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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demonstrated that chitin is the major component of cell walls of dermatophytes and fungi causing systemic infections of higher animals, and it was of interest to determine the chemical nature of cell walls of fungi that trap and parasitize nematode worms. Three organisms were employed: *Arthrobotrys conoides*, *Dactylaria thaumasia*, and *Dactylella ellipsospora*. *A. conoides* and *D. thaumasia* trap nematodes in networks of adhesive hyphal loops, whereas *D. ellipsospora* forms adhesive knobs for the capture of prey. The majority of known species of nematode-trapping hyphomycetes are included in the genera tested.

The fungi were cultivated in 4-l Fernbach flasks. Each flask contained 500 ml of a broth with the following % composition: glucose, 3.0; yeast extract, 0.3; $NaNO_3$, 0,366; K_2HPO_4, 0.1; $MgSO_4 \cdot 7H_2,O$ 0.05; KCl, 0.05; and $FeSO_4 \cdot 7H_2O$, 0.001. After inoculation flasks were incubated without agitation for 2 weeks at 28°. The pellicle formed was harvested, washed, and mascerated (Servall Omni-Mixer) in a known volume of distilled water. An aliquot of the resulting suspension was used to determine the dry weight of fungus tissue treated by procedures similar to those employed by FULLER AND BARSHAD[4] for identification of the cell-wall constituents of *Rhizidiomyces* species and briefly described below.

To extract everything but cellulose and chitin, the mycelial fragments were boiled in 4.5 % KOH until little or no cytoplasm was discernible within the cell walls when examined microscopically. The KOH was decanted and the fungus tissue washed with boiling distilled water until neutral. The cell walls were then treated with 2 % acetic acid to remove chitosan and subsequently with boiling water to eliminate the acid. To solubilize and thereby separate cellulose from chitin, the preparation was extracted with Schweitzer's reagent for each of two 12-h periods. The insoluble residue was collected, washed with distilled water until neutral, dried, and X-rayed. The Schweitzer's reagent was neutralized with conc. HCl to precipitate mercerized cellulose.

Samples were prepared for X-ray analysis by grinding the dried material with a mortar and pestle and the fraction that passed through a 325 mesh screen was packed in 0.5-mm diameter glass capillaries and mounted in a 2-radian Debye-Scherrer powder camera. Exposures were made for 3 h with Ni-filtered radiation from a copper-

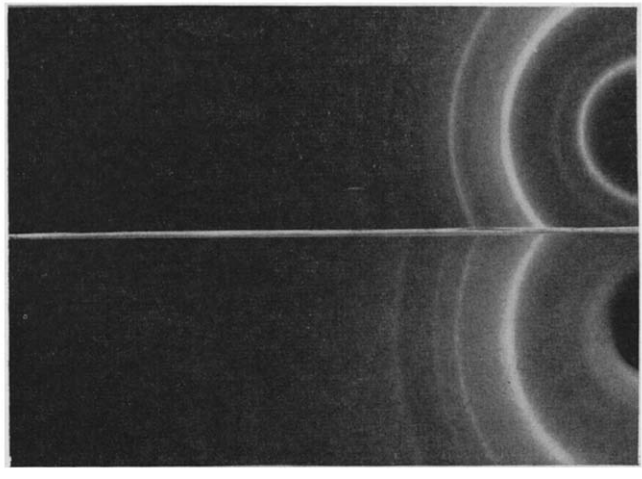

Fig. 1. X-ray diffraction patterns of chitin standard (top), and the cell walls of *A. conoides* (bottom).

target X-ray tube operated at 35 kV and 16 mA. The samples were rotated at a speed of 1.2 rev./min during exposure.

Two or three 1-h extractions in changes of boiling 4.5 % KOH were required to remove cytoplasm and obtain "clean" cell walls. These were insoluble in Schweitzer's reagent and found by X-ray diffraction to be chitin. In no case did neutralization of Schweitzer's reagent cause precipitation of cellulose and no maxima characteristic of cellulose were observed in X-ray-diffraction patterns of cell-wall preparations that were not extracted with Schweitzer's reagent. The X-ray-diffraction maxima for each of the three fungi examined were identical to each other and to a chitin standard prepared by Dr. D. M. REYNOLDS from crab exoskeleton[5]. The most prominent maxima are listed in Table I, and Fig. 1 shows the X-ray-diffraction patterns of the chitin standard and the cell walls of *A. conoides*. The lines at 1.5 and 15 Å are due to a copper–ammonium complex in Schweitzer's reagent.

The data provided unequivocal evidence that a major component of the cell wall of nematode-trapping hyphomycetes is chitin. The chitin content of *A. conoides*, *D. thaumasia*, and *D. ellipsospora* was estimated at 16, 18, and 8 % respectively and was within the range of 2.6 to 26.2 % of dry wt. reported for fungi by BLUMENTHAL AND ROSEMAN[6].

TABLE I

X-RAY DIFFRACTION LINES OF CHITIN

d-Spacing (Å)	*Intensity**
9.5	v.s.
7.0	m.
4.6	v.s.
4.25	w.
4.00	v.w.
3.75	v.w.
3.52	v.w.
3.40	s.
2.77	v.w.
2.58	v.w.
2.30	v.w.

* m, medium; s, strong; v, very; w, weak.

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