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SYNTHESIS OF TRITIUM LABELLED 24-EPIBRASSINOLIDE

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SUMMARY

Deuterium and tritium 5,7,7-tris-labelled 24-epibrassinolide were prepared by base catalyzed exchange reaction using 24-epicastasterone tetraacetate **1** or bis-isopropylidenedioxy-24-epicastasterone **8** and labelled water. Baeyer-Villiger oxidation of the obtained labelled 6-ketones **2** and **3** with CF_3CO_3H gave after alkaline deacetylation of the resulting **4** and **5** the desired tris-labelled 24-epibrassinolides **6** and **7**, respectively, or starting from **9** under simultaneous oxidation and deprotection in one step the same final products.

Keywords: Brassinosteroids, [5,7,7-$^3H_3$]-24-epibrassinolide, specific labelling, plant growth regulator, tritium, deuterium

INTRODUCTION

Since the discovery of brassinolide [1] as the first member of a new group of steroidal plant hormones with high growth stimulating activity as well as antistress properties, many efforts were done to discover new brassinosteroids, to synthesize native members and synthetic analogs, and to get deeper insight in the mode of action of such compounds [2,3]. For investigations of biosynthesis, metabolism, transport and distribution especially the preparation of radioactive labelled brassinosteroids is important, whereas only few studies in this direction have been done hitherto.Thus, starting from dolicholide via catalytically tritiation [24,28-$^3H_2$]-brassinolide and [24,28-$^3H_2$]-24-epibrassinolide were prepared [4]. Also a synthetic pathway leading to [4-^{14}C]-24-epibrassinolide has been published [5].

In this communication we report a convenient method for the preparation of [5,7,7-$^3H_3$]-24-epibrassinolide (**7**) starting from 24-epicastasterone tetraacetate (**1**) or bis-isopropylidene-dioxy-24-epicastasterone (**8**). Compounds **1** and **8** are readily available key intermediates of the 24-epibrassinolide synthesis from ergosterol [6].

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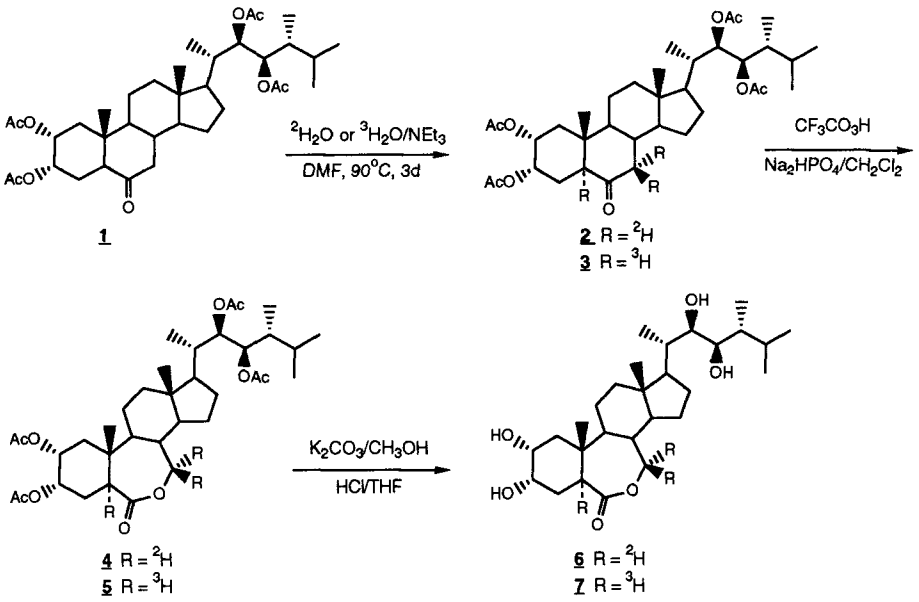
Our procedure has the advantage of specific introduction of labelling at a late stage, whereas three tritium atoms were introduced simultaneously in a stable position at C5 and C7.

SYNTHESIS

Starting from **1** at first the labelling sequence was realized using deuterium to check optimal reaction conditions. A procedure published by Allevi et al [7] was not convenient because of the used high excess of labelled water and methanol as well as the necessity of separation of side chain epimers.



