SYNTHESIS OF BETAMETHASONE-1,2-$^3H_2$ 17-BENZOATE

T. Kobari, S. Watanabe and S. Ikegami
Institute of Biological Science, Mitsui Pharmaceuticals, Inc.,
1900-1, Toga, Mobara, Chiba, Japan.
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SUMMARY

A new convenient method for the preparation of the title compound is described. The method consists of selective tritiation of betamethasone 17-benzoate (9α-fluoro-11β,17,21-trihydroxy-16β-methyl-1,4-pregnadiene-3,20-dione 17-benzoate) (1) with the use of tris(triphenylphosphine)rhodium chloride as catalyst, followed by dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).

A favorable overall radiochemical yield is obtained.

INTRODUCTION

The synthesis of tritium labelled betamethasone 17-benzoate (9α-fluoro-11β,17,21-trihydroxy-16β-methyl-1,4-pregnadiene-3,20-dione 17-benzoate) in five steps from betamethasone 21-acetate has been reported by Merrill et al.[1] In this method eight Ci of tritium gas was used for catalytic reduction of the ring A diene but the total radiochemical yield and the specific activity of the final compound were unfavorably poor. In order to accomplish metabolic studies of this glucocorticoid, betamethasone 17-benzoate(1), especially with whole-body autoradiogram, the labelled compound having a relatively high specific activity should be required. However, if it is prepared according to Merrill's method, tritium gas of very high activity has to be used for obtaining a labelled compound of high specific activity and there are many difficulties for the synthesis using such a high activity tritium gas. We thus intended to establish a new convenient method consisting of only two steps as illustrated in Scheme 1 to minimize the loss of tritium in the course of the synthesis.

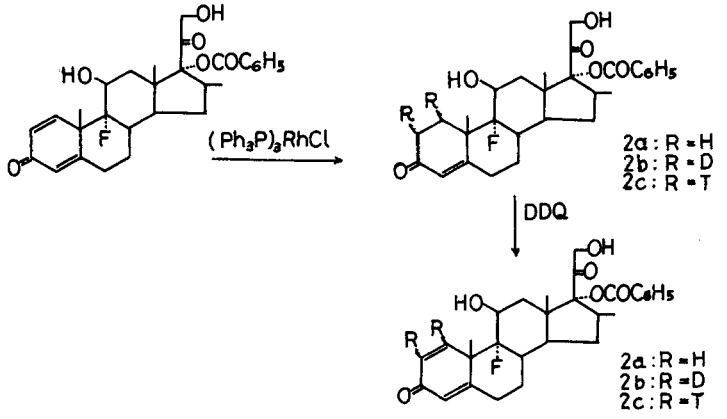
RESULTS AND DISCUSSION

The necessity for protection of 21-hydroxy group by acetylation, as used in the reported method[1], may be based on the fact that this group is

susceptible to oxidation under dehydrogenation conditions and may result in a poor final yield of the dehydrogenated product.[2,3] Besides, deacetylation is usually run in acidic or alkaline medium where undesirable exchange of tritium and hydrogen and/or other side reaction(s) sometimes may occur. Therefore, it seems much more convenient that betamethasone 17-benzoate(1) is directly tritiated and regenerated without any protection of the 21-hydroxy group if these reactions can favorably proceed in a good yield. Furthermore, as previously mentioned, Merrill, et al. introduced tritium into the steroid nucleus at C-1, C-2, C-3 and C-4 by the catalytic reduction of the ring A diene of 1.[1] But, obviously, Δ^4-3-ketosteroids are more easily dehydrogenated by DDQ-oxidation to $\Delta^{1,4}$-3-ketosteroids than saturated 3-ketosteroids each of which carries 5α-hydrogen.[2,4] A large excess of DDQ is required to convert the latter compounds to $\Delta^{1,4}$-3-ketosteroids and generally, the yield is quite low. On the other hand, the presumed side reaction of 21-hydroxy group under DDQ-oxidation condition may be controlled by decreasing the amount of DDQ. Accordingly, it was conceivable that the application of selectively 1,2-dihydrogenated betamethasone 17-benzoate(2a) would minimize the side reaction in DDQ-oxidation.

