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SYNTHESIS AND CERTIFICATION OF SALMETEROL AND SALMETEROL-D_{12} STANDARDS

W. Karpiesiuk[1], A. F. Lehner[2], and T. Tobin[2]

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Salmeterol is a potent long-acting selective β_2-adrenergic agonist. It is widely used as a bronchodilator for maintenance treatment of asthma and reversible airway obstruction. Due to these effects, its administration to racing horses has the potential to improve their performance. As such, a reliable analytical method for salmeterol in equine serum and urine is necessary.

Salmeterol is marketed by Glaxo Wellcome (England) as its xinafoate (hydroxy-naphthoate) salt. Salmeterol or salmeterol xinafoate are not available as certified reference standards from official or equivalent sources; we therefore initiated the in-house synthesis and certification of salmeterol and its deuterated analog – salmeterol-d_{12}.

These syntheses are analogous, starting from commercially available substrates and consisting of six synthetic steps. The key step in the synthesis is the coupling of a derivative of α-bromoacetophenone to a long chain benzylamine. Final reduction and deprotection steps yield salmeterol and/or salmeterol-d_{12}. The obtained salmeterol and salmeterol-d_{12} are then transformed into their xinafoate salts.

Both products are now undergoing purity certification, such that they can be used as primary standards in analytical methods for salmeterol. The process consists of rigorous characterization of each product to ensure its identity and purity, and will include two general steps: 1) confirming the structure of each standard, and 2) establishing the purity of each standard. Verification of the structures of salmeterol xinafoate and salmeterol-d_{12} xinafoate are made by ^{1}H-NMR, ^{13}C-NMR, MS, elemental analysis and IR. Data for establishing of the purity of both compounds are obtained using gas chromatography (GC), high performance liquid chromatography (HPLC), and thin layer chromatography.

The synthesis of salmeterol xinafoate and salmeterol-d_{12} xinafoate, as well as the certification process involving both compounds will be presented.

This project was supported by grants from the Kentucky Racing Commission and the Kentucky Equine Drug Research Council.

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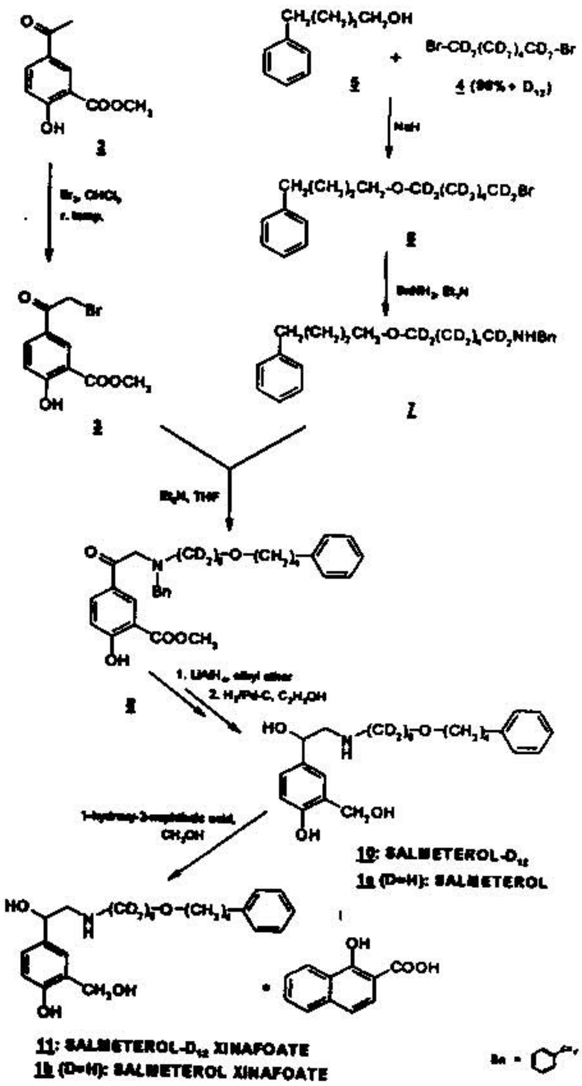
• EXPERIMENTAL APPROACH – SYNTHESIS

