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Synthesis and characterization of the *O*-desisopropyl metabolite of bisoprolol

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Abstract

Bisoprolol **(1)**, like other beta blockers, can be misused in certain sports, for example shooting and archery because of their performance enhancing properties. By using beta blockers athletes can control effects of performance anxiety such as nervousness, hand tremor and high heart rate [1,2]. Beta blockers are on the list of prohibited substances and methods published by WADA. As a part of an ongoing WADA-funded research project, we synthesized *O*-desisopropyl bisoprolol **(2)** for doping analytical purposes. The synthesized metabolite was purified and characterized by chromatographic and spectroscopic methods. Preparation and characterization of the bisoprolol metabolite is presented. The metabolite synthesized within this project will be made available without charge to all the WADA-accredited anti-doping laboratories.

Introduction

Bisoprolol belongs to the beta blocker class of antihypertensive drugs, which was originally developed for the treatment of *angina pectoris* [3]. Presently, they have several other indications such as coronary artery disease, congestive heart failure, ischemic heart disease and the management of cardiac arrhytmia [4-6]. Beta blockers are beta receptor antagonists that prevent the binding of adrenaline in adrenergic beta receptors. There are three different subclasses of beta receptors which reside in various tissues. The effect produced by the drugs varies depending on the type of receptor a drug binds to. The mechanism of action of beta blockers is not fully understood and it is probable that it even differs between different drugs [7].

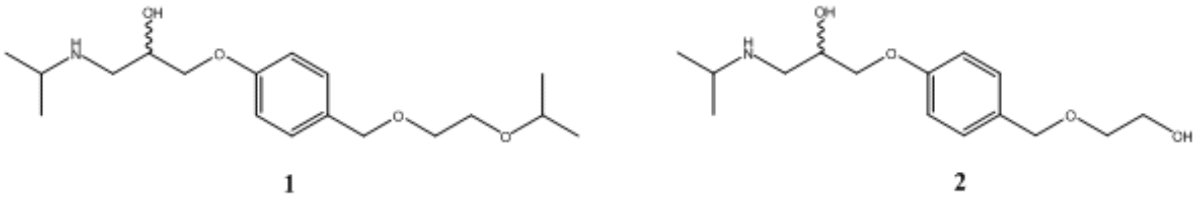

Figure 1. Bisoprolol (1) and *O*-desisopropyl bisoprolol (2)

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Experimental

Synthesis

O-desisopropyl bisoprolol (**2**) can be prepared using the synthetic route shown in Figure 2. The phenolic hydroxy group of 4-OH-benzylalcohol is benzylated with benzyl bromide. The benzylic alcohol is then converted to a bromide with phosphorus tribromide. The resulting bromide is reacted with ethylene glycol in the presence of sodium hydride to give the corresponding glycol ether. The phenol is deprotected using palladium-catalyzed hydrogenation and the resulting free phenol is subsequently treated with potassium carbonate and epichlorohydrin. This epoxy-containing intermediate is reacted with isopropylamine to give *O*-desisopropyl bisoprolol as the desired final product.

