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A new synthesis of brassinosteroids with a cholestane framework based on a highly functionalized starting material

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ABSTRACT

A new route to the synthesis of minor brassinosteroids with a cholestane framework (28-norcastasterone and 28-norbrassinolide) has been proposed. It makes use of commercially available 24-epicastasterone as a starting material. In addition, [26,26,26-$^2H_3$]-28-norcastasterone and [26,26,26-$^2H_3$]-28-norbrassinolide have been prepared as tools for analytical applications. The key steps were regioselective manipulations of functional groups in 24-epicastasterone, oxidative cleavage of 22,23-diol group and Claisen rearrangement.

1. Introduction

Chemical synthesis of any organic compound requires a solution of two major problems: (i) construction of the proper carbon framework and (ii) introduction of the necessary functional groups. Evidently, when the starting and final compounds have identical carbon skeletons, the implementation of the synthetic task is greatly facilitated making the corresponding product much more available and affordable for practical use. Compounds **1, 3, 4, 5a** (Fig. 1) are a good example from the field of plant steroidal hormones (brassinosteroids) [1]. Whereas 24-epibrassinolide **1** and 28-homobrassinolide **3** have already found practical application in agriculture [2,3], brassinolide **4** and 28-norbrassinolide **5a** until now are available for scientific purposes only. The reason is the availability of ergosterol and stigmasterol and a shortage of crinosterol and 22-dehydrocholesterol with carbon skeletons identical of those of 24-epibrassinolide **1**, 28-homobrassinolide **3**, brassinolide **4** and 28-norbrassinolide **5a**, respectively.

A characteristic feature of the current studies of BS is that they are not limited to plants but are being extended into animal models. Compounds originally known as phytohormones were shown to exhibit potentially useful medicinal effects such as anabolic, adaptogenic, anticancer, and/or antiviral activities [4–6]. Most studies on non-plant organisms have been and are being carried out with more available BS having an ergostane or stigmastane carbon skeletons. However, steroids with such frameworks are not typical constituents of non-plant organisms. In this respect, better attention should be paid to BS with a carbon skeleton identical to that of major animal sterol cholesterol. To date, there have been practically no papers dealing with medicinal aspects of such compounds, primarily because of their low accessibility. The known syntheses of 28-norbrassinolide from available commercial sterols are quite long because of the need, in addition to the side chain construction, to introduce the required functionality into AB-rings [7–10]. The solution of the problem could be the synthesis of the target BS starting from compounds, which already contain the necessary functional groups in the cyclic part. Recently, in connection with practical application of epibrassinolide **1** in agriculture [3], this compound as well as its synthetic intermediate epicastasterone **2** became available in bulk quantities and could be used as starting material for the preparation of some minor BS. As an example of such an approach, synthesis of 3,24-diepicastasterone and 3-dehydro-24-epibrassinolide (each differing from the parent compound by a set of functional groups) can be mentioned [11]. The aim of the present work was the development of a synthetic methodology to be applied to the preparation of minor BS, differing from the starting material not only by functional groups but also by a carbon framework. BS with a cholestane

Abbreviations: BS, brassinosteroids; $(DHQD)_2AQN$, hydroquinidine (anthraquinone-1,4-diyl) diether; DMAP, 4-dimethylaminopyridine.