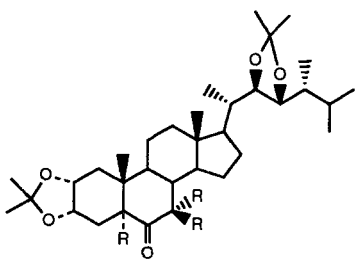
We found that reaction of compound **1** with ^2H_2O in the presence of triethylamine in dimethylformamide resulted in a smooth incorporation of labelling leading to the tris-deuterated compound **2** as the main product. The exchange rate was determined on the basis of the mass spectroscopic data given in **Table 1**. The position of the introduced deuterium followed from the NMR spectrum of **2** which lacked the signals for the 5α- and 7α,7ß- protons at 2,57 and 2,33 ppm, respectively [8]. Baeyer-Villiger oxidation of **2**, using CF_3CO_3H in CH_2Cl_2 gave the 7-oxa-6-oxo-lactone **4** besides approximately 10% of the undesired epimeric 6-oxa-7-oxo-lactone, separated by SiO_2 chromatography.

Saponification of **4** with K_2CO_3 in methanol/water followed by acidification with HCl in THF yielded the [5,7,7-$^2H_3$]-24-epibrassinolide **6**, whereas no exchange of deuterium was observed (**Table 1**).

Table1 Exchange rates of deuterium in % based on mass spectroscopic data

Compound	d_0	d_1	d_2	d_3	d_4-d_6
2	1,5	7,4	27,6	55,5	8,0
4	3,5	6,4	23,5	56,1	10,5
6	1,6	12,9	27,9	55,8	1,8

This procedure can be used also as a simple method for introduction of tritium using ^3H_2O as labelling reagent. Thus, reaction of compound **1**, solved in dimethylformamide, with ^3H_2O in the presence of triethylamine at 90°C followed by Baeyer -Villiger oxidation with CF_3CO_3H and alkaline hydrolysis afforded without isolation of the intermediates the desired [5,7,7-$^3H_3$]-24-epibrassinolide **7** in an overall yield of 60 % and with a specific radioactivity of 222 MBq/mmol. The reaction was monitored by TLC combined with radioisotopic measurements.



8 R= H
9 R=^{3}H

In further studies as a more convenient starting compound the bis-isopropylidene protected 24-epicastasterone **8** was used for the tritiation procedure. Also in that case the reaction with ^3H_2O and triethylamine in dimethylformamide afforded the corresponding 5,7,7-tris-labelled intermediate **9** which was without isolation oxidized with CF_3CO_3H to give under simultaneous deprotection directly the desired tris-tritiated lactone **7** with a specific radioactivity of 232 MBq/mmol. Therefore, starting from **8** this modification represents a smooth pathway for labelling of the biologically important 24-epibrassinolide in only two steps.

EXPERIMENTAL

[5,7,7-$^2H_3$]-24-epicastasterone tetraacetate (**2**)

To 157 mg 24-epicastasterone tetraacetate (**1**), solved in 500 µl anhydrous dimethylformamide in a glass ampoule were added successively 200 µl triethylamine and 200 µl deuterated water (98%) under argon. The closed ampoule was heated up to 90°C for three days. The reaction mixture was then evaporated under reduced pressure, exchangeable deuterium was removed with methanol, and the reaction product was purified by column chromatography over SiO_2 (Merck Kieselgel 60). Elution with hexane/chloroform 1:1 yielded 149 mg (94,9%) **2**.

[5,7,7-$^2H_3$]-24-epibrassinolide tetraacetate (**4**)

To a stirred mixture of **2** (94 mg) and Na_2HPO_4 (500 mg) in 3 ml CH_2Cl_2, 5 ml of a freshly prepared homogenous solution of CF_3CO_3H in CH_2Cl_2 (0,5 ml 70 % H_2O_2, 3 ml $(CF_3CO)_2O$, 2ml CH_2Cl_2) were added dropwise. When the exothermic reaction was subsided, the mixture was heated up to 40°C under stirring for 2 hrs. After cooling water was added, the mixture was extracted with $CHCl_3$, neutralized with $NaHCO_3$, washed and dried. After evaporation the residue was chromatographied over SiO_2. Elution with hexane/chloroform 1:1 yielded 79,4 mg (84 %) **4** (mp. 155-158°C from ether /hexan) besides nearly 8,2 mg of the epimeric 7-oxo-6-oxa-lactone .

