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Identification of 5α-Androst-1-ene-3*β*,17*β*-diol in the Fat of *Sus scrofa* L.: A "Nutritional Supplement" Not Found Previously in the Food Supply

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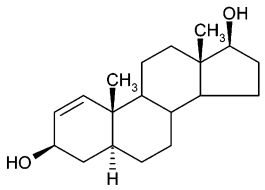
Abstract: 5α-Androst-1-ene-3*β*,17*β*-diol (**1**) was detected in extracts from fat of *Sus scrofa* L. (pig) by comparison with the commercially available synthetic compound, using gas chromatography−mass spectrometry. This observation is unprecedented because **1** is currently sold as a nutritional supplement, yet has not been previously reported as naturally occurring in the food supply.

Numerous hormonal compounds have been made available as over-the-counter nutritional supplements in the United States as a result of the Dietary Supplement Health and Education Act (DSHEA) of 1994. Although many hormonal supplements (e.g., androst-5-en-3*β*-ol-17-one (DHEA) and melatonin, in particular)[1,2] have been well-documented with regard to source, safety, and potential benefit, some other compounds have not. An esoteric member of this category, 5α-androst-1-ene-3*β*,17*β*-diol (**1**), has not been demonstrated to be present in the food supply and may, therefore, not be considered a nutritional supplement.

Recently, similarly unique members of the estren series of steroids (e.g., 17*β*-hydroxy-19-nor-androst-4-en-3-one; nortestosterone) have been identified in edible porcine tissues[3] and could be a source of positive athletic doping tests.[4] As with **1**, the estren steroids were previously not thought to be naturally occurring. This indicated that **1** may, indeed, be a naturally occurring compound and might be identified in porcine tissues. Fat has previously been shown to exhibit greater steroid concentrations than other tissues;[5] therefore, we hypothesized that **1** may be present in porcine fat in detectable amounts using GC−MS.



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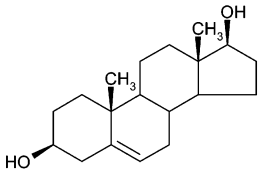

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A standard preparation of **1** was extracted from a commercially available product.[6] The TMS derivative of the extracted **1** was generated and analyzed using a Varian 2100 ion trap GC−MS (Walnut Creek, CA). Chromatography was performed on a Rtx-5ms, 30 m × 0.25 mm column (Restek, Bellefonte, PA) programmed from 120 to 300 °C. Mass spectral analysis was performed in full-scan mode generating the following spectrum: EIMS *m*/*z* 436 (10), 434 (70, M^+), 419 (15), 405 (100), 377 (16), 347 (21), 329 (10), 319 (12), 291 (11), 272 (32), 257 (42), 254 (11), 239 (11), 202 (22), 195 (21), 187 (25), 182 (12), 169 (16), 162 (10), 159 (18), 149 (16), 147 (12), 145 (21), 143 (97). Due to the complex nature of the matrix in the biological samples, it was anticipated that unit selected ion storage (uSIS) would eliminate matrix interference and facilitate identification of the analyte, if present. Therefore, uSIS was used on the abundant high-mass ions *m*/*z* 434 and 405 for analysis of biological samples. To determine whether **1** is indeed present in the food supply, fat[7] from *Sus scrofa* L. (pig) was extracted according to previous reports[8] to remove fatty acids and other interfering material. The TMS derivatives of the resulting steroid fraction were generated using BSTFA + 1% TMCS prior to GC−MS analysis in full-scan and uSIS modes.

In full-scan mode, a peak was identified with a spectrum fit of greater than 80% and a retention time matching the standard **1** obtained from extracts of the over-the-counter supplement. Using the uSIS mode on *m*/*z* 405 and 434, peaks were identified in all samples with retention times corresponding to the reference standard, clearly indicating the presence of **1** in this tissue. Quantitatively, it was

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determined that 0.03 and 0.46 μg/kg of **1** were obtained from kidney and flank fat, respectively. The concentrations of **1** determined in the current study are approximately 100-fold lower than those reported for the estren series compounds.[4] International sporting bodies have banned **1**; therefore, it remains to be determined if levels of **1** identified in the current study would be sufficient to elicit a positive doping test following the ingestion of these tissues, as demonstrated for estren compounds.[3]

The metabolism of exogenously administered 1-ene steroid isomers has been studied previously in humans,[9,10] but there has been only one previous report, specifically, of **1** in the literature.[11] In this study, Delbeke et al. examined urinary excretion of steroid metabolites following administration of the same commercial product utilized for reference in this study. Despite the commercial availability of **1** as a nutritional supplement, there have been no reports of its natural occurrence in the food supply. Additionally, there have been no reports of metabolic pathways that would result in the formation of this compound under normal physiological conditions. It was, therefore, surprising to find this compound as a naturally occurring substance in pig fat commonly available as a food.

The source of **1** prior to its accumulation in pig fat has yet to be determined. This compound is analogous to the naturally occurring androst-5-ene-3β,17β-diol (**2**), an abundant metabolite of DHEA in many organisms. Therefore, it may be that an unidentified metabolic pathway exists for the generation of 1-ene metabolites of endogenous steroids in the pig. An alternative hypothesis derives from reports of 1-ene intermediates in bacterial and fungal steroid metabolism.[12,13] Accordingly, it is possible that a component of the pig diet contains **1**, or this compound is produced by normal flora of the gut during the digestive process and subsequently accumulates in tissues. Further work will be required to ascertain the verity of these hypotheses.

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(6) Ergo Pharm, Seymour, IL 61875; Lot# DC31002EX05. To separate steroids from the cellulose filler, three times 10 mL of H_2O was added to powder contents of each capsule in a centrifuge tube, vortexed, and centrifuged at 2500 rpm, and the supernatant was discarded. To the final pellet, 1.0 mL of H_2O was added and mixed well. This solution was extracted six times with 2.0 mL of ether. Following centrifugation, each ether layer was pipetted into a tared test tube and the ether was evaporated in a 45 °C water bath under nitrogen stream. Each ether extract yielded 5.99 ± 1.26 mg for a total of 35.92 mg of steroid from a 100 mg capsule. Further extractions would likely have yielded more steroid; therefore this yield is not indicative of the total content of each capsule.
(7) On two occasions, kidney and flank fat were obtained from the Southeast Michigan meat processing plant of a major food retailer. Tissues were collected at the time of slaughter and immediately placed on dry ice.
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