PARTIAL SYNTHESIS OF TRITIUM-LABELLED GIBBERELLIN-A_3 [1]

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SUMMARY

The epimeric gibberellin-A_3-7-aldehydes 1 and 2 were silylated, enolised with KH and labelled with ^3H_2O to give after desilylation the tritium-labelled epimeric gibberellin-A_3-7-aldehydes 4 and 5. Subsequent acetylation, oxidation and deacetylation afforded [6-^{3}H] gibberellin-A_3 (6) and [6-^{3}H]6-epi-gibberellin-A_3 (7). Thus, this route provides a convenient method for the preparation of [6-^{3}H]GA_3 of high specific radioactivity.

Keywords: Gibberellin-A_3, Tritium, specific labelling

INTRODUCTION

The gibberellins are naturally occurring diterpenoid acids exhibiting hormonal functions in higher plants. Thus, labelled gibberellins have been used extensively to investigate transport, localization, and interconversion of these hormones in the fungus Gibberella fujikuroi as well as in plants.[2] Tritium-labelled gibberellin-A_3 (GA_3) has been prepared by using either the Wilzbach method[3] or heterogeneously catalysed exchange reactions.[4,5] Base-catalyzed exchange reactions of GA_{12}-7-aldehyde and GA_{14}-7-

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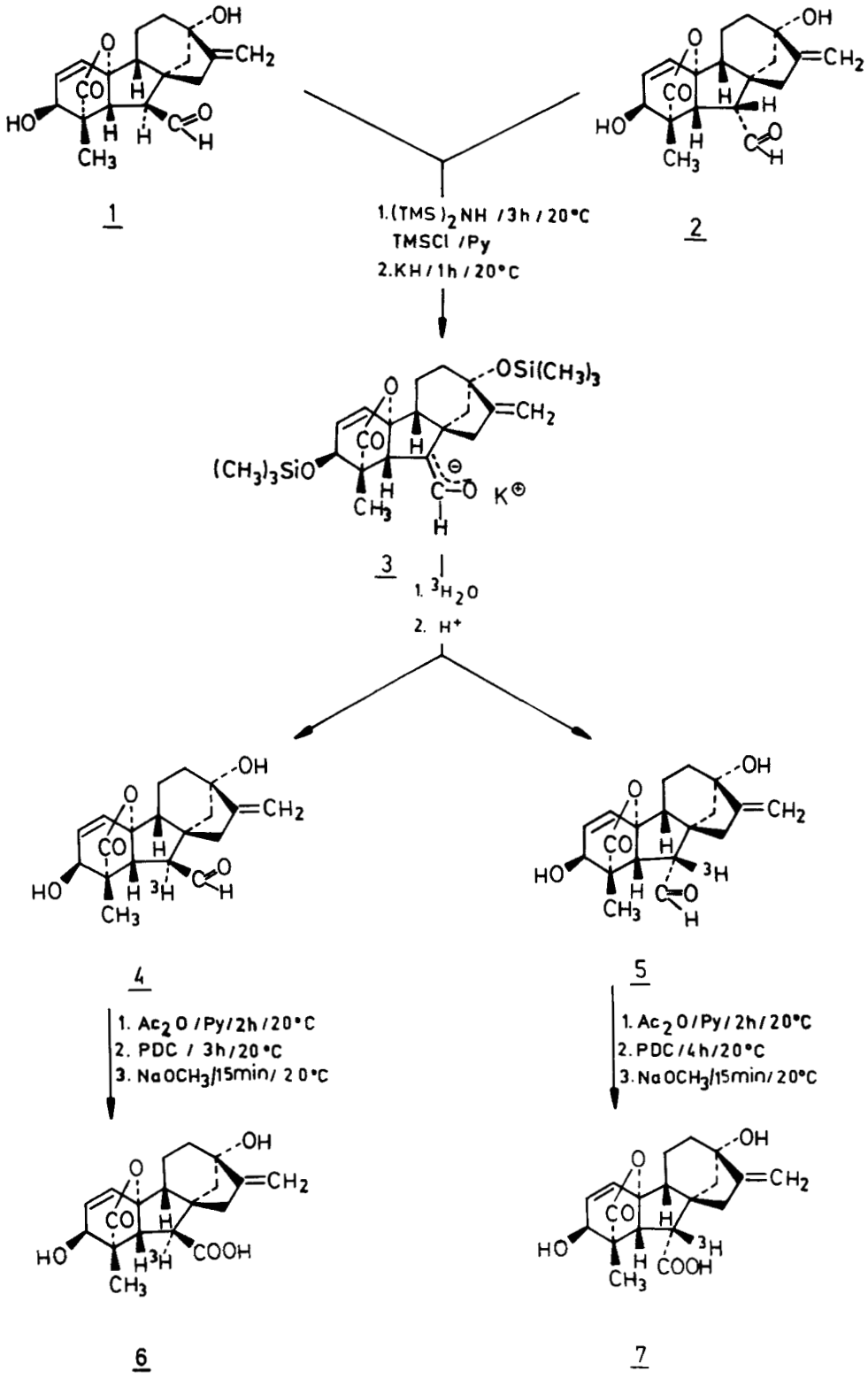
aldehyde in tritiated solvents afforded labelling of the aldehydes with tritium specifically in the 6-position[6,7].