[5,7,7-$^2H_3$]-24-epibrassinolide (**6**)

8,6mg **4**, 1,4 ml methanol and 40 mg K_2CO_3, solved in 0,6 ml H_2O were refluxed for three hrs. The cooled mixture was stirred for 1 hr, acidified with 6n HCl and heated for 1hr. The cooled reaction mixture was extracted with $CHCl_3$. The organic phase was neutralized, washed and dried. After evaporation the crude product was purified by column chromatography over SiO_2. Elution with chloroform and chloroform/methanol 95:5 yielded 6,0 mg (94,2%) **6** (mp. 255°C from EtOH; ref. [6] mp 256-258°C).

[5,7,7-$^3H_3$]-24-epibrassinolide (**7**)

90mg 24-epicastasterone tetraacetate **1**, 500 µl anhydrous DMF, 100 µl triethylamine and 100 µl tritiated water (750 MBq/mmol) were heated up to 90°C for 3 days in a closed glass ampoule. The reaction mixture was evaporated under reduced pressure, and exchangeable tritium was removed with methanol. The crude product was solved in 3 ml CH_2Cl_2, 500 mg Na_2HPO_4 and 5 ml freshly prepared CF_3CO_3H were added during stirring and cooling. The mixture was heated to 40°C for 3 hrs, water was added and the mixture extracted with $CHCl_3$. After neutralization, drying and evaporation of the organic phase the crude product was solved in 10ml CH_3OH and refluxed with 300 mg K_2CO_3 (solved in 5 ml H_2O).The cooled mixture was stirred for 1 hr, acidified with 6n HCl and heated for 1hr. After cooling the mixture was extracted with $CHCl_3$. The organic phase was neutralized, washed and dried. After evaporation the residue was purified by column chromatography. Elution with chloroform/methanol 98:2 yielded 42,3 mg (overall yield 64.0%) [5,7,7-$^3H_3$] - 24-epibrassinolide **7** with a specific radioactivity of 222 MBq/mmol, which was identical in its chromatographic behaviour with authentic 24-epibrassinolide.

[5,7,7-$^3H_3$]-2α,3α,22,23-bis-isopropylidene dioxy-24-epicastasterone (**9**)

40 mg 2α,3α,22,23-bis-isopropylidene dioxy-24-epicastasterone **8**, 100 µl anhydrous DMF, 100 µl triethylamine and 150 µl tritiated water (750 MBq/mmol) were heated up to 90°C for 2 days in a closed glass ampoule. The reaction mixture was evaporated under reduced pressure,

and exchangeable tritium was removed with methanol. The crude product was purified by column chromatography over SiO_2. Elution with chloroform/hexane 1:1 yielded 39 mg tritium labelled **9** (233MBq/mmol).

[5,7,7-$^3H_3$]-24-epibrassinolide (**7**)

39 mg **9** (233 MBq/mmol) were solved in 3 ml CH_2Cl_2, 300 mg Na_2HPO_4 and 3 ml freshly prepared CF_3CO_3H were added under stirring and cooling. The mixture was heated to 40°C for 1,5 hrs. Water was added, and after neutralization the solution was extracted with $CHCl_3$. After drying and evaporation the crude product was purified by column chromatography over SiO_2. Elution with chloroform/methanol 98:2 yielded 19 mg tritium labelled 24-epibrassinolide **7** (232MBq/mmol) identical with unlabelled material.

The progress of all reactions was monitored by TLC on silica gel plates from Merck. Hexane / chloroform 7:3 or chloroform / methanol 95:5 were used as developing reagents. Spots were detected by spraying with 85% H_2SO_4 followed by heating in combination with radioactivity measurement using a Beckman Liquid scintillation System LS 6000 TA and a Berthold Automatic TLC-Linear Analyzer. Incorporation rate was determined by mass spectrometry with a AMD 402 from the AMD Intectra GmbH. The ^1H NMR spectroscopic measurement were done with a Brucker WP 200 at 200 MHz in chloroform.

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