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Stereospecific synthesis of new steroidal isoxazoles in dry media

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ABSTRACT

An easy and fast procedure was developed for one-pot synthesis of steroidal isoxazoles starting from 23-acetylsapogenins derivatives in presence of P_2O_5/SiO_2 in dry media under microwaves irradiation is described. Substrates of the 25*S* and 25*R* series were used as raw materials, establishing that this new methodology is applicable to both series.

1. Introduction

During the past decade, the solid phase synthesis and the use of microwave irradiation have become important tools in organic syntheses. In the context of combinatorial synthesis, many types of reactions have been performed successfully in chemistry [1]. Microwave-assisted solid phase synthesis has attracted considerable attention and has been applied successfully in various fields of organic chemistry with significant rate enhancements and higher product yields [2]. In recent years, the synthesis of heterocyclic organic compounds has become important because of their biological activities; for instance as antibacterial [3], antiarrhytmic [4], antitumoral [5] and as anti-HIV [6]. The synthesis of steroids bearing diverse organic function at the side chain as those present in naturally occurring compounds (e.g. ecdysones, brassinosteroids, sterols, etc.) possess a challenging problem in the chemistry of steroids [7]. The matter of our ongoing research is the development of new synthetic strategies useful in the synthesis of steroid molecules possessing significant and potentially helpful biological activity.

The introduction of an isoxazole ring in the steroidal skeleton may lead to compounds that are of interest in pharmaceutical and agricultural industries [8]. For example, danazol® (**1**, Fig. 1), which it is an analog of ethisterone, a compound of interest due to its contraceptive activity [9]; or compound **2**, a precursor of brassinosteroids [8a].

Isoxazole derivatives represent another important class of nitrogen-containing heterocycles along with diverse useful bioactivities and are widely used as key intermediates in the preparation of natural products and related structures [10]. Intensive research has generated numerous approaches for the synthesis of isoxazoles [11], including reactions of hydroxylamine with 1,3-dicarbonyl compounds [12], α,β-unsaturated nitriles/carbonyl compounds [13,14], and ynones [15], reaction of an oxime-derived dianion with an ester/amide [16], and [3+2] cycloaddition reaction between an alkyne and a nitrile oxide [17]. An advantage of this procedure is that it ensures simultaneous formation of C–C bond and introduction of a number of functional groups under mild conditions, which is especially important for the synthesis of many natural steroids possessing poly-functionalized side chains [18].

Due to the importance of interest in isoxazole compounds, we have developed new methods of chemical synthesis to provide a wide variety of compounds for biological research. In order to generalize the rapid access to those heterocycles molecules, we have introduced an isoxazole moiety in the steroidal side chain, in a one-pot reaction, using P_2O_5/SiO_2 in solid phase under microwave irradiation, starting from 23-acetylsapogenins.

2. Experimental

2.1. General methods

Reactions were performed using a SEV (600 W max) focused microwave apparatus. 1D and 2D ^1H and ^{13}C NMR spectra (DEPT, COSY, and HSQC) were recorded on a Varian Mercury 400

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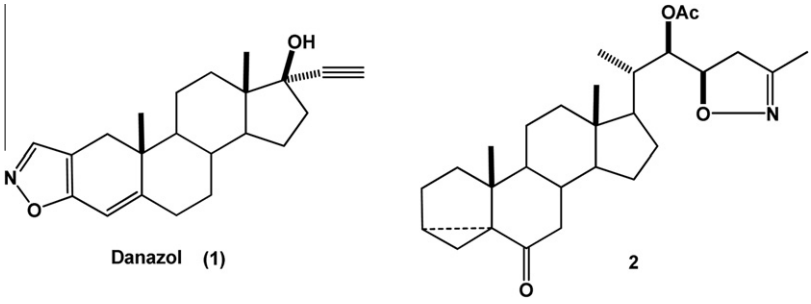

Fig. 1. Examples of isoxazole structures.

spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are stated in ppm (δ) and are referred to the residual ^{1}H signal (δ = 7.27) or to the central ^{13}C triplet signal (δ = 77.0) for $CDCl_3$. IR spectra were acquired on a Shimadzu FT-IR spectrophotometer using KBr pellets ($\bar{\nu}_{max}$, cm^{-1}). Mass (MS) spectra were obtained on a HP 5989A spectrometer using electron impact ionization. Optical rotations were determined on a Perkin–Elmer 241 polarimeter at room temperature using ethanol or methanol as solvent. Melting points were obtained in open capillary tubes on a Melt Temp apparatus and were not corrected. Analytical TLC were performed on silica gel ALUGRAM® SIL G/UV-252 plates and column chromatographies were carried out on silica gel Davisil™ grade 633 (200–400 mesh).

