one of six bases, pyridine, lutidine, triethylamine, N,N-diisopropylethylamine, dimethylaminopyridine (DMAP) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), were added. These bases were purchased from Aldrich Chemical Co. Inc., and except for DMAP, they were distilled from calcium oxide under N_2 prior to use. Thereafter 140 μmol of acetyl chloride (Aldrich Chemical Co. Inc.) was added dropwise into the stirred mixture and reacted for 3 hours at room temperature under N_2 (Scheme 1).



Scheme 1. Acetylation of 7-deacetylforskolin using acetyl chloride

The progress of the reaction was checked by TLC as described in Ref. 1. The solution was poured into ice-cold ethyl acetate/saturated aqueous sodium chloride solution, and the organic layer was washed twice with saturated aqueous sodium chloride. The separated organic layer was dried with Na_2SO_4 and concentrated. The product was purified by chromatography on silica gel with ethyl acetate:hexane (1:2). The purified product was fractionated into forskolin, 1-acetyl-7-deacetylforskolin and 6-acetyl-7-deacetylforskolin on a Shimadzu HPLC system (reversed phase column; Megapak SIL-C18T , 10 mm I.D.x 250 mm, Nihon Bunko Co. Ltd.; UV absorbance monitor at 310 nm with a Shimadzu UV detector SPD-6AV) by elution with acetonitrile:water (1:1), at a flow rate of 7 mL/min.

Identification of 1-acetyl-7-deacetylforskolin was achieved by means of IR, NMR and MS. ^1H NMR spectra of solutions in $CDCl_3$ were measured using a JEOL GSX-400V spectrometer (Me_4Si was the internal standard). IR spectra were recorded on a JASCO FT/IR-8000 and are reported in wavenumbers. Mass spectra were obtained using a JEOL SX-102A. All experiments were performed in duplicate and the results are expressed as the relative amounts of 1- and 7-acetylated products in mean percentages.

IR (nujol) 3524, 1754, 1719 cm^{-1}

^1H NMR δ 1.08 (3H, s, CH_3), 1.15 (1H, m, H-3), 1.28 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.66 (3H, s, CH_3), 2.03 (3H, s, acetyl), 2.11 (1H, d, J=2Hz, H-5), 2.15 (1H, m, H-2), 2.32 (1H, s, OH), 2.50 (1H, d, J=17Hz, H-12), 3.10 (1H, d, J=17Hz, H-12), 4.17 (1H, d, J=3Hz, H-7), 4.50 (1H, m, H-6), 4.95 (1H, s, OH), 4.97 (1H, dd, J=1,11Hz, vinylic-H), 5.16 (1H, dd, J=1,17Hz, vinylic-H), 5.58 (1H, t, J=3Hz, H-1), 6.09 (1H, dd J=11,17Hz, vinylic-H).

MS (EI) m/e 410 (M^+), 392 (M^+-H_2O).

HR MS, m/e ($C_{22}H_{34}O_7$-H_2O, M^+-H_2O) calcd. 392.2200, obsd. 392.2199.

The obtained regioselectivity of 7-deacetylforskolin acetylation was calculated from the data of analytical HPLC (reversed phase column, Finepak SIL-C18S, 4.6 mm I. D. x 150 mm, Nihon Bunkko Co. Ltd.; elution with acetonitrile:water 1:1; flow rate, 1 mL/min; monitored by UV absorbance at 310 nm) and reconfirmed from the ratio of proton peak between H-1(δ 5.58) and H-7(δ 5.48) in 1H NMR.

Labeled experiment

Based upon the results of the unlabeled experiment, two combinations of base and solvent were tested using [^{11}C]acetyl chloride. The combination of DMAP in toluene (1) was also tested.

