PREPARATION OF [15-^{3}H]GIBBERELLIN-A_3 [1]

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SUMMARY

The cyclobutane compounds 2, 3, 4 or 5, which represent in position-15 functionalized gibberellin derivatives, reacted with ^3H_2O under mild alkaline conditions in a retroaldol-like cleavage to give diacetyl[15-^{3}H]gibberellin-A_3-7-aldehyde (6) and the isomeric compound 7.

In respect of a second possibility of 15-tritiation, diacetyl-GA_3-7-aldehyde (1) was irradiated with UV-light in benzene/^3H_2O to form diacetyl[15α-^{3}H]GA_3-7-aldehyde (6a).

Oxidation and deacetylation of 6 afforded [15-^{3}H]gibberellin-A_3(8).

Keywords: Gibberellin-A_3, Tritium, Specific labelling

INTRODUCTION

The use of isotopically labelled gibberellins[2] is very important for studying transport, localization, metabolism, and mode of action in plants[3].

In particular tritium-labelled gibberellin-A_3 (GA_3) has been prepared by using either the WILZBACH method[4] or the exchange reactions by heterogeneous catalysis[5,6]. [6-^{3}H]GA_3 has been synthesized in our laboratory by chemical reactions[7].

In this communication we wish to describe a new preparation of tritium-labelled GA_3 based on a chemical route which is carried

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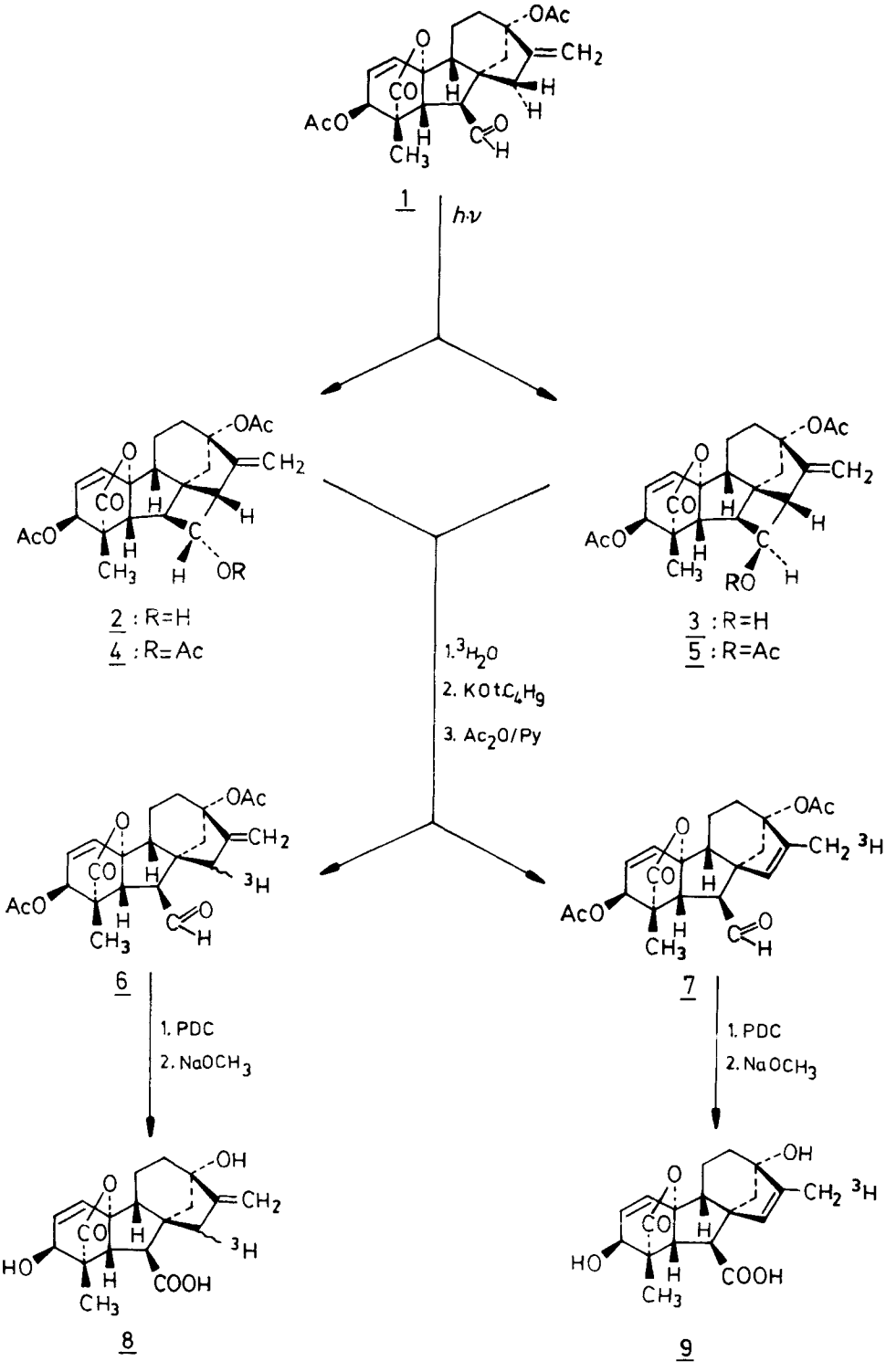

