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Synthesis of brassinosteroids with a keto group in the side chain

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ABSTRACT

The aim of this work was to prepare 24-epicryptolide and 22-dehydro-24-epibrassinolide as possible metabolites of 24-epibrassinolide. The main synthetic problem to be solved was the differentiation of functional groups in brassinosteroids. Distinguishing 2α,3α-diol function from another diol group in 24-epibrassinolide was achieved *via* selective hydrolysis of 2α,3α-cyclic carbonate or *via* regioselective reaction of boric acid with the functional groups in the side chain. The hydroxyl at C-23 was more reactive than the 22-OH in the oxidation with bromine in the presence of bis(tributyltin) oxide and in the benzylation reaction that resulted in the predominant formation of the corresponding α-hydroxy ketone derivatives with the ratio ranging from 4:1 to 1.5:1.

1. Introduction

The metabolic transformations of brassinosteroids (BS) proceed *via* various pathways, including dehydrogenation of one of the hydroxyl groups [1]. The evidence for the presence of this deactivation process for BS in plants is the identification of cryptolide **3** [2,3] in Japanese cedar pollen and anthers and of 23-dehydro-2-epicastasterone **4** from immature seeds of *Phaseolus vulgaris* [4] as metabolites of brassinolide **1** and castasterone **2**, respectively (Scheme 1). However, this process is still poorly studied, especially with respect to metabolic transformations of the side chain. Similar 22-dehydrogenation has not been described at all so far for BS, although steroidal 23-hydroxy-22-ketones are known as natural compounds [5–7].

In spite of various approaches are available for the synthesis of these compounds, none of them can be considered as practical or fully reliable. Thus, 23-dehydro-24-epibrassinolide was isolated as an impurity (0.11%) from commercially available 24-epibrassinolide [8]. An efficient method was proposed to build cryptolide side chain [9]. It is expedient for the preparation of steroids with a campestane carbon skeleton, but too cumbersome for making ergostane derivatives. Perhaps the easiest way to solve the problem is to use 22*R*,23*R*-diols as starting compounds. Such an approach implies selective protection of one of the two hydroxyls followed by oxidation of the remaining free hydroxyl group. This strategy was applied for the preparation of cryptolide [3] and 22- and 23-oxo derivatives of 28-homocastasterone [10]. In both cases, partial acetylation of 22*R*,23*R*-diols was used to distinguish the hydroxyl groups. Apart from the lack of regioselectivity at the acetylation step, possible epimerization in the course of deacetylation of intermediate acetoxyketones is another disadvantage of this approach.

The aim of the present work was therefore to develop a simple and reliable methodology for the preparation of steroids with an α-ketol in the side chain.

2. Experimental

2.1. General

Melting points were recorded on a Boetius micro-melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 500 (Bruker Biospin, Rheinstetten, Germany) spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shift values are given in δ (ppm) relative to the residual solvent peaks: δ_H 7.58 and δ_C 135.91 for C_5D_5N; δ_H 7.26 and δ_C 77.00 for $CDCl_3$, and coupling constants are reported in Hz. Mass spectra were performed on an LCQ Fleet mass spectrometer (Thermo Electron Corporation, USA) with an APCI source. HRMS/MS-spectra were acquired in positive electrospray ionisation mode with an Agilent 6550 iFunnel QTOF (Agilent Technologies, USA). Chemicals were purchased from Aldrich and Fluka and used as received. 24-Epibrassinolide (**1**)

Abbreviations: BS, brassinosteroids; TBAB, tetrabutylammonium bromide; DIPEA, diisopropylethylamine; DMP, Dess–Martin periodinane.

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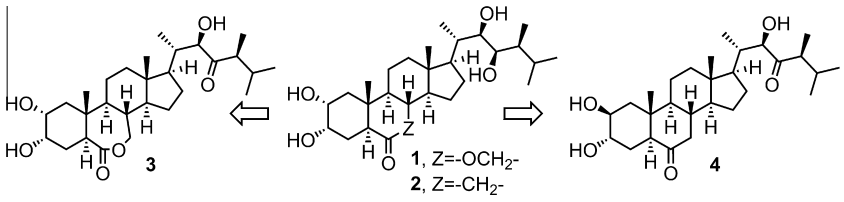

Scheme 1. 23-Dehydrogenation of brassinolide **1** and castasterone **2**.

was prepared according to the procedure described in [11]. All solvents were purified according to standard methods [12]. Reactions were monitored by TLC using aluminium sheets, silica gel 60 F_{254} precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734) (see Table 1).

