Synthesis of [2-^{14}C] Griseolic Acid 9'-(4-Acetoxy-3-Methoxybenzyl)Ester, A Antiglaucoma Agent

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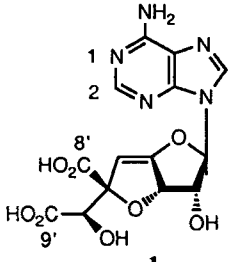
SUMMARY

Starting from [^{14}C] ethyl orthoformate and amidine derivative 3 derived from griseolic acid in 6 steps , the synthesis of [2-^{14}C] griseolic acid 9'-(4-acetoxy-3-gethoxybenzyl)ester [14C]-2 , a promising antiglaucoma agent with its regioisomer [2-^{14}C] griseolic acid 8'-(4-acetoxy-3-gethoxybenzyl)ester 9 and [2-^{14}C] griseolic acid [14C]-1 has been achieved.

Key Words : [^{14}C] Griseolic Acid ,[^{14}C] Benzylmonoester , Antiglaucoma Prodrug

INTRODUCTION

Griseolic acid 1, discovered in microbial culture broth of *Streptomyces griseoaurantiacus* SANK 63479 , has been reported to have an extremely strong c-AMP accumulation activity by inhibiting c-AMP PDE[1] and also a potent lowering effect on intraocular pressure.



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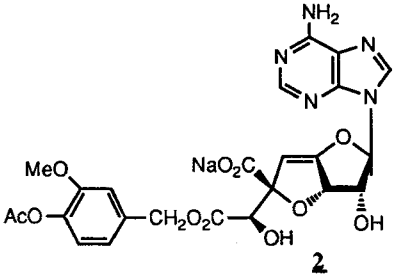

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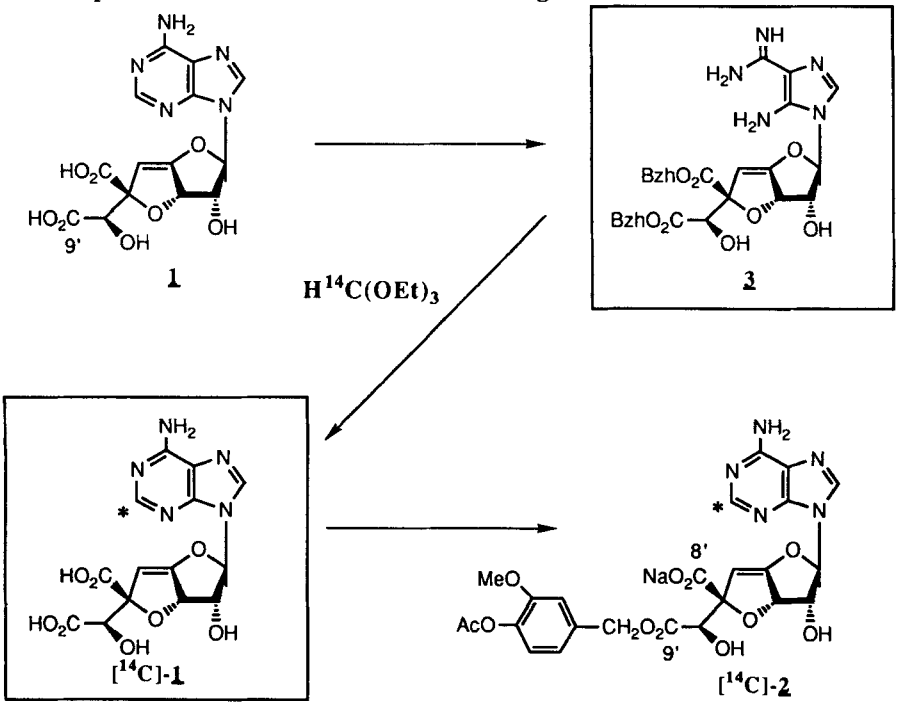
However owing to poor permeability of the cell-membrane , it does not exhibit any activity by instillation whereas griseolic acid 9'-(4-acetoxy-3-methoxybenzyl)ester 2 , a prodrug of griseolic acid , is considered to be able to cross the ophthalmic membrane and is easily hydrolyzed to griseolic acid and exhibits activity. After instillation of a 2.0% solution into rabbit eyes, The latter compound prevented water load-induced elevation of intraocular pressure, lowered normal intraocular pressure and inhibited the rise of intraocular pressure during darkness [2]. It therefore shows promise as a antiglaucoma agent.

