Formation of testosterone acetate by *Saccharomyces fragilis*

We recently reported that various steroids have an antibiotic effect on the yeast *S. fragilis* during growth[1]. It was further observed that androst-4-en-3,17-dione, such an inhibitor of yeast growth, was converted by intact yeast cells to a less polar compound. In the course of this work, a mutant resistant to steroids was isolated and also found to be capable of performing this transformation. Evidence is presented in this communication that this non-polar steroid is testosterone acetate.

Wild type *S. fragilis* (ATCC 10022) was grown on glucose–salts medium for 48 h at room temperature in the presence of $4.55 \cdot 10^{-4}$ *M* androst-4-en-3,17-dione, as described[1]. The cells were collected by centrifugation, washed 3 times with water, and extracted twice with 80% ethanol for 5 min at 50°. Both ethanolic extracts were combined, concentrated by evaporation and chromatographed on Whatman paper No. 1 in a modification of a solvent system devised by BUSH[2], consisting of cyclohexane–methanol–water (10:10:1). This revealed 3 u.v.-quenching areas with the mobilities of testosterone, androst-4-en-3,17-dione, and testosterone acetate. When androst-4-en-3,17-dione was omitted from the growth medium, none of these three spots was seen.

To establish further the relationship of the added steroid to the two new spots, [4-^{14}C]androst-4-en-3,17-dione, $1.54 \cdot 10^7$ counts/min, was added with unlabeled carrier so that the final concentration of steroid in glucose–salts medium was $1.4 \cdot 10^{-5}$ *M*. At this concentration of steroid, the growth of wild type and mutant were substantially the same. Cultures of wild type and steroid-resistant mutant were harvested at 50 h and extracted as before. The extracts were chromatographed and the radioactivity located by means of a Forro windowless, gas-flow, strip counter and a Nuclear ratemeter model 1614B coupled to an Esterlene-Angus recording ammeter. Radioactivity was found in three areas corresponding to the added steroid, testosterone, and testosterone acetate, indicating that the latter two were metabolites of the added steroid rather than compounds somehow elicited by it.

Although the mobility of the new non-polar compound corresponded exactly to that of testosterone acetate, it migrated only slightly more slowly than did testosterone propionate. The following studies were therefore undertaken to confirm its identity as the acetate.

The compound could be satisfactorily hydrolyzed to a more polar steroid in 24 h in a saturated solution of ethanolic bicarbonate which suggested that it contained an ester linkage. For convenience, it was usually hydrolyzed in 0.5 *N* methanolic KOH at 60° for 30 min. When the radioactive material, tentatively identified as testosterone acetate, was eluted from paper, hydrolyzed and re-chromatographed, there was quantitative conversion to an u.v.-quenching compound with the mobility of testosterone. When this latter substance was eluted from the paper, oxidized with chromic acid[3], and re-chromatographed, the radioactivity and u.v. absorption were now in a location corresponding to androst-4-en-3,17-dione, the expected product of testosterone oxidation. This compound was again eluted and enzymically reduced with microsomal Δ^4-3-ketosteroid reductase (5α) (ref. 4). The radioactive product had the mobility of the predicted compound, 5α-androstane-3,17-dione.

Thus the steroid moiety of the non-polar compound appeared to be testosterone. To identify the acyl moiety, the original material was hydrolyzed as above, acidified

with conc. H_2SO_4, and the volatile products steam distilled. The first 5 ml of distillate were collected, kept alkaline with NH_4OH, concentrated by evaporation and chromatographed in the ascending system of 95 % ethanol and 1 % conc. NH_4OH, described by KENNEDY[5]. The ammonium salt of the steam-volatile material was detected by spraying the chromatogram with bromphenol blue and had the mobility of ammonium acetate.

For further identification of the non-polar steroid, it was again chromatographed on paper together with standard samples of testosterone propionate and testosterone acetate. After development, the three steroids were eluted from the paper, concentrated, and their infrared spectra examined in the solid state by the KBr-pellet method. The spectrum of the unknown corresponded closely with that of the acetate and was clearly different from the propionate spectrum.

It has thus been demonstrated that *Saccharomyces fragilis* can convert androst-4-en-3,17-dione to testosterone acetate. This is a beneficial conversion for the yeast since it is killed by the former steroid while its growth is not affected by the latter[1].

This transformation is also of interest since no steroid-17-esters have previously been isolated from natural sources*.

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* While this report was in preparation, T. E. WEICHSELBAUM AND H. W. MARGRAF[6] presented evidence for a C-21 steroid acetate in human plasma.

X-ray-diffraction analysis of cell walls of nematode-trapping fungi*

The nematode-trapping fungi are a remarkable group of microorganisms capable of capturing living worms by means of specialized hyphal structures formed in response to the presence of their prey[1,2]. Since trapped nematodes struggle violently but seldom escape, the mycelium of these fungi is endowed with great tensile strength. BLANK[3]

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