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# Synthesis of sulfated brassinosteroids

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ABSTRACT

A number of water soluble sulfates of 24-epibrassinolide including the 2α,3α-disulfate and all possible monosulfates were synthesized. The target compounds were isolated in crystalline form as the corresponding sodium salts. Pyridine–sulfur trioxide complex was used as sulfating agent followed by transformation of the resulting pyridinium salts into the sodium sulfates by treatment with NaOH. The control of the regioselectivity was achieved by an appropriate use of acetyl and benzyl protecting groups.



## 1. Introduction

Brassinosteroids (BS) are an important group of plant bioregulators [1–4]. A wide range of biological effects have been documented for them including growth promotion, increased resistance to unfavorable biotic and abiotic environmental factors, crop yield increase, and shortening the period of vegetative growth [5]. As for many other hormones, BS homeostasis is strictly controlled through a variety of biosynthetic and metabolic processes [6–9]. At least 8 pathways of BS metabolic transformations have been described to date [8]. However, detailed knowledge is far from complete, in particular for the sulfation process. The sulfation of the C-22 hydroxyl group by sulfotransferases was supposed to be one of the mechanisms of BS inactivation [10,11]. Similar considerations were made with regard to other steroidal hormones (estrogens, androgens, etc). Their sulfated derivatives were considered first as inactive metabolites [12]. Only later the biological role of steroid sulfation has been recognized for the local supply of estrogens and androgens *via* the so-called sulfatase pathway [13].

It should be noted that our current knowledge of the BS sulfation is mainly based on the results of experiments with steroid sulfotransferases which were carried out without the characterization of sulfated BS in plant extracts [10]. Obviously, the corresponding studies would be greatly facilitated by the availability of authentic samples. The main purpose of the present work is, therefore, to prepare a set of BS derivatives as standards for studies of the sulfation process. Another potential interest of sulfated BS is related to possible medicinal application of this class of plant hormones [14]. Sulfation of similar polyhydroxysteroids was shown to endow them with new biological properties [15–20]. Here we report the synthesis of the 2-, 3-, 22- and 23-sodium monosulfates and the 2,3-disodium disulfate of 24-epibrassinolide.

## 2. Experimental

### 2.1. General

Melting points were measured using a Boetius 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 500.13 MHz for $^{1}H$ and 125.77 MHz for $^{13}C$. Chemical shift values are given in $\delta$ (ppm) relative to the residual solvent peaks: $\delta_H$ 3.31 and $\delta_C$ 49.00 for $CD_3OD$; $\delta_H$ 2.50 and $\delta_C$ 39.52 for DMSO-$d_6$; $\delta_H$ 7.26 and $\delta_C$ 77.16 for $CDCl_3$, and coupling constants are reported in Hz. COSY, HSQC, HMBC, and NOESY experiments were carried out with the use of the standard Bruker program package. HRMS/MS-spectra were acquired in positive electrospray ionization mode with an Agilent 6550 iFunnel QTOF

*Abbreviations:* BS, brassinosteroids; DMAP, *N*,*N*-dimethylaminopyridine; EBl, 24-epibrassinolide; DIPEA, *N*,*N*-diisopropylethylamine; TBAF, tetra-*n*-butylammonium fluoride; TBAI, tetra-*n*-butylammonium iodide; TBSCl, *tert*-butyldimethylsilyl chloride; TFA, trifluoroacetic acid.

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(Agilent Technologies, USA). Chemicals were purchased from Aldrich and Fluka and used as received. Solvents were dried and freshly distilled according to standard procedures [21]. All reactions were performed under positive argon pressure. TLC was performed on precoated aluminum backed TLC sheets (silica gel 60 F254) and visualized by spraying with anisaldehyde–$H_2SO_4$ reagent followed by heating. Column chromatography was conducted with Merck silica gel 60: 70–230 mesh.

### 2.2. Chemical synthesis

#### 2.2.1. General procedure 1: the preparation of pyridinium sulfates

Sulfur trioxide pyridine complex (2 eq.) was added to a 0.15 M solution of steroid (1 eq.) in dry $CH_2Cl_2$ and the resulting mixture was stirred at room temperature for 17 h. Then it was cooled to 0 °C, and the precipitate was filtered through a fine glass filter. The solvent was evaporated to give a solid, which was of sufficient purity to be used in the next step without additional purification.

#### 2.2.2. General procedure 2: the sodium monosulfate preparation and deprotection of the acetates

Sulfur trioxide pyridine complex (1 eq.) was added to a 5% methanolic solution of sodium hydroxide (12.5 eq.) and the resulting solution was stirred at room temperature for 4 h. Then it was diluted with water and extracted with EtOAc or $CHCl_3$. The extracts were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated. The resulting product needed no further purification.

