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Labelling of Biogenetic Brassinosteroid Precursors

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SUMMARY

A base catalyzed exchange procedure was employed to introduce tritium and deuterium, respectively, into enolizable positions of the steroidal ring system of several putative intermediates of 24*R*-type brassinosteroids. The method is suitable both for derivatives with protected hydroxyl groups as well as for non-protected compounds.

Key words: Brassinosteroids, plant hormones, deuterium, tritium, specific labelling.

INTRODUCTION

The brassinosteroids are a class of native plant growth regulators which have recently been established as new phytohormones.[1,2] Recent molecular genetic studies confirmed the essential role of brassinosteroids in plant growth and development.[3] Consequently, investigations on the function and the biochemical mode of action have been graetly intensified. The discovery of alternative late and early C-6 oxidation pathways[4] and the formation of different types of conjugates[1] suggested that the pattern of biogenetic reactions of brassinosteroids and their transformation is more complex than hitherto assumed. For such studies, the need for labelled brassinosteroids and brassinosteroid precursors was significantly increased.

A variety of labelling procedures of brassinosteroids with deuterium and tritium, respectively, in the side chain and in the ring system have been described.[5] For example, several [26,28-$^2H_6$]brassinosteroids were prepared in a multi-step synthesis starting from a 20-formylpregnane derivative.[6] The 24-methylene group of dolicholide was catalytically tritiated to [24,28-$^3H_2$]brassinolide and [24,28-$^3H_2$]24-*epi*-brassinolide.[7] [5,7,7-^{3}H]- and [5,7,7-^{2}H]24-*epi*-brassinolide, respectively, were synthesized by Bayer-Villiger oxidation from labelled acetyl

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or isopropylidene protected 24-*epi*-castasterone which had been prepared by catalytic exchange of enolizable protons adjacent to the carbonyl group using labelled water.[8] A synthesis of [4-^{14}C]24-*epi*-brassinolide was also published.[9]

In this paper we report on the preparation of [5,7,7-^{3}H]24-*epi*-teasterone (**3**), [2,2,4,4,5,7,7-^{2}H]3-dehydro-24-*epi*-teasterone (**5**), [5,7,7-^{2}H]- and [5,7,7-^{3}H]6-oxo-24β-methyl-22-dehydro-cholestanol (**7** and **8**) as well as [5,7,7-^{3}H]6-oxo-24-*epi*-campestanol (**10**), specifically labelled with deuterium or tritium.

RESULTS AND DISCUSSION

Most of the biogenetic and metabolic reactions of the brassinosteroids take place at the side chain. For example, hydroxylation may occur in several positions and even side chain degradation has to be expected.[1] Since these processes might be accompanied by loss of side chain labels, the ring system was selected as the preferred site for labelling.

The keto group in the C-6 position represents a suitable structural feature of the brassinosteroid precursors to be labelled in this work. 3-Dehydro-24-*epi*-teasterone (**4**)[10] contains an additional carbonyl group at C-3. Thus, base-catalyzed exchange of the enolizable protons in positions adjacent to the keto groups has been employed to introduce deuterium and tritium, respectively, into the steroid ring system.

As previously decribed, the isopropylidenedioxy group is convenient for protecting the vicinal hydroxyls of brassinosteroids during base-catalyzed exchange employing deuterium oxide or tritiated water.[8] Thus, we used 22,23-isopropylidenedioxy-24-*epi*-teasterone (**2**) to introduce ^{3}H into positions 5 and 7 of 24-*epi*-teasterone (**1**). The 3β-hydroxyl group remained unprotected during this isotopic exchange procedure. The poor yield of 45% of the desired [5,7,7-^{3}H]24-*epi*-teasterone (**2**) was due to the deprotection under relatively harsh acidic conditions.

Therefore, the labelling of further compounds was carried out without protection of the hydroxyl groups. 3-Dehydro-24-*epi*-teasterone (**4**) was directly subjected to the exchange procedure employing MeOH-d_4 as solvent instead of DMF, the other experimental conditions being essentially the same as decribed for labelling of **2**. In such a manner [2,2,4,4,5,7,7-^{2}H]3-Dehydro-24-*epi*-teasterone (**5**) was obtained in 83% yield. The deuterium content, shown in Table 1, was calculated from mass spectrometric data.

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Table 1 Deuterium content of [2,2,4,4,5,7,7-^{2}H]3-dehydro-24-*epi*-teasterone (**5**) and [5,7,7-^{2}H]6-oxo-24β-methyl-22-dehydrocholestanol (**7**) (in %).

