A MAJOR IMPURITY IN ^{3}H–17α–HYDROXYPREGNENOLONE

Martin I. Shapiro and Bhagu R. Bhavnani*
Department of Pediatrics, University of Montreal, Montreal, Canada.
Received on October 23, 1974.

SUMMARY

Evidence of a major impurity in commercially available ^{3}H-17α-hydroxypregnenolone is presented.

INTRODUCTION

17α-Hydroxypregnenolone (17αOH-Preg) is an important intermediate in steroidogenesis. Its labelled form (^{3}H-17αOH-Preg, commercially available) has been widely employed in many investigations.

Demonstration of the presence of a major impurity in commercially available ^{3}H-17αOH-Preg is the subject of this report.

MATERIALS

Commercial samples of 17αOH-Preg and 17α-hydroxypregnenolone-3-acetate (17αOH-Preg Ac) purchased from Sigma Chemical Co. (St. Louis, Mo.) were crystallized before use. Infrared spectra were identical with those of authentic material.

Acetone (chromatoquality), benzene (spectrophotometric), acetic anhydride and pyridine were obtained from Canlab (Montreal, P.Q.).

Acetic anhydride and pyridine were refluxed with calcium carbide and barium oxide respectively and distilled.

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* Bhagu R. Bhavnani, Department of Experimental Medicine, McGill University, Montreal, Canada.

Reprint requests: M.I.S., Laboratoire d'Endocrinologie, Hôpital Sainte-Justine, 3175 Chemin Sainte-Catherine, Montréal, P.Q., CANADA, H3T 1C5.

Ethanol (U.S. Industrial Chemicals Co., New York, N.Y.) was used directly. All other solvents were either distilled or, more recently, purchased redistilled (A. & C. American Chemicals, Montreal, P.Q.).

Thin layer plates (Silica gel F-254, 0.25 mm thickness, E. Merck AG, Germany) were obtained from Brinkmann Instruments Ltd. (Toronto, Ont.). All chemicals used were of reagent grade.

7-^{3}H-17α-Hydroxypregnenolone was purchased from New England Nuclear Corporation, Boston, Mass. (Lots 531-91,-78,-148 and 636-101) and Amersham/Searle Corporation, Don Mills, Ont. (Batch 4). Attempts to establish purity by isotope dilution is the subject of this report.

METHODS

Radioactivity measurement and determinations of infrared spectra have been previously described (1). Counting efficiencies for ^{3}H were from 26-33%.

Solvent systems 1. chloroform:diethylether (75:25), 2. benzene:methanol (80:20) and 3. chloroform:methanol (95:5) were used for thin layer chromatography (TLC). Steroid zones on the chromatograms were detected using phosphomolybdic acid (10% w/v absolute ethanol). Radioactivity was located by scanning on Packard Radiochromatogram Scanner Models 7200 and 7201 (Packard Instrument Company Inc., Downers Grove, Ill.).

Acetates were prepared by standard procedures (2 volumes of pyridine per volume of acetic anhydride).

^{3}H-17α-Hydroxypregnenolone was diluted in benzene:ethanol (9:1) within two weeks of receipt. To analyze for purity a suitable aliquot of the diluted ^{3}H-steroid was mixed in all but one case with μg quantities of unlabelled 17αOH-Preg, acetylated, mixed with mg quantities of 17αOH-Preg Ac and crystallized. The specific activities (SA) of the crystals (XL) and unfractionated mother liquors (ML) were determined. In Experiment 2, ^{3}H-17αOH-Preg was diluted initially with 49.4 mg of unlabelled 17αOH-Preg, acetylated and crystallized.

(For further details see foot-notes to Tables.) In Experiment 5, ^{3}H-17αOH-Preg was 'purified' sequentially on TLC systems 1, 2 and 3. Following system 3, radioactivity corresponding to standard 17αOH-Preg was eluted, diluted in benzene: ethanol (9:1) and an aliquot analyzed for purity by isotope dilution. (See foot-note to Experiment 5.)

RESULTS AND DISCUSSION

The extent of radiochemical impurity of ^{3}H-17αOH-Preg can be seen in Tables I and II. Four different lots of labelled steroid purchased over a two year period were analyzed. Since one normally accepts an impurity of 5% or less, all lots (Experiments 1-6, Tables I & II) are decidedly unacceptable. For example, in Experiment 1 specific activities of crystals and mother liquors differ considerably following two crystallizations. Impurity exceeds 40% and the SA of the second XL differs from the calculated value by more than 50%. This trend is consistent throughout (Experiments 1-6) - elevated SA of ML and large deviations from expected or calculated SA indicating the presence of a major impurity.