#### 2.2.3. General procedure 3: removal of the benzyl groups

Palladium on carbon catalyst (10% Pd) (10 mol% per each benzyl group) was added to a 0.05 M solution of steroid in MeOH. The resulting solution was purged with hydrogen and stirred under a hydrogen atmosphere for 17 h at room temperature. The mixture was filtered through a thin pad of Celite, and the solvent was evaporated to give the desired product, which required no further purification.

#### 2.2.4. (22R,23R,24R)-22,23-Diacetoxy-2α,3α-dihydroxy-B-homo-7-oxa-5α-ergost-6-one (**6**)

Acetic anhydride (4 mL, 42.4 mmol) and DMAP (48 mg, 0.396 mmol) were added to a solution of 24-epibrassinolide (**1**) (1.90 g, 3.96 mmol) in dry pyridine (8.5 mL). After stirring for 4 h at room temperature, MeOH (3 mL) was added and the mixture was stirred for 20 min. The solvents were evaporated, the residue was dissolved in EtOAc (50 mL) and successively washed with 0.1 N HCl (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 mL). After drying with anhydrous $Na_2SO_4$, evaporation of the solvent yielded tetraacetate **5** (2.57 g), which was used in the next step without further purification.

Sodium carbonate (2.0 g, 19 mmol) was added to a solution of the crude tetraacetate **5** (2.57 g) in MeOH (40 mL). The resulting mixture was stirred at room temperature for 30 min, diluted with water (200 mL), and extracted with EtOAc (2 × 100 mL). The extract was washed with brine (2 × 100 mL), and dried with anhydrous $Na_2SO_4$. The solvent was evaporated and the residue was purified by column chromatography on silica gel (petroleum ether/EtOAc, 2:1) to give diacetate **6** (2.15 g, 96%) as a colorless oil. $^1$H NMR ($CDCl_3$): $\delta$ 5.19 (d, $J$ = 7.3 Hz, 1H, 22-H), 5.03 (dd, $J$ = 7.3, 4.9 Hz, 1H, 23-H), 4.08–3.99 (m, 2H, 7-H), 3.97 (s, 1H, 3-H), 3.69–3.63 (m, 1H, 2-H), 3.08 (dd, $J$ = 12.2, 4.4 Hz, 1H, 5-H), 2.13–2.03 (m, 2H), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc) 1.95–1.86 (m, 2H), 1.86–1.78 (m, 2H), 1.76–1.57 (m, 5H), 1.50 (t, $J$ = 12.4 Hz, 1H), 1.41–1.05 (m, 8H), 0.95 (d, $J$ = 6.8 Hz, 3H), 0.90 (d, $J$ = 6.9 Hz, 3H), 0.87 (s, 3H, 19-H), 0.83 (d, $J$ = 6.8 Hz, 3H), 0.79 (d, $J$ = 7.1 Hz, 3H), 0.67 (s, 3H, 18-H). $^{13}$C NMR ($CDCl_3$): $\delta$ 176.6, 170.7 (×2), 77.6, 74.6, 70.5, 68.1, 68.1, 58.1, 53.0, 51.3, 42.6, 41.5, 41.0, 39.6, 39.2, 38.8, 38.3, 37.8, 31.2, 27.9, 27.0, 24.8, 22.5, 22.3, 21.01, 20.96, 17.2, 15.5, 13.4, 11.6, 10.9. IR (KBr) $\upsilon_{max}$, cm$^{-1}$: 3444, 2962, 2875, 1739, 1373, 1249, 1232. HRMS (ESI$^+$): calcd for $C_{32}H_{53}NaO_8$ $[M+Na]^+$ 587.3554, found 587.3553; calcd for $C_{32}H_{53}O_8$ $[M+H]^+$ 565.3735, found 565.3734.

#### 2.2.5. Dipyridinium (22R,23R,24R)-22,23-diacetoxy-2α,3α-dihydroxy-B-homo-7-oxa-5α-ergost-6-one 2,3-disulfate (**7**)