For ^{11}C acetylation, 5 mg (14 μmol) of 7-deacetylforskolin was dissolved in 0.5 mL of freshly distilled toluene, to which 66 μmol of DMAP or 33 μmol of pyridine was added. Thereafter, [^{11}C]acetyl chloride, produced from [^{11}C]carbon dioxide by the ^{14}N(p,α)^{11}C nuclear reaction using an AVF compact cyclotron (74 cm dia.; CYPRIS 370, Sumitomo Heavy Industries, Co., Ltd.) according to Le Bars et al. (5), was introduced into the mixture at room temperature as described (6). In another experiment, 5 mg (14 μmol) of 7-deacetylforskolin was dissolved in 0.5 mL of freshly distilled dichloromethane, to which 33 μmol of DBU was added, and the mixture was processed as described above. The solvent was evaporated and the residue was dissolved in a small volume of acetonitrile:water (1:1), and then purified on a Shimadzu HPLC system (reversed phase column; Megapak SIL-C18T, 10 mm I.D.x 250 mm, Nihon Bunkko Co. Ltd.; UV absorbance monitored by means of a Shimadzu UV detector SPD-6AV; radioactivity monitored with an ionization chamber; ICS-311, Aloka Co. Ltd) by elution with acetonitrile:water (1:1), at a flow rate of 7 mL/min. The retention times of [^{11}C]forskolin, [^{11}C]1-acetyl-7-deacetylforskolin and [^{11}C]6-acetyl-7-deacetylforskolin were confirmed by comparison with authentic species (forskolin, 6-acetyl-7-deacetylforskolin; Sigma Chemical Co. Ltd.) and synthesized non-labeled standard (1-acetyl-7-deacetylforskolin). To determine the quantity of [^{11}C]forskolin and its analogs, the absorbance of the UV detector (310 nm) in the analytical HPLC (reversed phase column; Finepak SIL-C18S; 4.6 mm I. D. x 150 mm; Nihon Bunkko Co. Ltd.; elution with acetonitrile:water 1:1; flow rate, 1 mL/min) was calibrated with known amounts of unlabeled forskolin and its analogs. The [^{11}C]forskolin and [^{11}C]1-acetyl-7-deacetylforskolin, obtained as described above, were evaporated to dryness and dissolved in 0.9 % NaCl as final products.

RESULTS AND DISCUSSION

In general, acylation of the 7-OH group on 7-deacetylforskolin proceeds after the

1-OH group is protected as 1-(tert-butyldimethylsilyl ether) (7) and 1,9-dimethylformamide acetal (4,8). However, this approach is too time-consuming for radiolabeling acylation with [^{11}C]acetyl chloride. We therefore acetylated the 7-deacetylforskolin directly with [^{11}C]acetyl chloride without protecting the 1-OH group.

Table 1 shows the acetylation of 7-deacetylforskolin between the 7- and 1-OH groups for various bases in the unlabeled experiment. Under each condition studied, up to 80 % of the 7-deacetylforskolin was acetylated at either the 1- or the 7-position. The regioselectivity to the 7-OH group was highest in the presence of N,N-diisopropylethylamine in toluene. However, this regioselectivity was lowered in dichloromethane and dichloroethane. Pyridine did not show the highest regioselectivity to the 7-OH group compared with other bases, but it was always effective in toluene, dichloromethane or dichloroethane, to selectively synthesize forskolin. On the other hand, DBU effectively produced 1-acetyl-7-deacetylforskolin in combination with dichloromethane and dichloroethane but not with toluene. Among the reaction solvents tested, toluene was the most suitable for the selective acetylation of the 7-OH group with any of the bases. We therefore decided on the following conditions: pyridine in toluene, DBU in dichloromethane and DMAP in toluene and studied the regioselective acetylation of 7-deacetylforskolin using [^{11}C]acetyl chloride. A comparison of the selectivity between 1- and 7-OH group [^{11}C]acetylation showed that the yield of forskolin was higher than that of 1-acetyl-7-deacetylforskolin (70:30) using pyridine in toluene, and that 1-acetyl-7-deacetylforskolin was preferentially synthesized (98:2) using DBU in dichloromethane. When DMAP was the base in toluene, the ratio of [^{11}C]forskolin to [^{11}C]1-acetyl-7-deacetylforskolin was 36:64 (Fig. 1).