The chemical syntheses of salmeterol and salmeterol-d_{12} were performed analogously and consisted of three general steps. First, the preparation of the proper bishydroxymethylphenol reagent 3; second, the preparation of 6-(4-phenyl-butoxy)hexylamine 7; and third, a coupling of these two units. The first step was easily achieved after direct bromination of methyl 5-acetylsalicylate (2) with bromine in chloroform (scheme). The preparation of the deuterated amine 7 was carried out in two steps. First, 1,6-dibromohexane-d_{12} (4) was reacted with 4-phenylbutanol (5) in the presence of sodium hydride and catalytical amounts of sodium iodide and tetrabutylammonium bromide in THF, which led to bromoether 6 with moderate yield. Bromoether 6 was next transformed to the corresponding amine 7 by reaction with benzylamine. After that followed the key step of the synthesis - the coupling of the α-bromoacetophenone derivative 3 with the long chain benzylamine 7 (Goodwin 2000). Reduction of both carbonyl and carboxylic groups in ketoester 8 with lithium aluminum hydride, followed by catalytic hydrogenolysis of the protective benzyl group, gave with good yield deuterated salmeterol-d_{12} (10) (or salmeterol 1a in an analogously provided synthesis using 1,6-dibromohexane instead of its deuterated analog). Next, salmeterol and salmeterol-d where transformed into their xinafoate salts after reaction with 1-hydroxy-2-naphthoic acid in methanol.



10: SALMETEROL-D_{12}
1a (D=H): SALMETEROL

11: SALMETEROL-D_{12} XINAFOATE
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• RESULTS

Both products have had to undergo a process of certification, so they can be used as primary standards for our development of an analytical method for salmeterol. This process consisted of a thorough characterization to ensure its identity and purity, and can be divided into two general processes: 1) confirmation of the proper structure, and 2) establishing of the purity of the standards. The following characterization information was completed for salmeterol xinafoate:

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Mass Spectrometry – the following spectra were recorded: EI-MS, ESI(+)-MS and ESI(-)-MS, as well as EI-MS of N-TMS-tris-O-TMS and tris-O-TMS derivatives of salmeterol. All spectra are consistent with the published data (ESI(+)-MS – McInally 2002, TMS-derivatives - Damasceno 2000).

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REFERENCES

Goodwin, T. E.; Zhou, Z.; [illegible]; Braun, P.; Andersen, R. J.; Miller, C. P.; [illegible], C. M.: Journal of Labelled Compounds and Radiopharmaceuticals 43 (2000), [illegible].

Breen R.A., Ley S.V., [illegible] S., and [illegible] P.A. Journal of the Chemical Society, Perkin Transactions I (2002), [illegible].

Damasceno L., Ventura R., Ortuno J., and Segura J.: Journal of Mass Spectrometry 35, 11 (2000), [illegible].

McInally T.J., [illegible] S.R., Journal of Labelled Compounds and Radiopharmaceuticals 45, (2002) [illegible].

Tong H.H.Y, [illegible] B.Y., York P., Chow A.H.L., Pharmaceutical Research 18, 6 (2001), [illegible].

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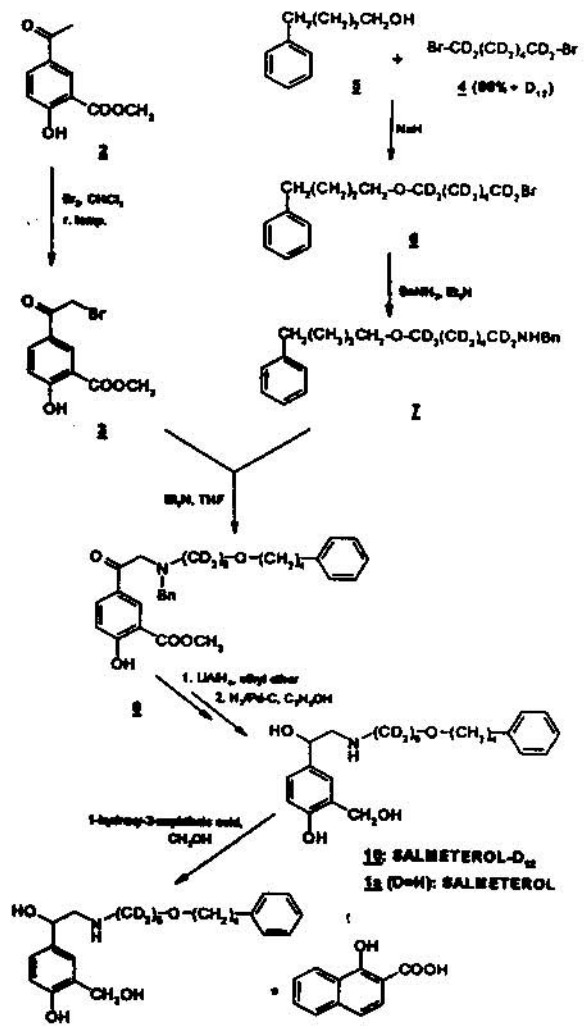

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REFERENCES

Goodwin, T. M.; Shaw, A.; Wells, P. A.; Brown, P.; Anderson, P. J.; Miller, C. P.; Concepción, C. M.; *Journal of Labelled Compounds and Radiopharmaceuticals* 43 (2000), 65-76.