For use in metabolic and disposition studies of 2 in the development stages , it was necessary to prepare a [^{14}C] labelled compound. Considering the fact that griseolic acid is a natural product having a complex structure and has not yet been synthesized [3], the place where [^{14}C] carbon can be introduced is very limited except for the adenine nucleus . Therefore we attempted to cleave the adenine nucleus at the pirimidine ring and then introduce a [^{14}C] carbon unit at the C-2 position to reconstruct the adenine ring.



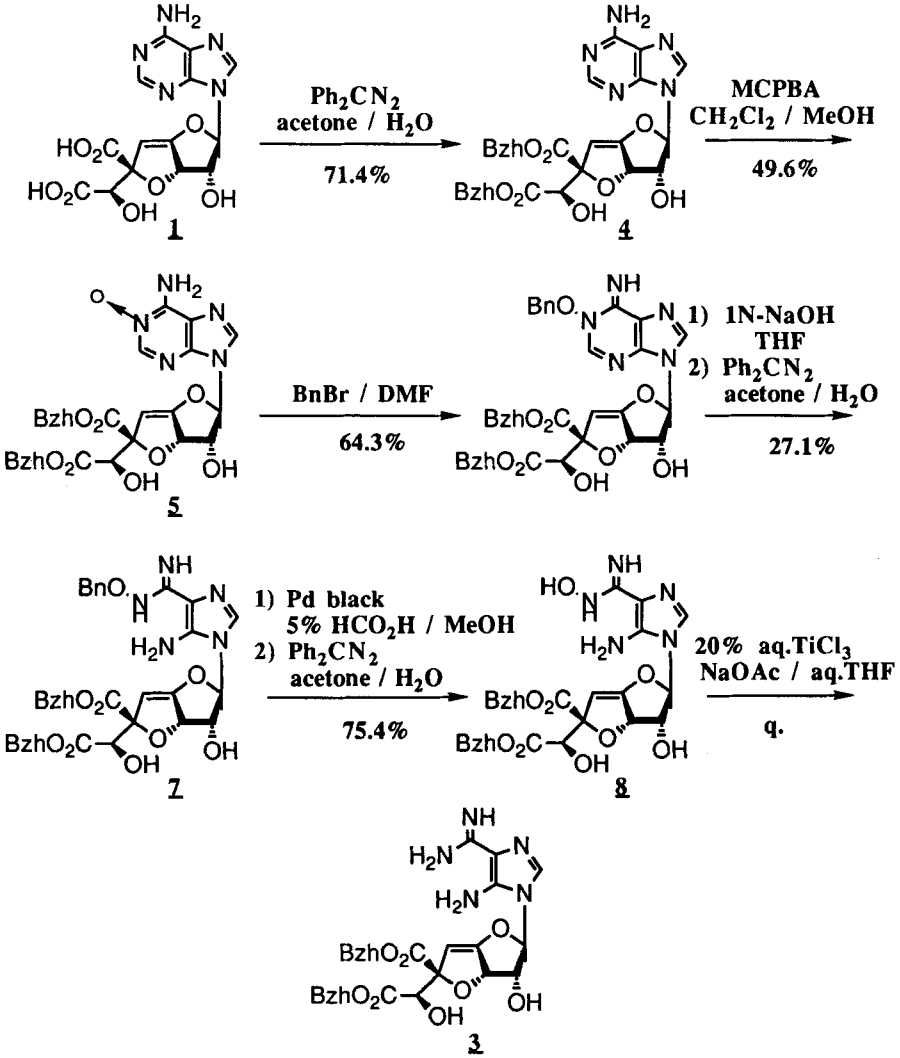
SCHEME 1

Our synthetic strategy is shown in Scheme 1. Firstly griseolic acid 1 is converted to the key intermediate 3 ,then incorporated [^{14}C] ethyl orthoformate as ^{14}C-carbon unit to regenerate [2-^{14}C] griseolic acid , prior to producing [2-^{14}C] griseolic acid 9'-(4-acetoxy-3-gethoxybenzyl)ester [^{14}C] -2 .