For a 14.1 MeV bombardment with 30 μA protons for 30 min in the ^{11}C production, the average yield of [^{11}C]forskolin using pyridine in toluene during three trials was 105±28 MBq, that of [^{11}C]1-acetyl-7-deacetylforskolin using DBU in dichloromethane during three trials was 641±179 MBq and those of [^{11}C]forskolin and [^{11}C]1-acetyl-7-deacetylforskolin using DMAP in toluene during five trials were 141±32 and 394±96 MBq respectively, at about 45 min after the end

Table 1. The regioselective acetylations of 7-deacetylforskolin with acetyl chloride in the presence of various bases and solvents.

Base	Regioselectivity upon acetylation (1-acetyl/7-acetyl; %)		
solvent	toluene	dichloromethane	dichloroethane
pyridine	14/86	15/85	12/88
lutidine	16/84	42/58	52/48
triethylamine	9/91	17/83	25/75
N, N-diisopropylethylamine	2/98	55/45	49/51
dimethylaminopyridine (DMAP)	13/87	53/47	21/79
1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)	7/93	95/5	81/19

A solution containing 5 mg of 7-deacetylforskolin (14 μmol) in 0.5 mL of each solvent was stirred with 9.7 μL of acetyl chloride (140 μmol) and the base (140 μmol) at room temperature for 3 hours. The regioselectivity of acetylation was calculated from the data in analytical HPLC and is expressed as the relative percentages of 1- and 7-acetylated products. The underlined conditions of base and solvent were tested in the labeled experiments.

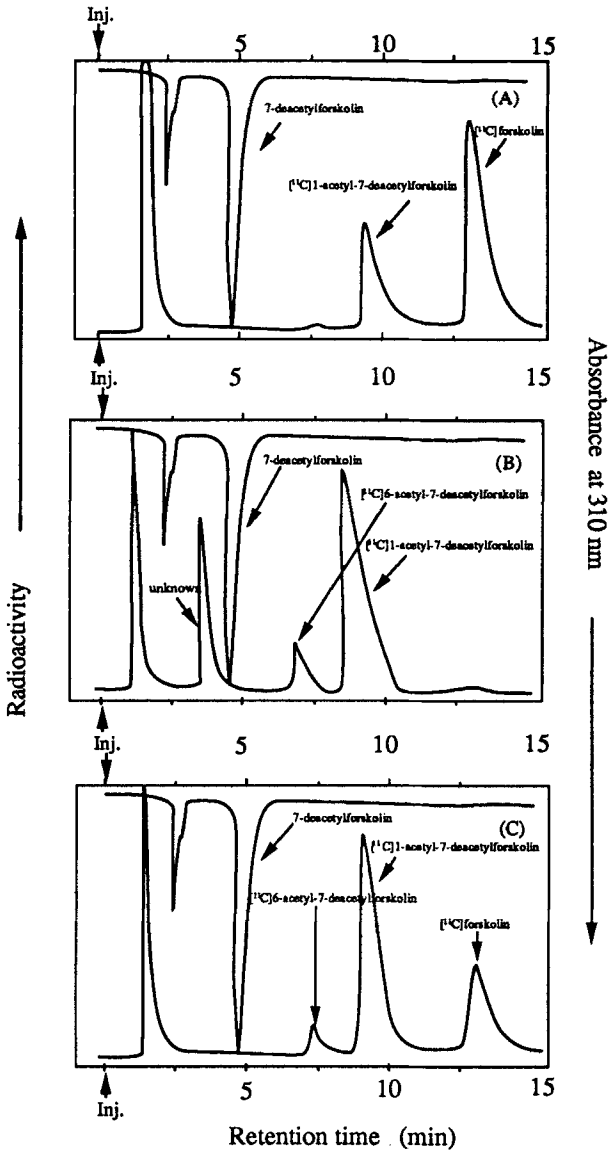


Figure 1. A comparison of the yield between [^{11}C]forskolin and [^{11}C] 1-acetyl-7-deacetylforskolin using preparative HPLC. ^{11}C Labeling was achieved by the acetylation of 7-deacetylforskolin (5 mg; 14 μmol) using [^{11}C] acetyl chloride with 33 μmol of pyridine in 0.5 mL toluene (A), with 33 μmol of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 0.5 mL dichloromethane (B) or with 66 μmol of dimethylaminopyridine (DMAP) in 0.5 mL toluene (C).