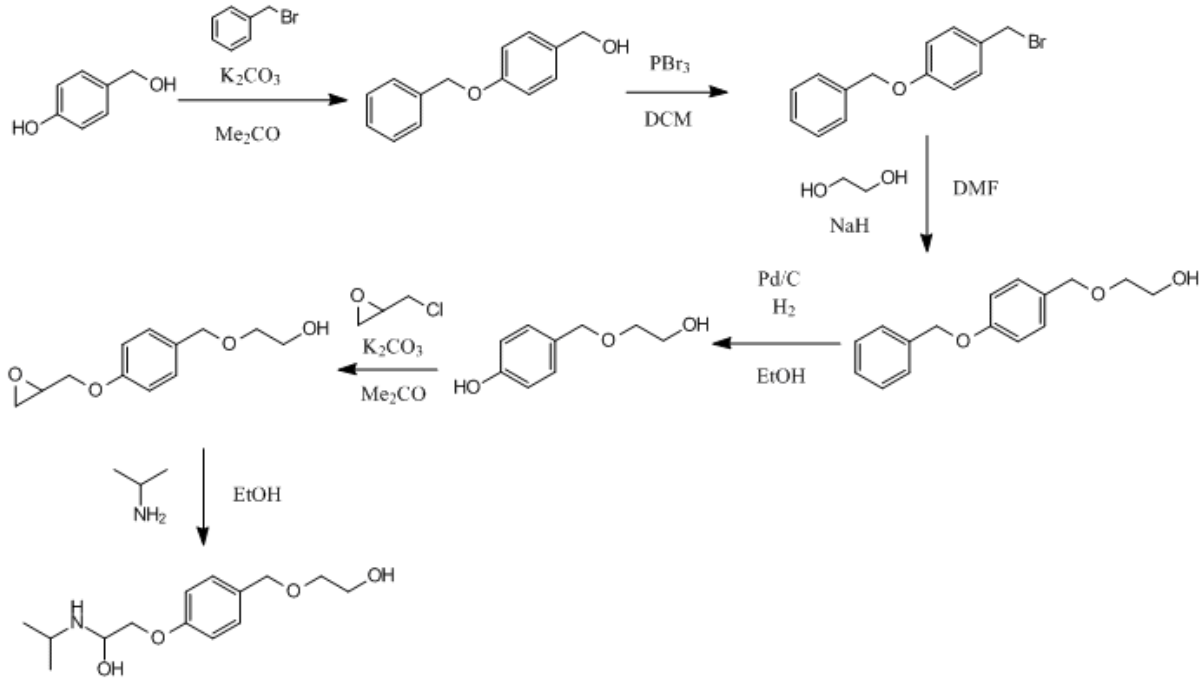

Figure 2. Synthesis of *O*-desisopropyl bisoprolol

Purity and characterization of the synthesized substance

Chromatographic purity of the synthesized bisoprolol metabolite was determined with an Agilent 1100 LC/UV-Vis system equipped with a Merck LiChroCART Purospher RP-18e (125 x 3 mm i.d., 3.5 µm) column. The mobile phase consisted of 2.5 mM ammonium acetate with 0.1% acetic acid, pH 4 (A) and methanol (B). A linear gradient run was applied; from 25 to 95% of B within 7 min at ambient temperature with a flow rate of 0.8 mL/min, and spectral data was collected (λ = 190-500 nm).
For accurate mass measurements a Bruker Daltonics micrOTOF mass spectrometer was used and the sample was infused directly to the ESI source. The capillary voltage was 4000 V in positive ion mode, and mass spectral data were recorded within the range of *m/z* 50-600. The average resolution at *m/z* 296 was 11000.
Tandem-mass spectrometric characterization was carried out using a ThermoFinnigan TSQ Quantum Discovery triple quadrupole instrument and direct infusion of the synthesized substance. Positive ion ESI (4000 V) was used for ionization, and product ion spectra of precursor *m/z* 284 were scanned from *m/z* 50 to 450.
NMR spectra were recorded using Varian Mercury Plus 300 spectrometer running at 300 MHz for proton and at 75 MHz for carbon measurements.

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Results and Discussion

Chromatographic purity and UV-Vis spectrum of the synthesized bisoprolol metabolite was determined using liquid chromatography. In the applied chromatographic conditions the substance eluted at 2.53 min, and the UV-Vis absorption spectrum showed an absorbance maximum at 224 nm as depicted in Figure 3. The spectrum was in good agreement with the earlier results on bisoprolol [8]. Chromatographic purity of the substance using wavelength 224 nm for detection was found to be 94.2%.

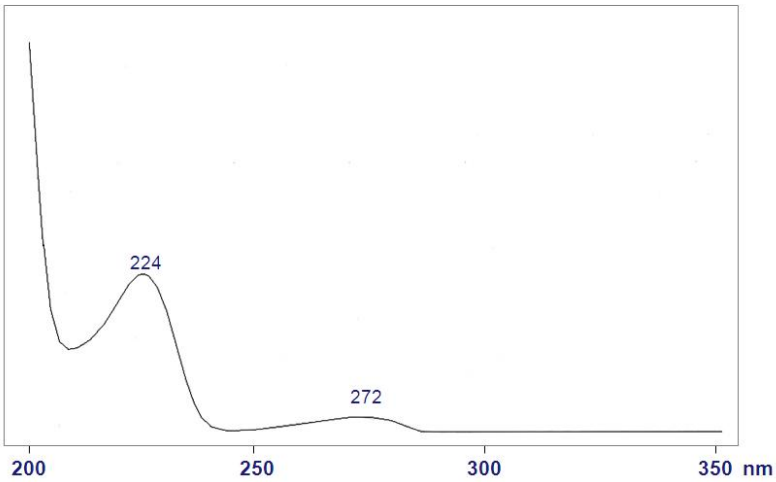

Figure 3. VU-VIS spectrum of the synthesized *O*-desisopropyl bisoprolol

The molecular weight of the synthetized substance was verified by high resolution and accurate mass measurents using TOFMS. The observed accurate mass for the protonated molecule ion was *m/z* 284.1842, with an acceptable mass error (-2.0 mDa) to the theoretical value.
In the MS/MS structural characterization ions *m/z* 266, 242, 207, 181, 145, 133, 116, 98, and 74 were formed as fragments of $[M+H]^+$ 284. Fragment *m/z* 266 and 242 indicate losses of water (-18 amu) and propene (-42 amu), respectively, whereas fragment *m/z* 116 has been earlier reported as a characteristic ion for terminal isopropyl group, and loss of 77 amu from $[M+H]^+$ (i.e. *m/z* 207) to be a common fragment of β-blockers and indicating the subsequent neutral losses of water and isopropylamine [9]. Fragment *m/z* 133 is a common ion with the spectrum of parent bisoprolol, but all the other main fragments (*m/z* 181, 145, 98 and 74) are different and therefore, proposed to involve the modified part of the molecule.

Following characteristic NMR signals were observed.
Spectral data:
^{1}H NMR (D_6-DMSO) 7.23 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.93 (bs, 1H), 4.59 (bs, 1H), 4.39 (s, 2H), 3.92 (m, 1H), 3.86 (m, 2H), 3.51 (m, 2H), 3.41 (m, 2H), 2.68 (m, 2H), 2.54 (m, 1H), 1,48 (bs, 1H), 0.97 (dd, *J*=6.2,1.0 Hz, 6H)

^{13}C NMR (D_6-DMSO) 158.8, 131.2, 129.8, 114.9, 72.4, 72.0, 71.5, 69.1, 60.9, 50.7, 48.9, 23.7

Conclusions

The *O*-desisopropyl metabolite of bisoprolol was synthesized and analyzed using LC/UV-VIS, ESI-TOFMS, ESI-MS and NMR techniques. The synthesized metabolite will be made available to all the WADA-accredited anti-doping laboratories.

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Acknowledgements

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