Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (11)

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S.W. WESTWOOD, S.R. DAVIES, G.J. TARRANT:
Preparation of Certified Reference Materials for Use in Doping Analysis for Steroid Prohormones and 19-Nor Steroids
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Preparation of Certified Reference Materials for use in doping analysis for steroid prohormones and 19-nor steroids

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Introduction

The NARL Pure Substance Reference Materials Team (PSRM) provides a range of steroid Reference Materials for sports drug testing. Previous work has focused on both endogenous steroids, including phase I and phase II metabolites, and exogenous steroids.[1,2]

This poster details the progress to date of two related pure substance Certified Reference Material (CRM) production projects which we have recently commenced.

Prohormone metabolite CRMs

The major project, funded by the World Anti-Doping Agency, aims to prepare materials for use in the detection of doping with steroid prohormones and 19-nor steroids.

In the initial phase of this project a questionnaire was distributed to the heads of IOC-accredited doping laboratories requesting their input to assist us to assign CRM priorities in this area. The candidate materials identified as a result of the consultation process and from our own literature review are detailed in table 1.

The second phase has been to commence "in-house" synthesis and call for tenders for an outsourcing component. The tenders for the outsourcing component have been awarded to three companies with demonstrated capabilities in steroid synthesis.

It is anticipated that the production of all candidate CRMs will be complete by the end of 2003 and the charaterisation completed three months thereafter. Once complete the CRMs will be available to approved laboratories.

Acknowledgement: This project has been carried out with the support of WADA.

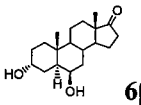
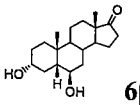
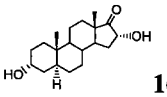
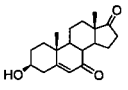
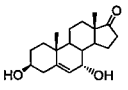
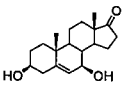
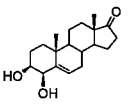
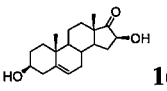
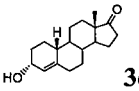
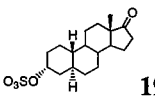
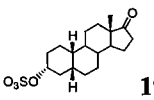
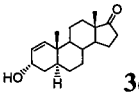
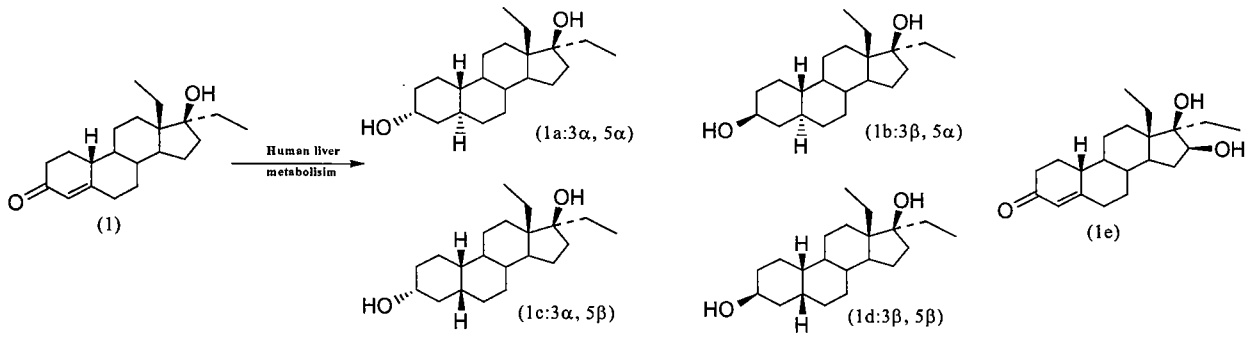
Steroid	Reference Material
Androstendione	 **6β -Hydroxyandrosterone**
	 **6β-Hydroxyetiocholanolone**
	 **16α-Hydroxyandrosterone**
Dehydroepiandrosterone (DHEA)	 **7-Keto DHEA**
	 **7α-Hydroxy DHEA**
	 **7β-Hydroxy DHEA**
	 **4β-Hydroxy DHEA**
	 **16β-Hydroxy DHEA**
19-Norsteroid (norandrostendione & "prohormones")	 **3α-Hydroxy-4-estren-17-one**
	 **19-Norandrosterone sulphate**
	 **19-Noretiocholanolone sulphate**
Testetherone & Equibolan	 **3α-Hydroxy-1-androsten-17-one**

Table 1: Candidate materials identified as a result of consultation with IOC laboratories