OAc
CH2
AcO
CO
H
CH3
C
O
H
1
hν
2 : R=H
4 : R=Ac
3 : R=H
5 : R=Ac
OR
RO
1. 3H2O
2. KOtC4H9
3. Ac2O/Py
3H
6
7
1. PDC
2. NaOCH3
OH
HO
COOH
8
9

out under mild alkaline conditions.

RESULTS AND DISCUSSION

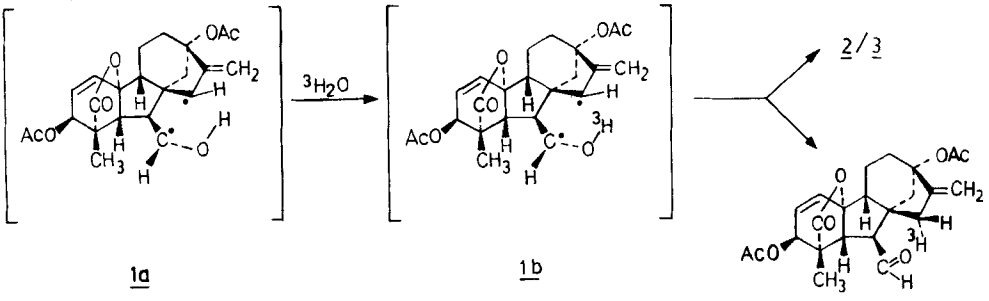
In a recent paper[8] we reported a procedure for the preparation of the cyclobutanols 2 and 3 starting from GA_3 via the following steps: diacetyl-GA_3, tetraacetyl-GA_3-anhydride, diacetyl-GA_3-7-alcohol and diacetyl-GA_3-7-aldehyde (1)[9]. Concerning the last step, the UV-irradiation of diacetyl-GA_3-7-aldehyde (1) afforded epimeric cyclobutanols 2 and 3 which represent in position-15 functionalized gibberellin derivatives[8,10]. As a result, 1 equivalent of pure 2 or 3 (or a mixture of 2 and 3 e.g. in proportions of 9 : 1)[8] was treated with an excess of ^3H_2O (6100 MBq/ml; 110 MBq/mmol) and 0.5 equivalent potassium tert. butoxide (free of alcohol). Selective cleavage of the cyclobutanol structure in a retroaldol-like cleavage gave, after reacetylation of the partial deacetylated 3-O-acetyl-group, diacetyl[15-^{3}H]GA_3-7-aldehyde (6) (64 %; specific activity 50 MBq/mmol) and diacetyl-16,17-dihydro-15,16-dehydro[17-^{3}H]GA_3-7-aldehyde (7) (30 %; specific activity 52 MBq/mmol).

According to the same method tritium labelling in the 15-position was also possible when starting with the acetylated cyclobutanols 4 or 5 (or a mixture of both)[11]. We obtained 6 (62 %; specific activity 55 MBq/mmol) and 7 (28 %; specific activity 54 MBq/mmol).