2.2. Synthesis of isoxazole derivative from microwave irradiation

General procedure. The isoxazole derivatives were prepared under the following standard procedure. A mixture of the acetylsapogenin **5** (2 mmol) or the furostene **7** (2 mmol), hydroxylamine hydrochloride (4 mmol) and P_2O_5/SiO_2 (1.0 g) [19] were ground thoroughly in a mortar. An immediate color change was observed. The mortar was covered with a watch glass and irradiated by microwaves (300 W) during 3 minutes. The completion of the reaction was monitored by TLC; the mortar was cooled at room temperature and removed. Then 10 mL of 5% aqueous HCl was added and the resulting solution was extracted with CH_2Cl_2 (2 × 10 mL). The extracts were combined and dried over $CaCl_2$. The crude product was purified by column chromatography using silica gel (200–400 mesh) and eluent AcOEt/hexane (3:2). Evaporation of the solvent under vacuum gave the isoxazole steroid **6a** in 81%, or 79% in **6b**.

2.2.1. (20S,2″S)-20-[4′-(3″-Hydroxy-2″-methylpropyl)-3′-methylisoxazol-5-yl]-5β-pregnane-3β,16β-diol (**6a**)

White crystals, mp 227–228 °C (acetone); $[\alpha]_D$ −106.4° (*c* 1.0 EtOH); IR$\bar{\nu}_{max}$: 3386, 3355, 3321, 2933, 2821, 1629; ^{1}H NMR δ: 4.12 (^{1}H, s, H-3), 3.98 (^{1}H, ddd, *J* = 8.4, *J* = 7.6, *J* = 3.6 Hz, H-16), 3.51 (^{1}H, dd, $J_{3a''-2''}$ = 6.0, J_{gem} = 10.4 Hz, H-3″$_a$), 3.47 (^{1}H, dd, $J_{3b''-2''}$ = 6.0, J_{gem} = 10.4 Hz, H-3″$_b$), 3.30 (^{1}H, dc, J_{20-17} = 11.6, J_{20-21} = 6.8 Hz, H-20), 2.47 (^{1}H, dd, $J_{1a''-2''}$ = 8.0, J_{gem} = 14.0 Hz, H-1″$_a$), 2.16 (^{1}H, dd, $J_{1b''-2''}$ = 8.0, J_{gem} = 14.0 Hz, H-1″$_b$), 2.22 (3H, s, CH_3-1‴), 1.28 (3H, d, *J* = 6.8 Hz, CH_3-21), 0.98 (3H, s, CH_3-19), 0.95 (3H, d, *J* = 6.0 Hz, CH_3-1‴), 0.93 (3H, s, CH_3-18); ^{13}C NMR δ:29.9 (C-1), 26.6 (C-2), 67.1 (C-3), 36.5 (C-4), 36.2 (C-5), 27.9 (C-6), 26.3 (C-7), 35.3 (C-8), 36.5 (C-9), 36.5 (C-10), 20.9 (C-11), 40.4 (C-12), 42.7 (C-13), 53.9 (C-14), 33.5 (C-15), 72.5 (C-16), 58.9 (C-17), 13.3 (C-18), 24.0 (C-19), 29.1 (C-20), 17.1 (C-21), 159.8 (C-3′), 173.0 (C-4′), 110.0 (C-5′), 26.1 (C-1″), 39.8 (C-2″), 67.5 (C-3″), 19.5 (C-1‴), 10.7 (C-1⁗); Anal. Calcd. For $C_{29}H_{47}O_4N$, C 73.53, H 9.99, O 13.51, N 2.95, found C 73.52, H 9.98, O 13.55, N 2.95.