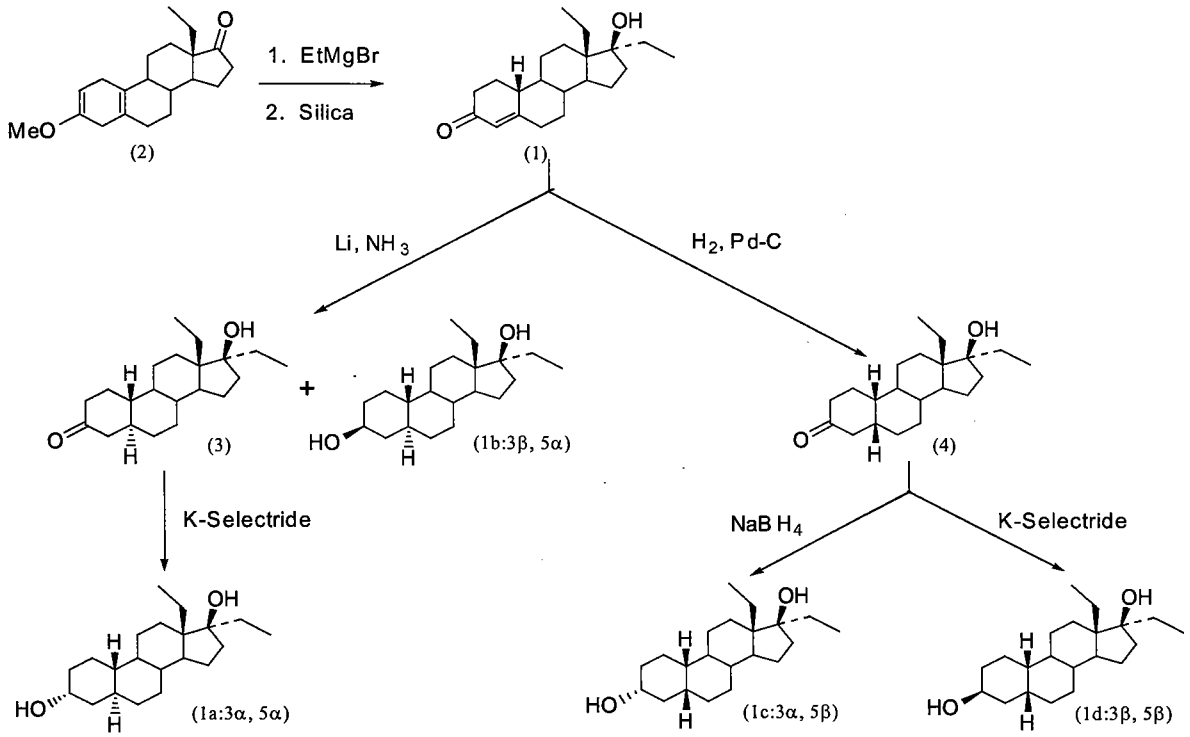
Norbolethone metabolite CRMs

Our second project, funded by the US Anti-Doping Agency, is to prepare authentic standards of possible phase I metabolites identified by Catlin *et al* as arising from doping with norbolethone (**1**).[3] Present evidence suggests that the metabolites of interest arise from tetrahydro reduction of the steroid A ring and oxidation of the D ring at C16 (Scheme 1).



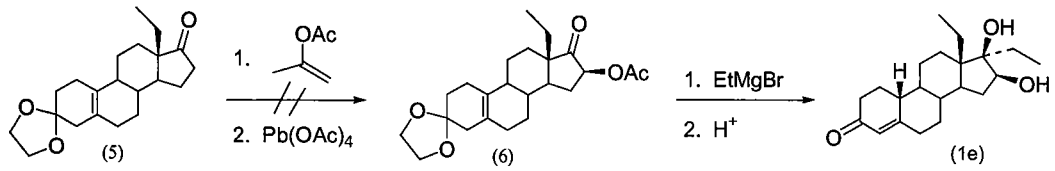
Scheme 1: Possible metabolites of norbolethone (1)

With the desired chemistry already well established on homologous compounds it proved to be a relatively facile process to produce all four possible tetrahydro metabolites (1a, b, c, d).[4, 5] Scheme 2 details the synthetic strategy used by NARL-PSRM to achieve this goal. Precursor (2) was converted to norbolethone (1) on a multigram scale. All four metabolites were prepared selectively, and used to confirm that metabolites 1a (3α-OH, 5α) and 1c (3α-OH, 5β) were those observed in human urine. A scaled up synthesis of 1a and 1c has now been completed providing 1a in > 99% purity and 1b in 97% purity (contaminated with 3% of the 3β-OH, 5α (1b) isomer).



Scheme 2: Synthetic strategy towards tetrahydo metabolites of norbolethone (1)

Attempts to prepare 16β-hydroxy-norbolethone (1e), using an established protocol for the introduction of a β–hydroxy at C16 (Scheme 3) have been unsuccessful due to the instability of the protected enone in the A ring under the reaction conditions.[5]



Scheme 3: Synthetic strategy towards the 16β-hydroxy metabolite (1e) of norbolethone

Acknowledgement: This project has been carried out with the support of the US Anti-Doping Agency.

References

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2. Steven Westwood, David Hancock, Christie Moule, Bruce Noble and Scott Starling; Progress in the Preparation of Anabolic Steroid Reference Materials and Certified Reference Materials, *Proceedings of the 18th Cologne Workshop on Dope Analysis*, Sport und Buch Strauß Köln, (2001), 109-118.

3. Don H. Catlin, Brian D. Ahrens and Yulia Kucherova; Detection of norbolethone, an anabolic steroid never marketed, in athletes' urine, *Rapid Commun. Mass Spectrom.* 2002, **16**, 1273-1275.

4. C.J.W. Brooks, A.R. Thawley, P. Rocher, B.S. Middleditch, G.M. Anthony and W.G. Stillwell; Characterisation of Steroidal Drug Metabolites by Combined GC-MS. *J. Chrom. Sci.*, 1971, **9**, 35.

5. Andrew R. McKinney and Damon D. Ridley; Synthesis of equine metabolites of norethandrolone: 19-nor-17-pregnane-3, 17-diols, 19-nor-17-pregnane-3, 16, 17-triols and 19-nor-17-pregnane-3, 17, 20-triols. Submitted for publication in *Aust J. Chem.*, 2003, **56**, 000-000.