The isomeric aldehydes 6 and 7 were separated by silica gel column chromatography using n-hexane/chloroform (6 : 4) as solvent. The structures of the radiochemically pure compounds 6 and 7 were established by physical data, e.g. IR, MS, and NMR. The labelling in 6 is located in 15α- or 15ß-position[12].

It was also possible to prepare specifically labelled diacetyl[15α-^{3}H]GA_3-7-aldehyde (6a). In this case UV-irradiation of 1 in a mixture of benzene/^3H_2O (110 MBq/mmol) resulted

in 15 α-hydrogen abstraction with the formation of a 7,15-biradical 1a, which has an exchangeable H-atom in the 7-OH function.



1a 1b 6a

The resulting tritium-labelled biradical 1b undergoes rearrangement or recombination to give 6a (specific activity 5.3 MBq/mmol)[12] and the cyclobutanols 2 and 3 (90 % 2 and 10 % 3; specific activity 35 kBq/mmol).

Oxidation of the tritiated aldehyde 6 (specific activity 50 MBq/mmol) was carried out using PDC[13] (3 h at 20 °C) to give the corresponding acid which on subsequent deacetylation with $NaOCH_3$ in methanol (4 h at 20 °C) gave 68 % [15-^{3}H]GA_3 (8) with a specific activity of 42 MBq/mmol.

The presented reaction conditions showed the labelling procedure is suitable even for the highly sensitive and chemically unstable GA_3-derivatives with 19⟶10 lactone ring and for the preparation of highly specific labelled GA_3. Thus, the extent of labelling depends only on the specific radioactivity of the tritiated water used. Using tritiated water of highest radioactivity available, [15-^{3}H]GA_3 (8) with specific activities in the range of 500 to 1000 GBq/mmol can be prepared suitable e.g. for the investigation of hormone-receptor-relationships. In this respect, 1 μmol of the acetylated cyclobutanol 4 or 5 (or a mixture of both) can react with about 1.5 μmol of high specific activity tritiated water under the influence of 1 μmol potassium tert. butoxide.

Tritium labelling in the 15-position is firmly bound because a solution of 8 in buffer solutions of pH 5, pH 7, and pH 8.6

can be left for several days at room temperature without tritium exchange.

Analogous oxidation, and deacetylation of the by-product 7 (specific activity 52 MBq/mmol) gave 16,17-dihydro-15,16-dehydro [17-^{3}H] GA_3 (9)[8] in 60 % yield with a specific activity of 45 MBq/mmol.

EXPERIMENTAL

Radioactive samples were counted in a Packard Tri-Carb liquid scintillation spectrometer model 3380 using a PPO/POPOP-toluene cocktail. Developed TLC plates were scanned on a radioscanner fitted with a model LB 2723 ratemeter (Fa. Berthold, Wildbad). The ^{1}H-NMR spectra were recorded on a Varian HA-100 spectrometer. The low resolution mass spectra were obtained using an electron attachment mass spectrograph (Research Institute "Manfred von Ardenne", Dresden).

Preparation of diacetyl [15-^{3}H] GA_3-7-aldehyde (6) and diacetyl-16,17-dihydro-15,16-dehydro [17-^{3}H] GA_3-7-aldehyde (7)

a) from 2 or/and 3:

The cyclobutanol 2 (or 3, or a mixture of 2 and 3)[8,10] (414.5 mg, 1 mmol) dissolved in anhydrous THF (3 ml) was treated with ^3H_2O (0.1 ml ≙ 5.55 mmol; specific activity 110 MBq/mmol). The mixture was left to stand for 10 min. at 20 °C and then evaporated under reduced pressure. To the residue were added again anhydrous THF (3 ml), ^3H_2O (0.1 ml ≙ 5.55 mmol) and subsequently potassium tert. butoxide (free of alcohol; 56.1 mg ≙ 0.5 mmol). After 15 min. at 20 °C the mixture was acidified with acetic acid (0.5 ml) and evaporated in vacuo. Reacetylation of the residue with acetic anhydride (4 ml) in pyridine (4 ml) at 20 °C for 2 h afforded labelled aldehydes 6 and 7. The solvents were removed in vacuo

and the residue chromatographed on silica gel. Elution with n-hexane/chloroform (6 : 4, v/v) gave at first in 30 % yield 125.1 mg 7 (specific activity 52 MBq/mmol)[10] and later in 64 % yield 265 mg 6 (specific activity 50 MBq/mmol), identical with unlabelled starting material 1, according to IR, ^{1}H-NMR and MS[9].