Based upon these considerations, investigations using hydrogen gas were tried prior to the actual labelling with tritium gas to determine whether the two step synthesis shown in Scheme 1 can serve as the preparation method of the target labelled compound.

Scheme 1



Homogeneous hydrogenation of 1 by the use of tris(triphenylphosphine) rhodium chloride resulted in the quantitative formation of 1,2-dihydrobetamethasone 17-benzoate(2a). Dehydrogenation of 2a was carried out at reflux

in dioxane using molar equivalent of DDQ. Thin layer chromatography (tlc) of the reaction mixture revealed that the yield of 1 was high enough and in the dehydrogenation step no other reaction products were found except 2a. Reaction time to complete the dehydrogenation depended on the molar ratio of DDQ and the starting material. Prolonged reaction time and the addition of a large excess of DDQ promoted further oxidation of the conjugate system to $\triangle^{1,4,6}$-betamethasone 17-benzoate.[5] As a result, direct dehydrogenation of the reaction mixture, without any purification or removal of the homogeneous catalyst,[6] proceeded in a good yield with no unfavorable side reactions by adding a slight excess of DDQ. In the preliminary experiments using deuterium gas, it was confirmed that the reaction in a mixture of deuterium and nitrogen gases depended upon a volumetric ratio of them. (Table 1) For example, in the presence of 0.03% of deuterium in nitrogen atmosphere, no reaction occurred. However, by increasing the amount of deuterium, the reaction proceeded successfully.

Table 1 - Results of the catalytic reduction of 1 with H_2 or D_2

Starting Material mg	$(Ph_3P)_3RhCl$ mg	Gas Phase	Reaction Time hr	Yield [a] of 2a or 2b %
50	100	H_2	20	100
50	100	H_2	5	60
50	100	$H_2/He=1$	14	100
100	200	$H_2/He=1$	14	75
25	50	D_2	20	100
25	50	$D_2/N_2=0.2$	21	55
25	50	$D_2/N_2=0.09$	22	40
25	50	$D_2/N_2=0.03$	21	0

a : This yield was determined from the ratio of 2a or 2b to 1 by mass spectrometry.

The total volume of the reaction flask and other glass tube connected was about 20 ml. If 1 Ci of tritium gas, whose volume is only 0.4 ml, is used for labelling, any reaction would not proceed because of very rare contact between the reactant gas and the substrate. With the proper addition of hydrogen as a carrier the reaction would proceed, but in this case the amount of added hydrogen should be controlled in order to obtain the labelled compound of an appropriate specific activity.

Finally, on the basis of these preliminary experiments, 100 mg of betamethasone 17-benzoate(1) was tritiated with 1.6 Ci of a tritium gas in the presence of 10 ml each of hydrogen and helium.[7] Successive treatment with

DDQ and the preparative fractionation of the resulting reaction mixture by tlc gave 20 mg of betamethasone-1,2-$^3H_2$17-benzoate(3b). The specific activity was 3.92 mCi/mg.

EXPERIMENTAL

Silica gel tlc plates (E. Merck, Darmstaht) were used to monitor the purity and the progress of the reaction. Preparative isolation of tritiated betamethasone 17-benzoate was performed on Silica gel F-254 plates (2 mm, E. Merck, Darmstaht) using a mixture of 80 parts by volume of benzene and 20 parts by volume of acetone as a developing solvent. Nmr spectra were measured by a JEOL PS-100 spectrometer in $CDCl_3$ using TMS as an internal standard. Mass and infrared spectra were recorded on a JEOL D-100 spectrometer and a Hitachi 285 infrared spectrophotometer, respectively.
The radioactivities of the tritiated compounds were determined by using a Nuclear Chicago Mark II liquid scintillation counter using a versatile toluene scintillator.