RESULTS AND DISCUSSION

Synthesis of Key Intermediate 3

As ring fissions of the adenine nucleus at a pyrimidine ring were extensively studied by T. Fujii group [4], we took advantage of their procedure to produce the key intermediate 3 as shown in Scheme 2.



SCHEME 2

Synthesis of [2-^{14}C] Griseolic Acid 9'-(4-Acetoxy-3-Methoxybenzyl)Ester [2-^{14}C]-2

At the beginning of the work , it was considered griseolic acid 1 would be converted to N1-oxide 5 by MCPBA through dibenzhydrylester 4 and benzylation of 5 would lead to benzyloxy derivative 6 which was thought to be easily susceptible to alkaline ring fission to afford 7 . Indeed alkaline hydrolysis of 6 followed by benzhydrylation gave 7 . After hydrogenolysis of 7 , reduction of carboxyamidoxime 8 to carboxyamidine 3 was difficult . However using 20% aq.$TiCl_3$ [5], the key intermediate 3 was successfully obtained in quantitative yield from 8 .

Incorporation of the C_1 unit to the key intermediate 3 was best realized by using ethyl orthoformate . However, owing to the presence of polyfunctional groups in 3 which may react with ethyl orthoformate , at least 2.5 eq. of reagent was necessary to achieve a satisfactory result. The reaction was carried out under reflux in a closed atmosphere and [^{14}C]-4 was obtained in 29.1% yield based on a [^{14}C] ethyl orthoformate . Excess volatile reagent was trapped as sodium [^{14}C] formate after sequential treatment with ethanol and 5N NaOH. Dibenzhydryl ester [^{14}C]-4 was efficiently hydrogenolyzed to [^{14}C]-1 with Pd-black / 5% HCO_2H-MeOH under a nitrogen atmosphere . In the monobenzylation reaction , it was found that Li_2CO_3 in DMF at room temperature for 7-8 h afforded the best result . [2-^{14}C]-2 and its regioisomer 9 was separated in a 3:1 ratio from the organic layer and unreacted [^{14}C]-1 was recovered from the aqueous layer (see Scheme 3 and experimental) .

Metabolic and disposition studies of [^{14}C]-2 will be the subject of a separate publication.

EXPERIMENTAL

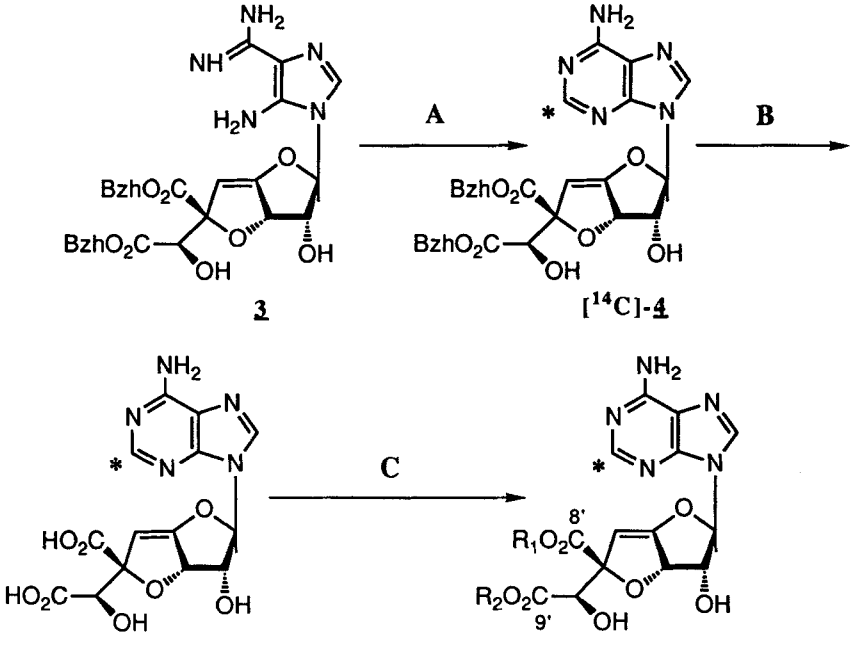
[14C] Ethyl orthoformate in THF (total activity 10.36GBq : specific activity 1.0GBq/mmol : radiochemical purity 98%) was purchased from Amersham International plc. All chemicals and solvents were analytical grade and were used without further purification unless otherwise noted . Proton magnetic resonance (NMR) spectra were recorded on a 270MHz JEOL GX or EX 270 spectrometer and are reported in δ (ppm) downfield from the internal standard tetramethylsilane (TMS). All NMR spectra were consistent with the structures assigned.