Brown S.H., Lay S.V., McDermott S., and Monaghan P.A. *Journal of the Chemical Society, Perkin Transactions 1* (2002), 2237-42.

Damasceno L., Montero R., Ortuno J., and Segura J.; *Journal of Mass Spectrometry* 35, 11 (2000), 1295-94.

Hellwich T.F., Stanley S.D., *Journal of Labelled Compounds and Radiopharmaceuticals* 45, (2002) 703-712.

Tong G.M.Y, Steinmetz S.Y., York P., Chow A.H.L, *Pharmaceutical Research* 18, 6 (2001), 853-859.

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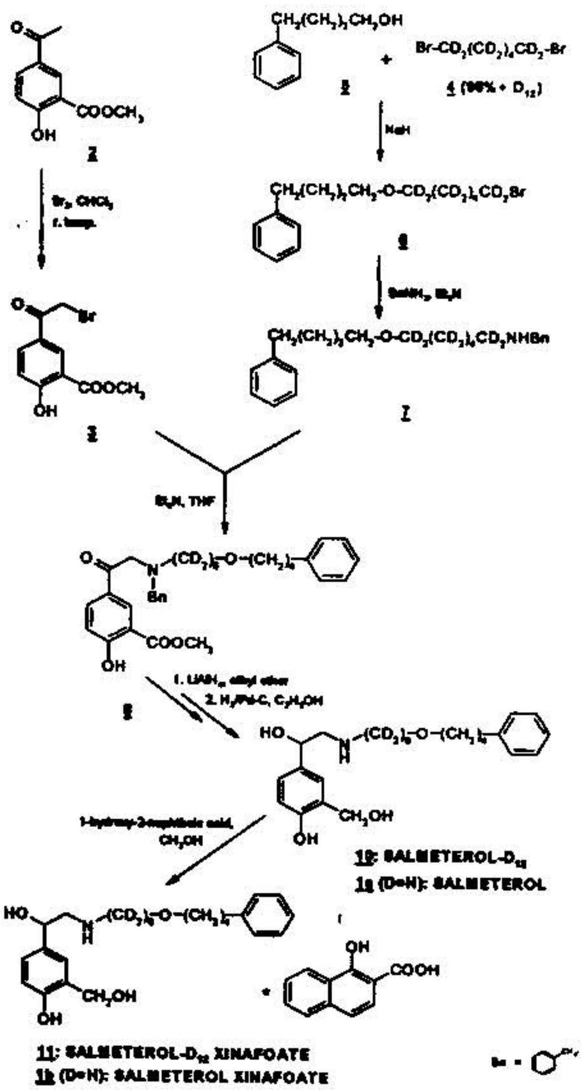

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Broom N.J.P., [illegible], and [illegible] P.A. *Journal of the Chemical Society, Perkin Transactions I* (2002), [illegible].

Damasceno L., Ventura R., Ortuño J., and Segura J.; *Journal of Mass Spectrometry* 35, 11 (2000), [illegible].

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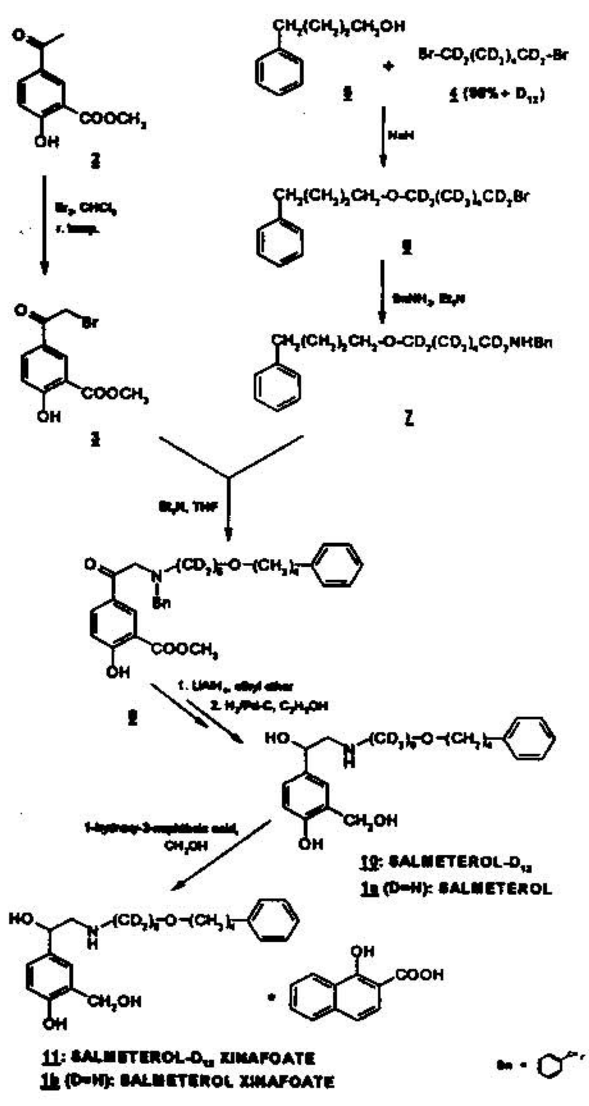

