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Synthesis of exemestane labelled with ^{13}C

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ABSTRACT

The synthesis of exemestane (Aromasin®), an irreversible steroidal aromatase inhibitor, specifically labelled with ^{13}C is reported. The preparation of [$^{13}C_3$]exemestane was achieved according to an eight-step procedure starting from the commercially available testosterone.

1. Introduction

Exemestane (Aromasin®, 6-methylenandrosta-1,4-diene-3,17-dione; molecular weight 296.41) (Fig. 1) is an orally active irreversible aromatase inhibitor which is clinically in use for the treatment of postmenopausal women with early or advanced breast cancer [1–3]. The initial analytical methods developed for the determination of exemestane in biological fluids involved the use of high-performance liquid chromatography (HPLC) combined with ultraviolet detection or with thermospray mass spectrometry or followed by specific radioimmunoassay [4–6]. However, a higher samples throughput as well as lower limits of quantitation were needed to assay exemestane in human plasma. Therefore, the preparation of a stable labelled version of the compound was required to be used as analytical internal standard (IS) to develop and validate a sensitive, specific and reliable method for the quantitation of exemestane in biological fluids using liquid chromatography–mass spectrometry (LC–MS). In this paper, the approach followed to prepare a suitable stable labelled IS of exemestane is described.

2. Experimental

Chemicals and materials: All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Testosterone (**1**) was purchased from Fluka, with a chemical purity >99%. [$^{13}C_2$]Acetyl chloride (99 atom % ^{13}C) and [^{13}C]formaldehyde (20% aqueous solution; 99 atom % ^{13}C) were obtained from Aldrich Chemical Co.

Instrumentation and equipment: Chemical purities were determined by HPLC using a series-200 pump (PerkinElmer) equipped with series-200 solvent degasser (PerkinElmer), series-200 autosampler (PerkinElmer) and a LC-235 UV diode array detector (PerkinElmer) connected with Totalchrom

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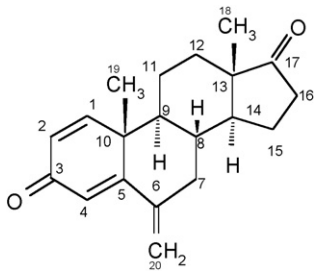

Fig. 1 – Structural formula of exemestane.

Client/Server (PENelson) as integrator *via* link 600 interface (PerkinElmer). Preparative-HPLC was performed at 25 °C using a PrepStar HPLC system (Varian). ^{1}H NMR data were recorded on Varian INOVA 400 spectrometer, equipped with a 5 mm 1H{15N-31P} *z*-axis pulse field gradient (PFG) indirect detection probe. ^{13}C NMR data were obtained by direct detection on a varian INOVA 500 spectrometer, equipped with a 3 mm broadband 15N-31P{1H-19F} probe optimized for 13C sensitivity and by heteronuclear correlation spectra (H-C) recorded on Varian Unity INOVA 500 spectrometer, equipped with triple resonance 1H{13C, 15N} z-axis pulse field gradient (PFG) indirect detection Cold-Probe. Chemical shifts were referenced with respect to the residual solvent signals (DMSO-d6: 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C).

Analytical method: HPLC: X-Terra Waters RP18 column (mm 4.6 × 100, 5 μm) eluting with H_2O:CH_3CN:HCOOH 90:10:0.1 by volume (A) and H_2O:CH_3CN:HCOOH 10:90:0.1 by volume (B): from 100% A to 0% A in 10 min; 5 min at 0% A; from 100% B to 100% A in 1 min; 4 min at 100% A. Flow rate: 1 ml/min. Column temperature: 25 °C. Analytical wavelength: 215 nm.

2.1. 17β–Benzoyloxy-testosterone (2)

A solution of benzoyl chloride (BzCl; 49 ml, 42 mmol) in dry toluene (60 ml) was slowly dripped into a stirred and cooled (0 °C) solution of testosterone (**1**) (10.08 g, 34.9 mmol) in dry toluene (100 ml). After addition of dry pyridine (14 ml, 173 mmol), the reaction mixture was stirred at reflux for 20 h then cooled to room temperature. An aqueous solution of 0.5 N HCl (60 ml) was added and the mixture was stirred for 1 h. After separation, the organic layer was treated with 0.5 N HCl (4 ml × 100 ml), 8% $NaHCO_3$ (3 ml × 100 ml), brine (2 ml × 100 ml) and dried over Na_2SO_4. The solvent was evaporated to dryness giving the intermediate (**2**) (14.03 g, 35.7 mmol), with a chemical purity of 91% (determined by HPLC; see analytical method; R_t = 14.0 min).