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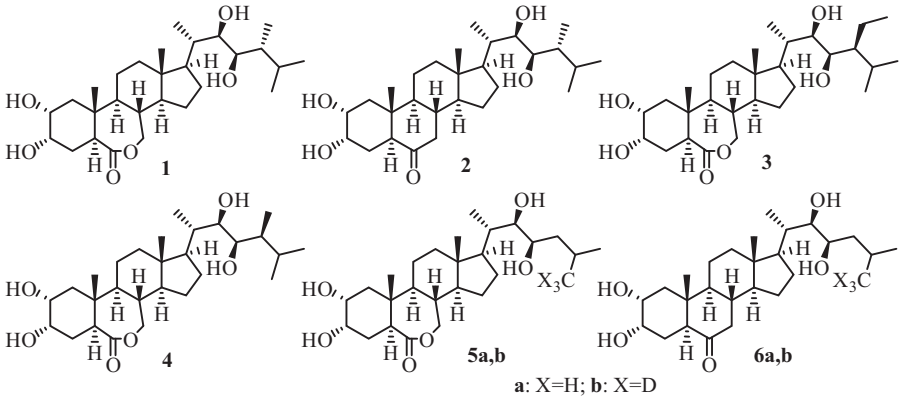

Fig. 1. Structures of compounds **1**–**6**.

carbon skeleton were chosen as target compounds to validate this new methodology. In addition to 28-norcastasterone **6a** and 28-norbrassinolide **5a**, their deuterated analogues **6b** and **5b** were prepared to be used for analytical purposes.

2. Experimental

2.1. General

Melting points were recorded on a Boetius micro-melting point apparatus and are uncorrected. IR spectra were taken on a Michelson Bomem 100 FTIR spectrophotometer and reported in cm^{-1}. ^1H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 500 (Bruker Biospin, Rheinstetten, Germany) spectrometer operating at 500 MHz for ^1H 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 a LCQ Fleet mass spectrometer (Thermo Electron Corporation, USA) with an APCI source. Chemicals were purchased from Aldrich and Fluka and used as received. 24-Epicastasterone **2** was prepared according to the procedure described in [12]. All solvents were purified according to standard methods [13]. Reactions were monitored by TLC using aluminum sheets, silica gel 60 F_{254} precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

2.2. Synthesis of the compounds

2.2.1. (20S)-2α,3α-Isopropylidenedioxy-5α-pregnan-6-one-20-carbaldehyde (*14*)

A solution of 24-epicastasterone **2** (11.0 g, 23.7 mmol) and boric acid (1.61 g, 26.1 mmol) in THF (280 mL) was stirred at ambient temperature for 1 h, then 2,2-dimethoxypropane (29.4 mL, 237 mmol) and $TsOH{\cdot}H_2O$ (902 mg, 4.74 mmol) were added and stirring was continued for 1.5 h. After adding triethylamine (9.9 mL, 71.1 mmol), the mixture was kept for 10 min while stirring, and then water (95 mL) and sodium periodate (15.2 g, 71.1 mmol) were added. The mixture was left stirring for 17 h, and then diluted with water (360 mL) and EtOAc (140 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 70 mL). The combined organic extract was dried with Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (petroleum ether-EtOAc = 5:1) to give the aldehyde **14** (8.53 g, 89%) as white crystals. Mp 184–187 °C (EtOAc). IR (KBr): ν_{max} 3430, 2945, 2875, 1715, 1700, 1460, 1380, 1240, 1215, 1055. ^1H NMR ($CDCl_3$) δ: 0.67 (s, 3H, C18-H), 0.69 (s, 3H, C19-H), 1.12 (d, J = 6.9 Hz, 3H, C21-H), 1.33 (s, 3H, Me_2C<), 1.49 (s, 3H, Me_2C<), 2.54 (dd, J = 12.6, 3.9 Hz, 1H, C5-H), 4.09 (ddd, J = 11.4, 6.8, 5.0 Hz, 1H, C2-H), 4.27 (td, J = 4.4, 1.6 Hz, 1H, C3-H), 9.57 (d, J = 3.1 Hz, 1H, C22-H). ^{13}C NMR ($CDCl_3$) δ: 12.3, 12.7, 13.4, 21.0, 22.5, 24.3, 26.5, 26.9, 28.6, 37.4, 39.0, 41.1, 42.4, 43.4, 46.8, 49.4, 50.9, 51.4, 53.3, 55.9, 72.1, 72.2, 107.9, 204.7, 211.1. MS (APCI$^+$) m/z (%): 403.1 ([M+H]$^+$, 50), 345.3 ([M−C_3H_6O+H]$^+$, 100).