We obtained about equal yields and specific activities of 6 and 7 when starting from 3, or different mixtures of 2 and 3.

b) from 4 or/and 5:

A solution of the acetylated cyclobutanol 4 (or 5, or a mixture of 4 and 5)[10,11] (456.5 mg ≙ 1 mmol) in anhydrous THF (5 ml) was treated with ^3H_2O (0.1 ml ≙ 5.55 mmol; specific activity 110 MBq/mmol). Within the course of 1 h at 20 °C, alcohol-free potassium tert. butoxide (112.2 mg ≙ 1 mmol) was added in portions. The mixture was acidified with acetic acid (0.5 ml) and then evaporated in vacuo. The residue was reacetylated with acetic anhydride (4 ml) in pyridine (4 ml) at 20 °C for 2 h and evaporated. Chromatography as described previously gave in 62 % yield 256.4 mg 6 (specific activity 55 MBq/mmol) and in 28 % yield 116 mg 7 (specific activity 54 MBq/mmol).

Preparation of diacetyl[15α-^{3}H]GA_3-7-aldehyde (6a)

A solution of 1 (41.5 mg ≙ 0.1 mmol) in anhydrous benzene (4 ml) was mixed with ^3H_2O (0.055 ml ≙ 3 mmol; specific activity 110 MBq/mmol) and then irradiated with UV-light (mercury high pressure lamp) for 8 h at 30 °C (cooling with an air blower) in a quartz flask under an atmosphere of N_2. The labile tritium was removed by repeated evaporation in vacuo, after the addition of methanol. The residue obtained was chromatographed on silica gel. Elution with n-hexane/chloroform (1 : 1, v/v) gave in 20 % yield 8.3 mg 6a (specific activity 5.3 MBq/mmol)[9,12]. With n-hexane/

chloroform (3 : 7, v/v) a mixture of 2 and 3 (specific activity 35 kBq/mmol; consisting of 90 % 2 and 10 % 3)[8,10] was obtained in 70 % yield (29.1 mg).

Preparation of [15-^{3}H]GA_3 (8)

6 (207.2 mg ≙ 0.5 mmol; specific activity 50 MBq/mmol) was oxidized with PDC[13] (376.2 mg ≙ 1 mmol) in DMF (1 ml). After 3 h at 20 °C, HCl (1 %; 10 ml) was added, the mixture extracted with ether (2 x 30 ml), the ether solution dried with Na_2SO_4, the ether evaporated, and the residue deacetylated with a 0.2 N solution of $NaOCH_3$ in CH_3OH (10 ml) at 20 °C for 4 h. Following addition of acetic acid (1 ml) and evaporation, the residue was mixed with H_2O and separately extracted with ethyl acetate (3 x 30 ml). The organic phase was dried (Na_2SO_4) and evaporated. Chromatography on silica gel by elution with chloroform/ethyl-acetate (4 : 6, v/v) gave in 68 % yield 117.8 mg 8 (specific activity 42 MBq/mmol) which was identical with unlabelled material in all respects[7].

Preparation of 16,17-dihydro-15,16-dehydro[17-^{3}H]GA_3 (9)

Oxidation and deacetylation of 7 (103.6 mg ≙ 0.25 mmol; specific activity 52 MBq/mmol) as described for 8 yielded after chromatography by elution with chloroform/ethyl acetate (4 : 6, v/v) in 60 % yield 51.8 mg 9 (specific activity 45 MBq/mmol; identical with unlabelled material[8]).

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