Homogeneous catalytic hydrogenation of betamethasone 17-benzoate(1) - The reaction flask containing a solution of 25 mg (0.05 mmol) of (1) and 25 mg (0.03 mmol) of tris(triphenylphosphine)rhodium chloride in 1 ml of distilled dioxane was evacuated and then an appropriate amount of deuterium (or hydrogen) was introduced into the flask by the aid of a Toepler pump. After stirring for a short time, nitrogen was introduced to adjust the pressure to an atmospheric pressure. Then, the mixture was stirred for 20 hrs at room temperature and submitted to the DDQ-dehydrogenation, as described later, without purification. When the reaction was run under a sufficient amount of hydrogen atmosphere (about 20 ml), 1,2-dihydrobetamethasone 17-benzoate(2a) was obtained in a quantitative yield. The spectral data of 2a are as follows; ir(KBr) 3440, 3230, 1730, 1702, 1640, 1610, 1280, 1100-1060, 860, 710 cm^{-1}; nmr ($CDCl_3$) 7.30-8.12(m,5), 6.95(d,1,J=10Hz), 6.20(d,1,J=10Hz), 4.30 (broad,1), 4.15(s,2), 1.55(s,3), 1.45(d,3,J=6Hz), 1.00(s,3) ; mass m/e 498(M^+), 469, 345, 318(base), 122, 105, 77.

DDQ-dehydrogenation of 2b - In a three necked flask fitted with a condenser and a nitrogen gas inlet glass tube were placed the reaction mixture obtained above and added 50 mg of DDQ.
The reaction mixture was refluxed for 6 hrs at 105°C under nitrogen. After the reaction was completed, the solvent was removed in vacuo and a residue was dissolved in 50 ml of chloroform, which was washed with 1% sodium hydroxide solution and water, dried and evaporated. The residue was then purified by preparative tlc to afford 3a (or 1). By mass spectrometry, it was confirmed

that at least one deuterium remained in 3a. The spectral data and melting point of the non-labelled product (1) were identical with those of an authentic sample.

Tritiation of betamethasone 17-benzoate(1) - A reaction flask containing a mixture of 100 mg each of betamethason 17-benzoate(1) (0.2 mmol) and tris(triphenylphosphine)rhodium chloride (0.13 mmol) in 2 ml of distilled dioxane was cooled in a liquid nitrogen bath and under reduced pressure and 1.6 Ci of carrier-free tritium gas was then introduced.
In order to achieve sufficient diffusion of tritium gas in the reaction flask, freezing and melting the dioxane solution was repeated in vacuo.
Then 10 ml of hydrogen gas was introduced into the reaction flask as carrier by the aid of a Toepler pump. After stirring for 1.5 hrs, the pressure was adjusted to atmospheric pressure by introducing helium gas (about 10 ml). The reaction was run for 48 hrs at room temperature. During the reaction, additional helium was supplied to maintain atmospheric pressure at 2,4 and 24 hrs after the initiation of the reaction. The reaction mixture was then frozen in vacuo, and all the remaining gases were transferred to a breakable ampoule containing activated charcoal to adsorb the unreacted tritium gas. The reaction mixture was used directly in the following DDQ-oxidation without any purification.

Betamethasone-1,2-$^3H_2$ 17-benzoate(3b) - To the above mixture 200 mg (0.88 mmol) of DDQ and 10 ml of dioxane were added and the solution was refluxed for 5 hrs. The solvent was removed and a residue was extracted with 250 ml of chloroform. The organic layer was washed with 1 % sodium hydroxide solution and water, dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in acetone and purified by preparative tlc. The appropriate radioactive area was scraped from the plates and eluted with acetone. The eluate was filtered and evaporated at 40°C. After drying in vacuo, 3b weighed 20 mg (overall yield from 1, 20 %) and its radiochemical purity was greater than 99 %. The specific activity was found to be 3.92 mCi/mg. The mass spectrum of this compound was almost identical with that of the non-labelled compound (1).

REFERENCES AND NOTES

1. Merrill E. G. and Vernice G. G. - J. Label. Compds., 4, 509(1968).
2. Walker D. and Hilbert J. D. - Chem. Rev., 67, 153(1967).
3. Burn D., Kirk D. N. and Petrow V. - Proc. Chem. Soc., 84, 3766(1960).
4. Turner A. B. and Ringold H. G. - J. Chem. Soc., 1720(1967).
5. This was confirmed by mass spectral analysis.

6. Usual purification method using alumina column could not be carried out because of decomposition of the product by alumina.
7. Helium was preferably used in this labelling experiments because easier diffusion of tritium in helium than in nitrogen was expected.