2.2.2. (22E)-2α,3α-Isopropylidenedioxy-5α-cholest-22-en-6-on-26-oic acid ethyl ester (*16*)

To a cooled to −78 °C solution of the aldehyde **14** (7.61 g, 18.9 mmol), 1 M vinylmagnesium bromide solution in THF (24.6 mL, 24.6 mmol) was added dropwise keeping the temperature below −60 °C. The mixture was stirred at -78 °C for 1.5 h, then NH_4Cl (2.8 g, 52.3 mmol) was added and the temperature was raised to ambient. The mixture was diluted with water (100 mL) and EtOAc (60 mL). The water phase was separated and extracted with EtOAc (2 × 35 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue (9.09 g) was dissolved in benzene (430 mL), then triethyl orthopropionate (21 mL, 106 mmol) and propionic acid (1.6 mL, 21 mmol) were added. The mixture was refluxed under argon for 4 h. After cooling to room temperature, pyridine (6.6 mL) was added and solvents were evaporated *in vacuo*. The residue was chromatographed on silica gel (petroleum ether-EtOAc = 9:1) to give the ester **16** (5.11 g, 53%) as an oil. $[\alpha]^{20}$ = +20.7° (*c* 1.01, $CHCl_3$). IR (film): ν_{max} 2940, 2870, 1733, 1712, 1460, 1380, 1368, 1242, 1215, 1175, 1163, 1056. ^1H NMR ($CDCl_3$) δ: 0.64 (s, 3H, C18-H), 0.66 (s, 3H, C19-H), 0.979, 0.981 (d, J = 6.6 Hz, 3H), 1.102, 1.104 (d, J = 6.9 Hz, 3H), 1.24 (t, J = 7.4 Hz, 3H, $C\underline{H}_3CH_2O$–), 1.33 (s, 3H, Me_2C<), 1.49 (s, 3H, Me_2C<), 4.05–4.15 (m, 2H, $CH_3C\underline{H}_2O$– and C2-H), 4.26 (td, J = 4.5, 1.6 Hz, 1H, C3-H), 5.19–5.33 (m, 2H, C22- and C23-H). ^{13}C NMR ($CDCl_3$) δ: 12.1, 12.6, 14.2, 16.5, 20.6, 21.1, 22.5, 23.9, 26.5, 28.2, 28.3, 28.6, 36.5, 36.6, 37.5, 39.2, 39.7, 39.8, 40.0, 41.1, 42.5, 42.7, 46.9, 51.4, 53.3, 55.6, 56.7, 60.1, 72.1, 72.3, 107.8, 124.2, 124.3, 139.1, 176.3, 211.5. MS (APCI$^+$) m/z (%): 457.1 ([M−C_3H_6O+H]$^+$, 100).

2.2.3. (22E)-2α,3α-Isopropylidenedioxy-6-(1,3-dioxolan-2-yl)-5α-cholest-22-en-26-oic acid ethyl ester (*17*)

A solution of **16** (4.52 g, 8.78 mmol) and $TsOH{\cdot}H_2O$ (167 mg, 0.88 mmol) in 2,2-dimethyl-1,3-dioxolane (25 mL) was kept at 110 °C for 2 h, then pyridine (1 mL) was added and solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether-EtOAc = 5:1) to give compound **17** (4.24 g, 86%) as an oil. $[\alpha]^{20}$ = +20.8° (*c* 1.03, $CHCl_3$). IR (film): ν_{max} 2938, 2870, 1734, 1460, 1378, 1368, 1242, 1215, 1176, 1088, 1058. ^1H NMR ($CDCl_3$) δ: 0.65 (s, 3H, C18-H), 0.82 (s, 3H, C19-H), 0.97 (d, J = 6.6 Hz, 3H),