2.2. Synthesis of the compounds

2.2.1. (22R,23R,24R)-2α,3α:22,23-Bis[carbonylbis(oxy)]-B-homo-7-oxa-24-methyl-5α-cholestan-6-one (**6**)

A solution of triphosgene (124 mg, 0.42 mmol) in CH_2Cl_2 (1.7 mL) was added to a stirred solution of epibrassinolide (**5**) (200 mg, 0.42 mmol) and pyridine (0.81 mL, 10 mmol) in CH_2Cl_2 (1.3 mL) at −80 °C. The cooling bath was removed, and the mixture was stirred at room temperature for 12 h. The excess of triphosgene was decomposed with a few drops of saturated NH_4Cl, and the water phase was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic phases were consecutively washed with 1 N HCl and saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was chromatographed on SiO_2 ($CHCl_3$–EtOAc = 9:1 → 4:1) to give dicarbonate **6** (194 mg, 89%) as white crystals. Mp 266–267 °C (acetone). $[\alpha]_D^{20}$ +140 (c 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 0.71 (s, 3H, C18-H), 0.76 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.93 (s, 3H, C19-H), 1.02 (d, *J* = 6.6 Hz, 3H), 2.35 (dd, *J* = 15.8, 5.1 Hz, 1H), 3.03 (dd, *J* = 9.5, 5.4 Hz, 1H, C5-H), 4.04–4.17 (m, 3H, C7- and C23-H), 4.42 (d, *J* = 4.8 Hz, 1H, C22-H), 4.85–4.90 (m, 1H, C2- or C3-H), 4.94–4.98 (m, 1H, C3- or C2-H). ^{13}C NMR ($CDCl_3$) δ: 8.6, 11.3, 11.8, 16.3, 18.9, 20.6, 22.9, 24.4, 26.5, 27.1, 27.7, 33.5, 36.0, 39.0, 39.1, 40.5, 40.6, 42.8, 42.9, 51.0, 51.7, 55.0, 71.0, 73.9, 74.8, 81.1, 82.2, 154.4, 154.8, 174.5. MS ($APCI^+$) *m*/*z* (%): 532.9 ($[M+H]^+$, 100). HRMS (ESI^+): calcd. for $C_{30}H_{44}NaO_8$ $[M+Na]^+$ 555.2928, found 555.2917.

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **17** and **18**[a,b].

Position	**17**		**18**	
	δ, C	δ, H (α/β)	δ, C	δ, H (α/β)
1	33.5	2.32 dd (15.7, 3.2)/1.11	33.5	2.31 dd (15.6, 3.6)/1.11
2	73.1	4.35	73.0	4.35
3	72.4	4.37	72.4	4.37
4	27.6	1.78	27.7	1.80
5	40.2	3.28	40.2	3.28 dd (10.5, 4.1)
6	176.6		176.6	
7	71.1	4.08	71.2	4.07 dd (12.7, 9.6)/4.11 dd (12.9, 2.6)
8	39.3	1.80	39.4	1.80
9	54.6	1.73	54.6	1.74
10	35.9		35.9	
11	22.9	1.33[c]/1.83[c]	22.9	1.33[c]/1.82[c]
12	39.7	1.28/2.01	39.7	1.32/1.97
13	43.2		42.9	
14	51.7	1.15	51.7	1.21
15	24.8	1.21[c]/1.59[c]	24.5	1.21[c]/1.61[c]
16	28.6	1.35[c]/1.90[c]	27.5	1.30[c]/2.00[c]
17	52.9		52.4	
18	11.7	0.71 s	12.0	0.70 s
19	19.6	0.88 s	19.6	0.87 s
20	41.4	1.54	40.8	1.60
21	13.6	1.02 d (5.9)	12.3	0.94 d (6.7)
22	81.2	3.53 d (2.8)	71.5	3.69 t (4.0)
23	75.0	3.26	83.7	3.37 dd (5.5, 3.4)
24	43.2	1.42	39.9	1.40
25	26.6	1.92	28.3	1.68
26	21.7	0.90 d (7.0)	22.2	0.93 d (6.5)
27	16.1	0.83 d (6.8)	19.6	0.94 d (6.2)
28	10.2	0.74 d (6.9)	12.1	0.97 d (7.0)
2,3-Me<u>C</u><	107.6		107.6	
2,3-<u>Me</u>C<	23.6	1.32 s	23.6	1.31 s
2,3-<u>Me</u>C<	26.6	1.54 s	26.5	1.51 s
1°	74.3	4.64 s	73.3	4.50 d (11.1)/4.69 d (11.1)
2°	138.4		138.3	
3°/7°	128.4	7.31	128.4	7.31
4°/6°	127.5	7.31	127.7	7.31
5°	127.7	7.31	127.7	7.31