of bombardment. DBU in dichloromethane was also useful for obtaining [^{11}C]1-acetyl-7-deacetylforskolin. However, pyridine in toluene did not confer any advantages upon the yield of [^{11}C]forskolin compared with DMAP in toluene.

The results for ^{11}C acetyl chloride acetylation largely agreed with those of the unlabeled acetylation. However, the yield of [^{11}C]forskolin using pyridine in toluene was lower than in the corresponding unlabeled experiment. We found that water in the reaction significantly interfered with the yield of ^{11}C acetylation. It is very difficult to obtain anhydrous pyridine, because it has a lower boiling point. We considered that water in the unlabeled reaction is consumed by acetyl chloride to become acetic acid, but in the labeled reaction this become critical, because the mass of acetyl chloride in the labeled experiment is considerably lower than that in the unlabeled procedure.

The 1-OH group on 7-deacetylforskolin was more acetylated in the labeled than the corresponding unlabeled experiment. From the standpoint of the correlation between the basicity and regioselectivity of acetylation, stronger bases preferentially acetylated the 1-OH group on 7-deacetylforskolin (Table 1). We consider that the preference for 1-OH acetylation in the labeled reaction is attributed to the high basicity; the molar ratio of acetyl chloride to base in the labeled reaction is considerably lower than in the unlabeled reaction (acetyl chloride/base, 1/1). Another difference between labeled and unlabeled reactions was the yield of 6-acetyl-7-deacetylforskolin, which was negligible with unlabeled acetyl chloride, but notable with [^{11}C]acetyl chloride (Fig. 1). The yield of [^{11}C]6-acetyl-7-deacetylforskolin was the highest in DBU (Fig. 1). The 6-OH group on 7-deacetylforskolin can only be trans-acetylated from the 7-OH group under alkaline conditions (7). Therefore the emergence of [^{11}C]6-acetyl-7-deacetylforskolin is also attributed to the high basicity of the base.

There are four OH groups, 1-, 6-, 7- and 9-OH, on 7-deacetylforskolin that can be [^{11}C]acetylated. Because the 9-OH group is the most sterically hindered among them (7), it is the least reactive in acetylation. The 6-OH group is also sterically hindered (9). In general, equatorial alcohols are less hindered than axial alcohols with respect to acylation (10). According to this law, the equatorial 7-OH is more reactive than the axial 1-OH group during the acetylation of 7-deacetylforskolin. Indeed, we

reported the selective acetylation of 7-OH on 7-deacetylforskolin with [^{11}C]acetic acid in the presence of dicyclohexylcarbodiimide (DCC) and DMAP (11,12). Kosley et al. have also reported (4) that the reaction of 7-deacetylforskolin with 4-morpholinoacetic acid in the presence of DCC and DMAP resulted in the predominant acylation of 7-OH group. Furthermore they suggested that bromoacetyl bromide in the presence of dimethylaniline was effective in the predominant bromoacetylation of the 1-OH group. However, this study using acetyl chloride was not always in accordance with Kosley's results using bromoacetyl bromide, and the selectivity of acetylation depended on the base and solvent. We do not know of a mechanism of selective acetylation that depends on these parameters. However, we suppose that the basicity and the steric state around the nitrogen atom among the six bases are contributing factors.

Controlling the regioselective acylation of 7-deacetylforskolin with respect to bases and solvents has not been reported as yet. Our procedures could become the optimal protocol for the labeled- and unlabeled acylation of 7-deacetylforskolin.

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