The title compound **7** (340 mg) was obtained as an amorphous solid in 93% yield from diacetate **6** according to the general procedure 1. $^1$H NMR ($CDCl_3$): $\delta$ 9.01–8.94 (m, 4H, pyridinium), 8.46 (t, $J$ = 7.8 Hz, 2H, pyridinium), 8.00 (t, $J$ = 6.8 Hz, 4H, pyridinium), 5.17 (d, $J$ = 7.1 Hz, 1H, 22-H), 5.05–4.96 (m, 2H, 3- and 23-H), 4.44 (d, $J$ = 11.9 Hz, 1H, 2-H), 4.07–3.95 (m, 2H, 7-H), 3.15 (dd, $J$ = 11.8, 3.8 Hz, 1H, 5-H), 2.35–2.25 (m, 1H), 2.08–1.99 (m, 2H), 1.99 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.89–1.75 (m, 2H), 1.68–1.53 (m, 5H), 1.37–1.00 (m, 7H), 0.92 (d, $J$ = 6.5 Hz, 3H), 0.90–0.86 (m, 6H), 0.81 (d, $J$ = 6.6 Hz, 3H), 0.77 (d, $J$ = 6.9 Hz, 3H), 0.63 (s, 3H, 18-H). $^{13}$C NMR ($CDCl_3$): $\delta$ 176.1, 170.6 (×2), 146.6 (×2), 142.4 (×4), 127.6 (×4), 77.5, 74.5, 74.2, 73.7, 70.4, 57.5, 52.9, 51.1, 42.5, 41.5, 39.8, 39.4, 39.2, 38.8, 38.5, 37.7, 29.7, 27.8, 26.9, 24.8, 22.5, 22.2, 20.98, 20.96, 17.2, 15.6, 13.4, 11.5, 10.8. IR (KBr) $\upsilon_{max}$, cm$^{-1}$: 3445, 3069, 2960, 2877, 1737, 1377, 1245, 1211. HRMS (ESI$^+$): calcd for $C_{32}H_{51}Na_2O_{11}S$ $[M-2PyH-SO_3+2Na]^+$ 689.2942, found 689.2944.

#### 2.2.6. Disodium (22R,23R,24R)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergost-6-one 2,3-disulfate (***8***)

Sulfur trioxide pyridine complex **7** (87 mg, 0.1 mmol) was added to a 5% methanolic solution of sodium hydroxide (2 mL, 2.5 mmol) and the resulting solution was stirred at room temperature for 4 h. Then it was diluted with water (10 mL) and extracted with *n*-BuOH (3 × 5 mL). The extract was washed with brine (3 × 10 mL), dried with anhydrous $Na_2SO_4$, and the solvent was evaporated. The residue was dissolved in 5 mL of *n*-BuOH and filtered through thin pad of Celite. The solvent was evaporated to give compound **8** (58 mg, 86%). Mp 216 °C (dec) (MeOH-EtOAc). $^1$H and $^{13}$C NMR data are given in Table 1. IR (KBr) $\upsilon_{max}$, cm$^{-1}$: 3454, 2962, 2870, 1713, 1637, 1468, 1253, 1236.

#### 2.2.7. Benzylation of (22R,23R,24R)-22,23-diacetoxy-2α,3α-dihydroxy-B-homo-7-oxa-5α-ergost-6-one (***6***)

A mixture of steroid **6** (740 mg, 1.31 mmol), $Bu_2SnO$ (326 mg, 1.31 mmol), TBAI (160 mg, 0.44 mmol), DIPEA (505 μL, 2.92 mmol), and BnBr (345 μL, 2.92 mmol) in dry toluene (2 mL) was stirred for 4 h at 90–110 °C. The solvents were evaporated and the residue was purified by column chromatography on silica gel (toluene/EtOAc, 10:1) to give:

(a) (22*R*,23*R*,24*R*)-22,23-diacetoxy-3α-benzyloxy-2α-hydroxy-B-homo-7-oxa-5α-ergost-6-one (**9**) as an oil (62 mg, 7%). $^1$H NMR ($CDCl_3$): $\delta$ 7.39–7.27 (m, 5H, —OC$H_2$Ph), 5.21 (d, $J$ = 7.2 Hz, 1H, 22-H), 5.05 (dd, $J$ = 7.2, 4.9 Hz, 1H, 23-H), 4.63 (d, $J$ = 11.9, 1H, —OC$H_2$Ph), 4.48 (d, $J$ = 11.9 Hz, 1H, —OC$H_2$Ph), 4.06 (d, $J$ = 11.9 Hz, 1H, 7-H), 3.95 (dd, $J$ = 12.4, 9.4 Hz, 1H, 7-H), 3.77 (br. s, 1H, 3-H), 3.64–3.55 (m, 1H, 2-H), 2.92 (dd, $J$ = 12.0, 4.5 Hz, 1H, 5-H), 2.17–1.97 (m, 5H), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.83 (dtd, $J$ = 9.6, 6.7, 2.7 Hz, 1H), 1.78–1.58 (m, 3H), 1.48 (t, $J$ = 12.5 Hz, 1H), 1.42–1.06 (m, 7H), 0.97 (d, $J$ = 6.7 Hz, 3H), 0.91 (d, $J$ = 6.9 Hz, 3H), 0.89 (s, 3H, 19-H), 0.84 (d, $J$ = 6.8 Hz, 3H), 0.81 (d, $J$ = 7.1 Hz, 3H), 0.68 (s, 3H, 18-H). $^{13}$C NMR ($CDCl_3$): $\delta$ 176.1, 170.6, 170.6, 138.5, 128.6 (×2), 127.93, 127.63 (×2), 77.5, 68.9, 74.6, 71.2, 70.5, 67.9, 58.3, 53.0, 51.3, 43.3, 42.6, 41.6, 39.6, 39.2, 38.87, 38.38, 37.8, 28.4, 27.9, 27.0, 24.8,