In Experiment 5, ^{3}H-17αOH-Preg was 'purified' sequentially on TLC systems 1, 2 and 3. The ^{3}H-17αOH-Preg was applied to each TLC in two portions (90% & 10%). After development and location of standard material, the 10% column was divided into 1 cm sections, placed in liquid scintillation vials and mixed with scintillation fluid prior to counting. Throughout the TLC procedures radioactivity exhibited a polarity identical to standard unlabelled 17αOH-Preg. Following system 3, radioactivity corresponding to standard 17αOH-Preg was eluted, diluted in benzene:ethanol (9:1) and an aliquot analyzed for purity by isotope dilution. The presence of a large impurity is evident (Table II, Experiment 5). Chromatographic evidence of purity, using those systems described, is thus inadequate.

Table III demonstrates that pure ^{3}H-17αOH-Preg is occasionally available commercially. Batch 4 analyzed in the manner described (foot-note, Table III)

T A B L E I. Proof of Radiochemical Impurity of 7-^{3}H-17α-Hydroxypregnenolone

	Specific Activity dpm/mg					
	Experiment 1* Lot 531-91		Experiment 2** Lot 531-91		Experiment 3*** Lot 531-78	
Crystallization	XL	ML	XL	ML	XL	ML
1	1,744	9,464	1,466	11,597	2,116	12,899
2	1,355	5,060			1,944	3,082
Calculated	3,010		3,490		3,932	

* ^{3}H-17αOH-Preg (172,415 dpm) was diluted with 668 μg of unlabelled steroid, acetylated, mixed with 57.3 mg of 17αOH-Preg Ac and crystallized.

** ^{3}H-17αOH-Preg (172,415 dpm) was diluted with 49.4 mg of unlabelled steroid, acetylated and crystallized.

*** ^{3}H-17αOH-Preg (205,604 dpm) was diluted with 593 μg of unlabelled steroid, acetylated, mixed with 52.3 mg of 17αOH-Preg Ac and crystallized.

TABLE II. Proof of Radiochemical Impurity of 7-^{3}H-17α-Hydroxypregnenolone

	Specific Activity dpm/mg					
	Experiment 4* Lot 531-148		Experiment 5** Lot 531-148 'Purified'		Experiment 6*** Lot 636-101	
Crystallization	XL	ML	XL	ML	XL	ML
1	2,162	13,877	1,517	8,536	2,965	16,779
Calculated	4,790		3,246		5,035	

* ^{3}H-17αOH-Preg (213,744 dpm) was diluted with 307 µg of unlabelled steroid, acetylated, mixed with 45.3 mg of 17αOH-Preg Ac and crystallized.

** Chromatographically 'Purified' (see Text) ^{3}H-17αOH-Preg (151,956 dpm) was diluted with 275 µg of unlabelled steroid, acetylated, mixed with 46.8 mg of 17αOH-Preg Ac and crystallized.

*** ^{3}H-17αOH-Preg (173,740 dpm) was diluted with 443 µg of unlabelled steroid, acetylated, mixed with 34.5 mg of 17αOH-Preg Ac and crystallized.

TABLE III. Proof of Radiochemical Purity of 7α-^{3}H-17α-Hydroxypregnenolone

	Specific Activity dpm/mg	
	Experiment 7* Batch 4	
Crystallization	XL	ML
1	9,771	11,210
2	9,991	10,192
Calculated	9,863	

* ^{3}H-17αOH-Preg (340,274 dpm) was diluted with 539 μg of unlabelled steroid, acetylated, mixed with 34.5 mg of 17αOH-Preg Ac and crystallized.

exhibits constancy at the second XL stage - i.e., the SA of XL 1 and 2 and ML 2 are identical and in agreement with the theoretical value. The calculated percent impurity being 1.3%, this material is suitable for experimental use.

In conclusion it is suggested that investigators verify the purity of commercial radioactive 17αOH-Preg by means similar to those described as chromatographic evidence of purity could be inadequate.

ACKNOWLEDGEMENTS

M.I.S. and B.R.B. are Medical Research Council of Canada Scholars. This study was supported by a grant from the Medical Research Council of Canada (MA-4039).

REFERENCE

1. Shapiro, M.I., STEROIDS 20, 1 (1972).