In this communication we report a convenient method for the preparation of [6-^{3}H]GA_3-7-aldehyde despite its sensitivity towards alkaline and acidic conditions. Oxidation of this aldehyde under mild conditions yielded [6-^{3}H]GA_3.

RESULTS AND DISCUSSION

The partial synthesis of GA_3-7-aldehyde has been realized starting from GA_3 via the intermediates diacetyl-GA_3, tetra-acetyl-GA_3-anhydride, diacetyl-GA_3-7-alcohol, and diacetyl-GA_3-7-aldehyde to give GA_3-7-aldehyde (1)[8]. Prior to the tritiation 1 was silylated with hexamethyldisilazane and trimethylchloro-silane in pyridine[9]. The resulting product was enolized with KH[10] to form the enolate 3 and the tritium labelling was carried out by addition of tritiated water (6100 MBq/ml, 110 MBq/mmol). Subsequent desilylation with acetic acid afforded a mixture of the labelled epimeric aldehydes 4 and 5. These were easily separated by SiO_2 column chromatography to give 30 % [6-^{3}H]GA_3-7-aldehyde (4) with a specific radioactivity of 51 MBq/mmol and 44 % of the epimeric [6-^{3}H]6-epi-GA_3-7-aldehyde (5) with a specific radioactivity of 54 MBq/mmol. Both compounds were radiochemically pure and their structures were established by physical data e. g. IR, MS, and NMR. Large differences were observed in the proton NMR spectra of 4 and 5 with regard to chemical shifts of the proton signals at C-5 with δ 3.30 (6ß aldehyde) and at δ 2.97 (6αaldehyde). Due to the protons at C-1 and C-3 an additional coupling constant of J = 2 cpm is observed in the proton NMR spectra of 5.

Prior to the oxidation of the aldehyde group in 4 to produce the carboxylic function in 6, the hydroxyl group at C-3 had to be


1
2
1.(TMS)$_2$NH /3h/20°C
TMSCl /Py
2.KH/1h/20°C
3
1. ^3H_2O
2. H$^+$
4
5
1. Ac$_2$O/Py/2h/20°C
2. PDC / 3h/20°C
3. NaOCH$_3$/15min/20°C
1. Ac$_2$O/Py/2h/20°C
2. PDC/4h/20°C
3. NaOCH$_3$/15min/20°C
6
7

protected by selective acetylation. The oxidation of the tritiated O(3)-acetyl-GA_3-7-aldehyde was carried out using PDC[11] to form the tritiated O(3)-acetyl-GA_3 which on subsequent deacetylation with $NaOCH_3$ in methanol at room temperature gave 81 % [6-^{3}H]GA_3 (6) with a specific radioactivity of 45 MBq/mmol.

Analogous acetylation, oxidation, and deacetylation of 5 afforded [6-^{3}H]6-epi-GA_3 (7)[12] in 54 % yield with a specific radioactivity of 48 MBq/mmol.

According to the same method tritium labelling in position 6 of the gibberellane skeleton is possible when starting with 6-epi-GA_3-7-aldehyde (2)[12]. As a result of the mild conditions the labelling procedure is suitable even for the chemically unstable gibberellin-7-aldehydes with 19⟶10 lactone ring.

In further experiments tritiated water of higher specific activity (660 GBq/ml) was used to produce the aldehydes 4 and 5 with specific radioactivities of 3590 and 3920 MBq/mmol respectively. Oxidation of the former in the described manner gave [6-^{3}H]GA_3 (6) with a specific radioactivity of 3880 MBq/mmol.