Griseolic acid dibenzhydrylester 4

To a suspension of griseolic acid 1 (30g , 79mmol) in 10% aq.acetone (1L) , diphenyldiazomethane (32.7g , 166mmol) was added at room temperature and vigorously stirred for 8h. As the reaction proceeded , the solution became clear , and a small amount of undissolved material was filtered off. The filtrate was

concentrated to 1/5 volume , to which n-hexane was added until white powder of 4 was precipitated. 40.0g (71.4%)

^{1}H-NMR(DMSO-d^6) δ_{ppm} : 8.18 , 8.37 (2H,2xs), 7.44-7.18(20H,m), 6.79,6.73(2H,2xs), 6.58(1H,s), 6.35(1H,dd,J=3.0,6.0Hz), 5.27(1H,d,J=3.0Hz), 4.93(1H,s), 4.68(1H,d,J=6.0Hz)



[^{14}C]-1 R_1 = Na R_2 = 4-acetoxy-3-methoxybenzyl : [^{14}C]-2
R_1 = 4-acetoxy-3-methoxybenzyl R_2 = Na : 9

A ; 1) 2.5eq. H^{14}C(OEt)$_3$ / p-TsOH / THF reflux 2) EtOH / reflux 3) distillation 4) column purification

B ; Pd-black / 5% HCO_2H - MeOH / reflux

C ; 1) 4-Acetoxy-3-methoxybenzylbromide / Li_2CO_3 / DMF 2) Na 2-ethylhexanoate 3) Lobar column purification for [^{14}C]-2 and 9 : HP-20 for [^{14}C]-1 4) freeze drying

SCHEME 3

The specific radioactivities and radiochemical purities are shown in the Table .

		[^{14}C]-2	[^{14}C]-1	9
Specific radioactivity	(MBq/mg)	1.40	1.82	1.43
Radiochemical purity	(%)	96.7	94.3	91.6
Amount	(mg)	195.5	250.0	68.0
Total radioactivity	(MBq)	278.3	456.1	97.4

TABLE

N^1-Oxygriseolic acid dibenzhydrylester 5

70% m-Chloroperbenzoic acid (MCPBA ,13.5g, 55mmol) was added to a mixed solution of 4 (30g,42mmol) in dichloromethane and methanol (600ml,5/1) at 0° C and stirred for 24h at room temperature. After the reaction ceased , the solvent was evaporated off *in vacuo*. The resulting residue was extracted with a mixed solvent of ethyl acetate and THF (1/1). The extract was sequentially washed with saturated sodium bicarbonate solution and brine, then dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was subjected to sillica gel column chromatography (methylene chloride/methanol = 10/1 elution) to afford the white powder of 5 (15.2g, 49.6%) .