2.2.2. (20S,2″R)-20-[4′-(3″-Hydroxy-2″-methylpropyl)-3′-methylisoxazol-5-yl]-pregn-5-ene-3β,16β-diol (**6b**)

White crystals, mp 210-211 °C (MeOH); $[\alpha]_D$ −98.6° (*c* 0.036 MeOH); IR$\bar{\nu}_{max}$: 3386, 3321, 3298, 2933, 2821, 1666, 1629; ^{1}H NMR δ: 5.34 (^{1}H, d, *J* = 5.2 Hz, H-6), 4.06 (^{1}H, ddd, *J* = 7.6, *J* = 7.2, *J* = 4.0 Hz, H-16), 3.53 (^{1}H, m, H-3), 3.47 (^{1}H, dd, $J_{3a''-2''}$ = 5.6, J_{gem} = 10.8 Hz, H-3″$_a$), 3.44 (^{1}H, dd, $J_{3b''-2''}$ = 5.6, J_{gem} = 10.8 Hz, H-3″$_b$), 3.34 (^{1}H, dc, J_{20-17} = 12.0, *J* = 6.8 Hz, H-20), 2.47 (^{1}H, dd, $J_{1a''-2''}$ = 8.0, J_{gem} = 14.4 Hz, H-1″$_a$), 2.20 (^{1}H, dd, $J_{1b''-2''}$ = 8.0, J_{gem}=14.4 Hz, H-1″), 2.22 (3H, s, CH_3-1‴), 1.28 (3H, d, *J* = 6.8 Hz, CH_3-21), 1.03 (3H, s, CH_3-19), 0.97 (3H, s, CH_3-18), 0.96 (3H, d, *J* = 6.8 Hz, CH_3-1‴); ^{13}C NMR δ: 31.7 (C-1), 31.6 (C-2), 71.7 (C-3), 42.2 (C-4), 140.8 (C-5), 121.3 (C-6), 36.7 (C-7), 35.3 (C-8), 50.0 (C-9), 36.5 (C- 10), 20.8 (C-11), 37.2 (C-12), 42.2 (C-13), 54.1 (C-14), 39.9 (C-15), 72.6 (C-16), 58.2 (C-17), 13.0 (C-18), 19.4 (C-19), 28.8 (C-20), 19.7 (C-21), 159.8 (C-3′), 173.3 (C-4′), 110.0 (C-5′), 25.9 (C-1″), 31.5 (C-2″), 66.7 (C-3″), 17.1 (C-1‴), 10.7 (C-1⁗); HRMS-FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{29}H_{45}O_4N$, 472.3427; found, 472.3426.

3. Results and discussion

In a previous work, we reported the preparation of the corresponding 23-acetylsapogenins **5** [20] through the hydrolysis of 22,26-epoxycholestanes **4** (Scheme 1), which in turn can be obtained from 25*R* and 25*S*-sapogenins **3** [21,22].

In this paper we describe the transformation of 23-acetylsapogenins **5** to an isoxazole framework, under a one-pot synthesis. The synthetic routes to introduce an isoxazole moiety in steroidal side chains are scarce [23].

Previous methods are tedious giving moderate yield and some ones being hazardous [11–17]. The goal of the present work was to introduce an isoxazole moiety in the steroidal side chain, in a one-pot sequence in dry media (Scheme 2). It is worth mentioning that our methodology shows greater qualities, faster, shorter, with a global yield from 76% for **6a** and 85% **6b** from sapogenins **3a** and **3b**. Despite the presence of a strong acidic medium, there is no epimerization of the stereogenic centers of the steroidal skeleton.

We observed that the reaction reached 5% conversion with the conventional conditions after 3 h and that longer reaction time did not improved this conversion. By contrast, heating the reaction medium via focused microwaves (300 W) led to complete conversion, and the isoxazole derivative was obtained. We could explain this observation by the fact that this 'instantaneous' increase of temperature is not possible with conventional heating due to slow heat transfer from the wall of the vessel to the solution. After workup and purification by column chromatography, the reaction furnished **6** as a white solid, which was characterized as indicated below.

Analysis by NMR spectroscopy in the ^{1}H NMR spectrum, indicated that the transformation of the spiroketal moiety at C-22 of 23-acetylsapogenins **5** into the corresponding isoxazole derivatives