[a] NMR chemical shifts (δ) and coupling constants (Hz) are from spectra obtained in $CHCl_3$ solution.
[b] Assigned by DEPT, COSY, HSQC, and HMBC experiments.
[c] May be reversed.

2.2.2. (22R,23R,24R)-22,23-Carbonylbis(oxy)-2α,3α-dihydroxy-B-homo-7-oxa-24-methyl-5α-cholestan-6-one (**7**)

A solution of LiOH (15.8 mg, 0.66 mmol) in water (0.4 mL) was added with stirring to a solution of dicarbonate **6** (175 mg, 0.33 mmol) in THF (4 mL). The mixture was stirred at room temperature for 40 min, then 0.1 N H_2SO_4 was added till pH 3 was achieved followed by extraction with $CHCl_3$ (3 × 5 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to give carbonate **7** (130 mg, 78%) as white crystals. Mp 259–260 °C (EtOAc). $[\alpha]_D^{20}$ −4.1 (c 0.49, $CHCl_3$). ^{1}H NMR δ: 0.70 (s, 3H, C18-H), 0.77 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.91 (s, 3H, C19-H), 0.94 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 3.12 (dd, *J* = 12.1, 4.5 Hz, 1H, C5-H), 3.71 (d, *J* = 10.1 Hz, 1H, C2-H), 4.02 (s, 1H, C3-H), 4.06–4.10 (m, 2H, C7-H), 4.12 (dd, *J* = 9.1, 5.0 Hz, 1H, C23-H), 4.43 (d, *J* = 5.0 Hz, 1H, C22-H). ^{13}C NMR δ: 8.6, 11.3, 11.5, 15.4, 16.3, 20.6, 22.1, 24.7, 27.2, 27.6, 31.0, 38.3, 39.2, 40.6, 40.8, 41.4, 42.5, 42.8, 51.0, 51.8, 57.9, 68.0, 68.0, 70.2, 81.2, 82.3, 154.9, 176.2. HRMS (ESI^+): calcd. for $C_{29}H_{47}O_7$ $[M+H]^+$ 507.3316, found 507.3309.

2.2.3. (22R,23R,24R)-2α,3α,22,23-Tetrahydroxy-B-homo-7-oxa-24-methyl-5α-cholestan-6-one 2,3-acetonide (**9**)

Variant A: A mixture of carbonate **7** (250 mg, 0.49 mmol), 2,2-dimethoxypropane (0.31 mL, 2.5 mmol), TsOH (5 mg) and THF (2 mL) was stirred at room temperature for 45 min. Then Et_3N (0.3 mL) was added, and solvents were evaporated under reduced pressure. The residue containing **8** was dissolved in MeOH (1.7 mL) and treated with K_2CO_3 (340 mg, 2.47 mmol). The mixture was stirred at room temperature for 5 days, diluted with water (3 mL) and extracted with $CHCl_3$ (3 × 5 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was chromatographed on silica gel (petroleum ether–EtOAc = 7:3 → 11:9) to give the acetonide **9** (150 mg, 62%) as an