Thus, the extent of labelling depends only on the specific radioactivity of the tritiated water used. For a complete reaction 1 µmol of 2 needs about 1.5 µmol of tritiated water. Using tritiated water of highest radioactivity available [6-^{3}H]GA_3 (6) with specific radioactivities in the range of 500 to 1000 GBq/mmol can be prepared suitable e. g. for the investigation of hormone-receptor-relationships.

The tritium atom at C-6 of the gibberellane skeleton is firmly bound because the labelled compounds 4, 5, and 6 in buffer solutions of pH 5, 7, and 8.6 can be left for several days at room temperature without tritium exchange. Even the [6-^{3}H]GA_3-7-aldehyde is stable during the usual purification procedures of plant extracts[1].

EXPERIMENTAL

Methods

Proton NMR spectra were obtained on a VARIAN HA-100 instrument, operating at 100 MHz.

The 70 eV spectra and mass measurements were obtained using an AEI MS 902 S mass spectrometer.

For measuring the specific activity we used a liquid scintillation spectrometer Tricarb model 3380.

Preparation of [6-^{3}H]GA_3-7-aldehyde (4) and [6-^{3}H]6-epi-GA_3-7-aldehyde (5)

a) from GA_3-7-aldehyde (1):

Hexamethyldisilazane (2 ml) and trimethylchlorosilane (2 ml) were added to GA_3-7-aldehyde (1) (330.4 mg ≙ 1 mmol) dissolved in anhydrous pyridine (15 ml). The mixture was left to stand for 3 h at 20 °C and then evaporated under reduced pressure. SiO_2-chromatography of the residue by elution with n-hexane /chloroform 8 : 2 (v/v) yielded bis-trimethylsilyl-GA_3-7-aldehyde [m.p. 179 - 183 °C (ether/n-hexane)]. This silylated GA_3-7-aldehyde was dissolved under argon in oxygen-free anhydrous THF (8 ml) and KH (44 mg ≙ 1.1 mmol) was added. The mixture was stirred at 20 °C for 1 h. Tritiated water (0.1 ml ≙ 5.55 mmol; specific radioactivity 110 MBq/mmol) was added to 3. The mixture was stirred for 3 minutes and subsequently mixed with 1 ml acetic acid. Labile tritium was removed by repeated evaporation in vacuo, with the addition of methanol. For removal of the protective groups, THF (8 ml), H_2O (2 ml) and acetic acid (1 ml) were added to the residue and the mixture left to stand overnight. After

evaporating the solution under reduced pressure a saturated aqueous solution of $NaHCO_3$ was added, followed by extraction with ethyl acetate (3 x 40 ml). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporating afforded a residue which was subsequently chromatographed on silica gel. Elution with chloroform/ethylacetate 8 : 2 (v/v) gave in 30 % yield [6-^{3}H]GA_3-7-aldehyde (4) (99 mg; specific radioactivity 51 MBq/mmol) identical with unlabelled material[8]. With chloroform/ethyl acetate 7 : 3 (v/v) in 44 % yield 145 mg of [6-^{3}H]6-epi-GA_3-7-aldehyde (5) were obtained: specific activity 54 MBq/mmol; m.p.: 185 - 187 °C (acetone/n-hexane); $[\alpha]_D^{26}$ + 2.4 (c = 0.35, ethanol); IR ($CHCl_3$): ν_{max} 3600 (OH), 2730 and 1725 (aldehyde), 1775 (γ-lactone-CO) and 1665 cm^{-1} (=CH_2); 70 eV MS (source 140 °C): m/e 330.1438 (M^+, $C_{19}H_{22}O_5$, calc. 330.1467); ^{1}H-NMR (acetone-d_6/TMS): 1.19 (s, 18-H_3), 2.75 (dd, J = 10 cpm, J' = 2.5 cpm, 6-H), 2.97 (d, J = 10 cpm, 5-H), 4.32 (m, 3-H), 4.89 and 5.21 (m, 17-H_2) 5.84 (dd, J = 9 cpm, J' = 2.5 cpm, 2-H), 6.28 (dd, J = 9 cpm, J' = 2 cpm, 1-H) and 9.80 ppm (d, J = 2.5 cpm, 7-H).