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1. Information to substantiate the proof of the chemical structure:

^{1}H-NMR – the spectrum was recorded in CD_3OD on 400 MHz and is consistent with the published data (Bream 2002): δ (p.p.m.) 1.3-1.4 (m, 4H), 1.46-1.75 (m, 8H), 2.58 (t, 2H), 2.86 (t, 2 H), 3.02-3.14 (m, 2H), 3.34 (t, 2H), 3.37 (t, 2H), 4.66 (s, 2H), 4.78 (d, 1 H), 7.10-7.28 (m, 7H), 7.36 (d, 1H), 7.38-7.44 (m, 1 H), 7.45-7.50 (m, 1H), 7.71 (broad d, 1H), 7.87 (d, 1H), 8.28 (broad d, 1 H).

^{13}C-NMR – recorded in CD_3OD on 100 MHz, the spectrum is consistent with the anticipated peak occurrence: δ (p.p.m.) 26.91, 27.16, 27.63, 29.36, 30.40, 30.63, 36.78, 55.20, 60.97, 70.22, 71.73, 71.93, 113.03, 116.14, 117.83, 124.61, 125.71, 126.72, 126.86, 127.16, 127.19, 127.96, 128.69, 129.75, 129.88, 129.42, 129.54, 133.02, 138.09, 143.82, 155.46, 161.16, 177.60.

Mass Spectrometry – the following spectra were recorded: EI-MS, ESI(+)-MS and ESI(-)-MS, as well as EI-MS of N-TMS-tris-O-TMS and tris-O-TMS derivatives of salmeterol. All spectra are consistent with the published data (ESI(+)-MS – Modnski 2002, TMS-derivatives - Damasceno 2000).

HR-MS – high resolution mass determination for the molecular ion [MH$^+$]: ESI 416.2798, calculated for $C_{25}H_{38}NO_4$ [MH$^+$] 416.2796.

Elemental Analysis – molecular formula for salmeterol xinafoate is $C_{36}H_{45}NO_7$ what corresponds to C 71.62%, H 7.51%, N 2.32 %. Obtained values are C 71.49%, H 7.57%, N 2.40%.

2. Data Establishing Purity:

TLC – thin layer chromatography was performed on silica gel with UV indicator, as eluent was used chloroform-methanol (4:1). One spot with Rf 0.45 was observed both under the UV lamp and after development in iodine.

Elemental Analysis – excellent elemental analysis with differences on carbon 0.13%, hydrogen 0.06%, and nitrogen 0.08% indicates high purity of the sample.

Melting point – salmeterol xinafoate was crystallized from ethyl acetate and its melting point was determined as 123-124 °C. Salmeterol xinafoate is known to exist in two crystalline polymorphic forms with melting points 122.7 °C and 137.6 °C. The more stable form is the first one with m.p. 122.7 °C (Tong 2001).

GC – as GC/MS. After derivatization two peaks of tris-O-TMS and N-TMS-tris-O-TMS derivatives of salmeterol were observed. The proportion of the tris-O-TMS peak to the peak of N-TMS-tris-O-TMS salmeterol depends on the derivatization conditions. The estimated purity of salmeterol xinafoate in this method was established as 99.5 %.

Establishing of water, moisture or residual solvents – a sample of 50 mg of salmeterol xinafoate was kept 72 hours in high vacuum over P_2O_5. No difference in mass was observed (<0.1 mg).

HPLC – one peak was registered on reverse phase column chromatography, equipped with UV detector. As eluent water-methanol was used. The purity of salmeterol xinafoate was established as 99% plus.

REFERENCES

[illegible], T. [illegible]; Zhou, X.; [illegible], P. A.; [illegible], R.; Anderson, P. J.; [illegible], C. P.; [illegible], C. M.; Journal of Labelled Compounds and Radiopharmaceuticals [illegible] ([illegible]), [illegible].

Bream R.N., Ley S.V., [illegible] B., and [illegible] P.A. Journal of the Chemical Society, Perkin Transactions I (2002), [illegible]-42.

Damasceno L., Ventura R., Ortuno J., and Segura J.; Journal of Mass Spectrometry 35, 11 (2000), 1285-94.

[illegible] T.F., [illegible] S.R., Journal of Labelled Compounds and Radiopharmaceuticals 45, (2002) [illegible].

Tong H.H.Y., [illegible] B.Y., York P., Chow A.H.L., Pharmaceutical Research 18, 6 (2001), [illegible].

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