^{1}H-NMR(DMSO-d^6) δ_{ppm} : 8.56,8.48(2H,2xs), 7.43-7.20(20H,m), 6.74,6.68(2H,2xs), 6.52(1H,s), 6.05(1H,dd,J=2.0,4.9Hz), 5.32(1H,d,J=2.0Hz), 4.89(1H,s), 4.62(1H,d,J=4.9Hz)

N^1-Benzyloxygriseolic acid dibenzhydrylester 6

To a suspension of 5 (15.2g, 21mmol) in DMF (200ml) , benzyl bromide (10.1ml, 84mmol) was added at room temperature and stirred for 24h. The solvent was evaporated under reduced pressure. After the resulting residual oil was solidified by adding sufficient of diethyl ether, the solid was filtered and purified using column chromatography (methylene chloride / methanol = 30 / 1 elution) to afford a light yellow powder of 6. 11.0g (64.3%)

^{1}H-NMR(DMSO-d^6) δ_{ppm} : 8.18,8.15(2H,2xs), 7.55-7.19(20H,m), 6.73,6.66(2H,2xs), 6.41(1H,s), 5.93(1H,dd,J=2.0,4.9Hz), 5.32(1H,d,J=2.0Hz), 5.24(2H,s), 4.88(1H,s), 4.53(1H,d,J=4.9Hz)

5-Amino-1β-(deadeninogriseolic acid dibenzhydrylester-1'-yl) imidazole-4-carboxyamido-O-benzyloxime 7

The reaction mixture of 6 (11.0g, 13mmol) in THF(200ml) and 1N NaOH solution (80ml) was stirred for 24h. The aqueous layer was separated and adjusted by adding conc.HCl solution to pH 3 to 4. After making a clear solution with acetone (300ml) , diphenyldiazomethane (5.6g, 28mmol) was added and the solution stirred for 5h. After evaporation of solvent, the resulting residue was extracted with mixed solvent of ethyl acetate and THF (1/1) which was then subsequently washed with saturated sodium bicarbonate solution and brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. the residue was purified using column chromatography (methylene chloride / methanol = 30 / 1 elution) to afford a yellow powder of 7. 3.0g (27.1%)

^{1}H-NMR(DMSO-d^6) δ_{ppm} : 7.41-7.19(21H,m), 6.73,6.67(2H,2xs), 6.17(1H,s), 5.56(1H,dd,J=2.4,4.9Hz), 5.25(1H,d,J=2.4Hz), 4.91(2H,s), 4.86(1H,s), 4.50(1H,d,J=4.9Hz)

5-Amino-1β-(deadeninogriseolic acid dibenzhydrylester-1'-yl) imidazole-4-carboxyamidoxime 8

Palladium black (600mg) was placed in a three necked flask charged with nitrogen gas. A solution of 7 (3.0g,3.7mmol) in 4.4% formic acid- methanol (120ml) was added and stirred for 6h at room temperature. The catalyst was carefully filtered off through a celite pad and the filtrate evaporated *in vacuo*. The resulting residue was dissolved in 10% aq. acetone (190ml) and to this reaction mixture was added diphenyldiazomethane (730mg, 3.6mmol) and the solution stirred for 5h at room temperature. After evaporation of solvent, the resulting residue was extracted with mixed solvent of ethyl acetate and THF (1/1) which was subsequently washed with saturated sodium bicarbonate solution and brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The residue was purified through column chromatography (methylene chloride / methanol = 20 / 1 elution) to afford a yellow powder of 9. 2.0g (75.4%)

^{1}H-NMR(DMSO-d^6) δ_{ppm} : 7.42-7.18(21H,m), 6.73,6.67(2H,2xs), 6.18(1H,s), 5.58(1H,dd,J=2.0,4.4Hz), 5.26(1H,d,J=2.0Hz), 4.87(1H,s), 4.53(1H,d,J=4.4Hz)

5-Amino-1β-(deadeninogriseolic acid dibenzhydrylester-1'-yl) imidazole-4-carboxyamidine 3

Under a nitrogen gas atmosphere, to the reaction mixture of 8 (2.0g, 2.7mmol) and sodium acetate (2.0g, 27mmol) in 20% aq.THF (60ml) with ice-cooling bath, 20% aq. $TiCl_3$ solution (4ml, 5.4mmol) was added and the solution stirred for 6h at room temperature. The reaction mixture was quenched by adding saturated sodium bicarbonate solution, precipitated inorganic material was filtered off by washing with ethyl acetate. The filtrate was extracted with ethyl acetate and washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The resulting residue was triturated with ethyl ether to afford a yellow powder of 3. 1.9g (quant.)