Analogous to this preparation, GA_3-7-aldehyde (1) (1.652 g ≙ 5 mmol) was silylated (10 ml hexamethyldisilazane, 10 ml trimethylchlorosilane, 75 ml pyridine), enolised (280 mg KH ≙ 7 mmol) and tritiated (1 ml ^3H_2O ≙ 55.5 mmol was distilled in; 660 GBq). After addition of acetic acid (3 ml) the solvents were then removed in vacuo and portions of methanol (3 x 15 ml) distilled into the reaction vessel and off again to remove exchangeable tritium. For desilylation the residue was dissolved in THF (40 ml), H_2O (10 ml) and acetic acid (5 ml). The mixture was left to stand overnight, evaporated and the residue chromatographed as before. The chromatographic working up gave in 29 % yield [6-^{3}H]GA_3-7-aldehyde (4) (480 mg; specific radioactivity 3590 MBq/mmol) and in 35 % yield [6-^{3}H]6-epi-GA_3-7-aldehyde (5) (580 mg; specific radioactivity 3920 MBq/mmol).

b) from 6-epi-GA_3-7-aldehyde (2):

According to the first preparation, 6-epi-GA_3-7-aldehyde (330.4 mg) was silylated, enolised (44 mg KH) and tritiated (0.1 ml ^3H_2O, specific radioactivity 110 MBq/mmol). Analogous working up and chromatography gave in 31 % yield [6-^{3}H]GA_3-7-aldehyde (4) (103 mg; specific radioactivity 55 MBq/mmol) and in 45 % yield [6-^{3}H]6-epi-GA_3-7-aldehyde (5) (148.3 mg; specific radioactivity 51 MBq/mmol).

Preparation of [6-^{3}H]GA_3 (6)

[6-^{3}H]GA_3-7-aldehyde (4) (330.4 mg ≙ 1 mmol); specific radioactivity 51 MBq/mmol) was acetylated (3 ml acetic anhydride/3 ml pyridine, 20 °C). After 2 h the mixture was evaporated under reduced pressure and the residue was oxidized with PDC[11] (752.4 mg ≙ 2 mmol) in DMF (2 ml) at 20 °C for 3 h. HCl (1 %) was added and the mixture was extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) and evaporated and the residue obtained was deacetylated with a 0.2 N solution of $NaOCH_3$ (10 ml) at 20 °C for 15 minutes. Following addition of acetic acid and evaporation, the residue was mixed with H_2O and separately extracted with ethyl acetate (3 x 50 ml). The organic phase was dried (Na_2SO_4) and evaporated. SiO_2-chromatography of the residue yielded by elution of chloroform/ethyl acetate 4 : 6 (v/v) in 81 % yield 280 mg [6-^{3}H]GA_3 (6): specific radioactivity 45 MBq/mmol; m.p. 232 - 235 °C (ethyl acetate); $[\alpha]_D^{25}$ + 84.2° (c = 0.48 in ethanol); IR (nujol): ν_{max} 3400 (OH), 1755 (γ-lactone-CO) and 1710 cm^{-1} (acid); ^{1}H-NMR (acetone-d_6/TMS): 1.20 (s, 18-H_3), 2.67 (d, J = 10 cpm, 6-H), 3.24 (d, J = 10 cpm, 5-H), 4.05 (d, J = 3.5 cpm, 3-H), 4.88 and 5.22 (2m, 17-H_2), 5.86 (dd, J = 9 cpm, J' = 3.5 cpm, 2-H), and 6.35 ppm (d, J = 9 cpm, 1-H).

Analogous to this preparation, [6-^{3}H]GA_3-7-aldehyde (450 mg; specific radioactivity 3590 MBq/mmol) was acetylated, oxidated

and deacetylated to give [6-^{3}H]GA_3 (102 mg ≙ 22 %, not optimized) with a specific radioactivity of 3880 MBq/mmol.

Preparation of [6-^{3}H]6-epi-GA_3 (7)

Acetylation, oxidation (the sterically hindered 6α-aldehyde function needed 4 hours for oxidation) and deacetylation of [6-^{3}H]6-epi-GA_3-7-aldehyde (5) (330.4 mg; specific radioactivity 54 MBq/mmol) as described for GA_3-7-aldehyde (4) yielded after chromatography by elution with chloroform/ethyl acetate 3 : 7 (v/v) in 54 % [6-^{3}H]6-epi-GA_3 (7) (187.2 mg; specific radioactivity 48 MBq/mmol) which was in all respects identical with unlabelled material[12].

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