Compound	d_0	d_1	d_2	d_3	d_4	d_5	d_6	d_7
5	16.3	4.9	4.3	5.8	9.5	16.7	21.2	19.5
7	5.1	13.2	37.4	44.3	-	-	-	-

In further experiments also 6-oxo-24β-methyl-22-dehydrocholestanol (**6**)[10,11] was labelled in positions 5 and 7 with ^{2}H and ^{3}H, respectively. Both [5,7,7-^{2}H]6-oxo-24β-methyl-22-dehydrocholestanol (**7**) and [5,7,7-^{3}H]6-oxo-24β-methyl-22-dehydrocholestanol (**8**) were readily obtained with over 80% yield.

Furthermore, the new 6-oxo-24–*epi*–campestanol (**9**), prepared by hydrogenation of compound **6** with Pd-C (10%) in dioxane was used for the labelling experiments. In such a manner, exchange of hydrogen by means of HTO afforded [5,7,7-^{3}H]6-oxo-24-*epi*-campestanol (**10**) which was purified by silica gel column chromatography to give the radiochemically and chemically pure compound in 47 % yield.

Metabolic studies with the so prepared labelled brassinosteroid precursors *via* feeding experiments on cell cultures are under way.

EXPERIMENTAL

Labelled chemicals: HTO (specific radioactivity 525 MBq mmol^{-1}) from Amersham, Braunschweig, Germany, and D_2O (99.9%) from Deutero GmbH, Kastellaun, Germany, were used.

General labelling procedure: Freshly destilled triethylamine and labelled water were successively added under nitrogen to a solution of the compound to be labelled in DMF or MeOH-d_4. If not otherwise indicated, the mixture was heated for 64 h in a sealed glass ampoule to 80°C. Then the reaction mixture was evaporated under a stream of nitrogen gas. Exchangeable tritium was removed with MeOH. The crude product was subjected to silica gel column chromatography (Merck Silica gel 60). *n*-Hexane - EtOAc was used as eluent in various ratios.

[5,7,7-^{3}H]24-epi-Teasterone (**3**): 22,23-Isopropylidenedioxy-24-*epi*-teasterone (**2**) was prepared from 24-*epi*-teasterone (**1**) by means of 2,2-dimethoxypropane and *p*-toluenesulfonic acid in dry ethyl acetate. Reaction mixture for labelling: triethylamine (100 µl), HTO (120 µl), compound **2** (91 mg), dry DMF (200 µl). The protecting group was removed with 1.5 ml 4 M HCl in 15 ml MeOH by heating for 3 h to 50°C. Column chromatography: stepwise elution with *n*-hexane - EtOAc 3 : 7 and 1 : 4; yield 41 mg (45%); specific radioactivity 56.7 MBq mmol^{-1}.

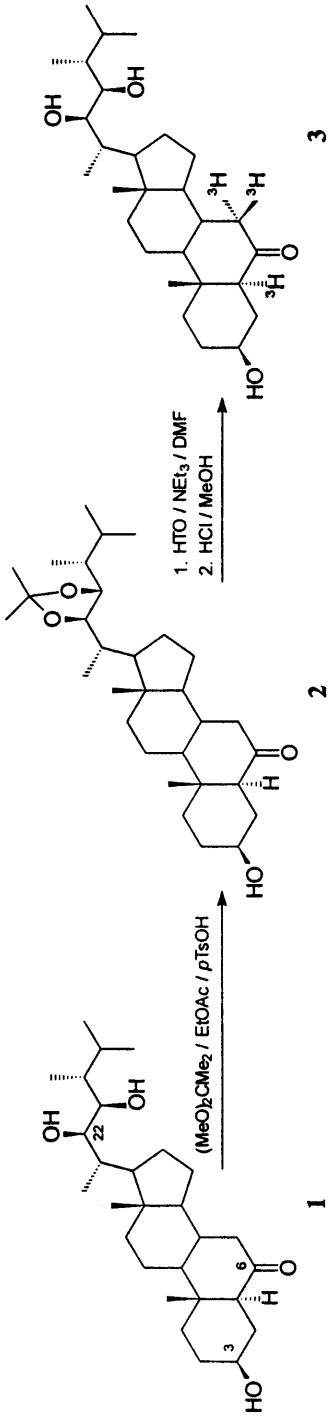
[2,2,4,4,5,7,7-^{2}H]3-Dehydro-24-epi-teasterone (**5**): Reaction mixture: triethylamine (100 µl), D_2O (400 µl), 3-dehydro-24-*epi*-teasterone (**4**)[10] (25 mg), MeOH-d_4 (300 µl); reaction conditions: 70°C, 50 h; column chromatography: *n*-hexane - EtOAc 1 : 1; yield 21 mg (83%). For deuterium content see Table 1.