2.2. 17β-Benzoyloxy-5-oxo-3,5-seco-4-norandrostan-3-oic acid (3)

An aqueous solution of 2M K_2CO_3 (32.6 ml) was added to a suspension of (**2**) (13.00 g, 33.2 mmol) in *tert*-butanol (325 ml). The reaction mixture was heated at 40 °C under stirring and two separate solutions of 0.13 M $KMnO_4$ (42.25 ml) and 0.75 M KIO_4 (419 ml) were contemporaneously introduced into the reaction flask. At the end of the addition (about 2 h) the mixture was stirred for 2 h at 40 °C then cooled to room temperature and evaporated to dryness. The obtained residue was dissolved with water (500 ml), transferred into a separating funnel and extracted with ethyl acetate (EtOAc 2 ml × 200 ml). After separation, the combined organic layers were back extracted with 8% $NaHCO_3$ (3 ml × 100 ml). The basic aqueous phases were combined with the aqueous phase previously obtained, then 6 N HCl was added up to pH 2. After extraction with EtOAc (4 ml × 250 ml) the obtained organic phases were combined, extracted with brine (2 ml × 300 ml) and dried over Na_2SO_4. The solvent was removed by evaporation, and the obtained intermediate (**3**) (11.09 g, 28.9 mmol) was used without further purification in the next step.

2.3. 3-Oxo-4-oxa-5-androsten-17β-yl benzoate (4)

The intermediate (**3**) (11.09 g, 28.9 mmol) was dissolved in dry EtOAc (480 ml) under nitrogen then a freshly prepared solution of acetic anhydride (94 ml) and 65% $HClO_4$ (0.24 ml) in dry EtOAc (480 ml) was introduced into the reaction flask. After stirring at room temperature for exactly 10 min, 8% $NaHCO_3$ (740 ml) was added, the mixture was stirred for 30 min then transferred into a separating funnel. After separation of the aqueous layer, the organic phase was extracted with 8% $NaHCO_3$ (3 ml × 300 ml), water (2 ml × 300 ml), brine (2 ml × 300 ml) and dried over Na_2SO_4. After solvent evaporation, the oily yellow residue was submitted to 3 crystallizations from mixtures of CH_3OH:H_2O 3:1 by volume. After drying under high vacuum at 40 °C, the intermediate (**4**) was recovered as a white powder (5.79 g, 14.7 mmol) with a chemical purity of 90% (determined by HPLC; see analytical method; R_t = 11.7 min). MS (ESI-MS): *m*/*z* 395 ($[MH]^+$).

2.4. 2-[1′,2′-$^{13}C_2$]Acetyl-3-oxo-4-oxa-5-androsten-17β-yl benzoate (5)

A solution of the intermediate (**4**) (1.51 g, 3.83 mmol) in dry tetrahydrofuran (THF; 34.4 ml) was slowly added under nitrogen to a cooled (−78 °C) and stirred 1 M solution of lithium bis(trimethylsilyl)amide (7.66 ml; 7.66 mmol) in dry THF. After stirring under nitrogen at −78 °C for 1 h, a solution of [$^{13}C_2$]acetyl chloride (308 mg, 3.83 mmol) in dry THF (2.7 ml) was added. The mixture of reaction was stirred at −78 °C for 90 min, then 1 N HCl (4.8 ml) and water (4.8 ml) were added and the mixture was allowed to reach room temperature then diluted with EtOAc (50 ml). After transferring into a separating funnel and separation, the organic layer was washed with brine (4 ml × 200 ml), dried over Na_2SO_4 and evaporated to dryness. The obtained crude intermediate (**5**) was purified by flash chromatography on silica gel using a mixture *n*-hexane:EtOAc 2:1 by volume as the eluting solvent system. The collected fractions were combined as appropriate and after solvent evaporation to dryness, the intermediate (**5**) (446 mg; 1.02 mmol) was recovered as a white solid with a chemical purity >95% (determined by HPLC; see analytical method; R_t = 13.8 min) and an isotopic purity of 99 atom % ^{13}C. MS (ESI-MS): *m*/*z* 439 ($[MH]^+$).