^{1}H-NMR(DMSO-d^6) δ_{ppm} : 7.69(1H,s), 7.42-7.18(20H,m), 6.74,6.68(2H,2xs), 6.29(1H,s), 5.58(1H,dd,J=2.4,4.4Hz), 5.33(1H,d,J=2.4Hz), 4.89(1H,s), 4.59(1H,d,J=4.4Hz)

[2-^{14}C]-Griseolic acid dibenzhydrylester [^{14}C]-4

The reaction mixture of amidine derivative 3 (1.5g, 2.14mmol), [^{14}C] ethyl orthoformate (5180MBq) and catalytic amount of p-toluenesulfonic acid (40mg) in THF (30ml) was refluxed for 1h. Excess [^{14}C] ethyl orthoformate was decomposed by adding ethanol (5ml) and refluxed for 30 min , then the solvent was distilled off. The distillate was treated with 5N NaOH solution (40ml) and

left for 24h to trap volatile [^{14}C] ethyl orthoformate as solid sodium formate (3145MBq). The residue was dissolved in a small amount of 10% methanol-methylene chloride solution and purified using column chromatography (8% methanol-methylene chloride elution) to afford the white powder of [^{14}C]-4 1290mg (1510.3MBq. 29.1%). In another experiment on the same scale, 1250mg (1446.5MBq, 27.9%) of [^{14}C]-4 was obtained. Two lots of [^{14}C]-4 (totally 2540mg, 2956.8MBq) was combined and used in the next step.

[2-^{14}C]-Griseolic acid 9'-(4-acetoxy 3-methoxybenzyl)ester [^{14}C]-2 and its regioisomer [2-^{14}C]-Griseolic acid 8'-(4-acetoxy 3-methoxybenzyl)ester 9 and [2-^{14}C]-Griseolic acid [^{14}C]-1

Palladium black (2.0g) was placed in a three-necked flask charged with nitrogen gas and to this a mixed solution of [^{14}C]-4 (2540mg, 2956.8MBq) in THF and 5% formic acid- methanol (30ml/200ml) was added and refluxed for 8h. The catalyst was filtered off through a celite pad and the filtrate was evaporated in vacuo to dryness. The resulting residue was used in the next step without further purification. The reaction mixture of the above residue, 4-acetoxy-3-methoxybenzyl bromide (2.0g, 7.7mmol) and lithium carbonate (740mg, 10.0mmol) in DMF(60ml) was stirred for 7h at room temperature. The reaction mixture was extracted with ethyl acetate and washed with saturated sodium bicarbonate solution. In the aqueous layer , griseolic acid and its benzylmonoesters were supposed to be involved. The aqueous layer was adjusted by adding conc.HCl solution to pH.3 and extracted with a mixed solvent of THF and ethyl acetate(1/1) several times.

THF and ethyl acetate(1/1) layer

The combined organic layer was washed with brine and evaporated in vacuo. The resulting residue was dissolved in THF and methanol mixed solution (30ml/15ml), to which 0.2M sodium 2-ethyl hexanoate methanol solution (7ml) was added to make the sodium salts , and evaporated in vacuo. The residue was dissolved in 10% acetonitrile- H_2O (10ml) and subjected to Lobar column chromatography (RP-8, 5%acetonitrile- H_2O elution) and freeze-dried to afford [^{14}C]-2 (195.5mg, 278.3MBq) and its regioisomer 9 (68.0mg, 97.4 MBq) .

Aqueous layer

The aqueous layer was again made alkaline by adding 5N NaOH solution and subjected to CHP-20 column chromatography (H_2O elution) to remove inorganic base and salts , then freeze-dried . The resulting residue was

further purified through reverse column chromatography (RP-8, 5%acetonitrile-H_2O elution) and freeze-dried to afford [^{14}C]-1 (250mg, 456.1MBq).

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