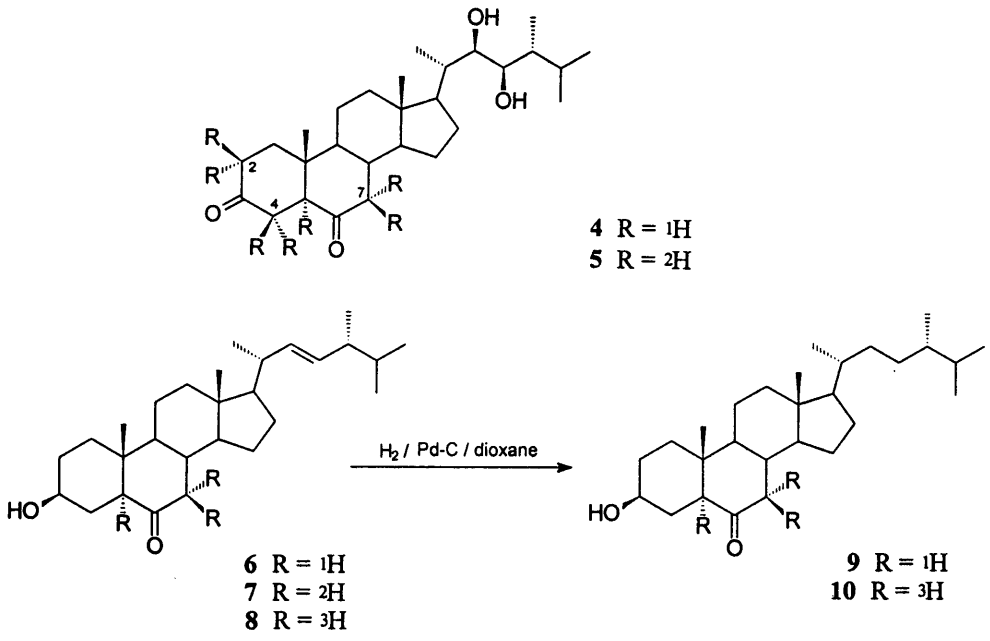
[5,7,7-^{2}H]6-Oxo-24β-methyl-22-dehydrocholestanol (**7**): Reaction mixture: triethylamine (50 µl), D_2O (50 µl), 6-oxo-24β-methyl-22-dehydrocholestanol (**5**) (8 mg), dry DMF (100 µl); column chromatography: *n*-hexane - EtOAc 3 : 2; yield 6.6 mg (83%). For deuterium content see Table 1.

[5,7,7-^{3}H]6-Oxo-24β-methyl-22-dehydrocholestanol (**8**): Reaction mixture: triethylamine (100 µl), HTO (120 µl), 6-oxo-24β-methyl-22-dehydrocholestanol (**6**)[10,11] (93 mg), dry DMF (200 µl); column chromatography: *n*-hexane - EtOAc 3 : 2; yield 76 mg (82%).

[5,7,7-^{3}H]6-Oxo-24-epi-campestanol (**10**): Pd-C (10 %) (8 mg) in dioxane (1 ml) was added under nitrogen to a solution of 6-oxo-24β-methyl-22-dehydrocholestanol (**6**)[10,11] (94 mg) in 1.5 ml dioxane. Then a stream of hydrogen was passed over the stirred mixture. After 2.5 hr the hydrogenation was finished. Pd-C was removed by filtration and 6-oxo-24-*epi*-campestanol (**9**) (mp. 138-138.5°C) was quantitatively obtained after evaporation under a stream of nitrogen. Reaction mixture for labelling: triethylamine (100 µl), HTO (120 µl), 6-oxo-24-*epi*-campestanol (**9**) (94 mg), dry DMF (200 µl); column chromatography: *n*-hexane - EtOAc 4 : 1; yield 44 mg (47%); specific radioactivity 129.6 MBq mmol^{-1}.

Analytical methods: Radioactive fractions were measured by liquid scintillation counting using a Beckman LS 6000 TA. The radiochemical purity of the tritiated compounds was analysed by TLC on silica gel plates developed with $CHCl_3$: MeOH 95 : 5. The radioactive zones of the TLC plates were detected with a Berthold Automatic TLC-Linear Analyzer. Spots of deuterated compounds were made visible by spraying with 85% H_2SO_4 followed by heating. Mass spectra (electron impact, positive ionisation, 70eV) were obtained with a AMD 402 spectrometer from AMD Intectra GmbH.





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