the sample reduces the size and heat capacity requirement of the surface from which rapid evaporation takes place.

Reproducibility of sample introduction was determined by comparing the calculated volumes of methyl laurate samples introduced into a chromatographic column with the response of the detector to each sample integrated with respect to time. The quotients obtained, measures of the apparent sensitivities of the detector, were reproducible to less than 10 per cent average deviation with volumes from 4×10^{-6} ml. to $0{\cdot}5 \times 10^{-3}$ ml.

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Physarosterol, a New Sterol from a Slime Mould

During the course of isolating the yellow pigment contained in the myxomycete, *Physarum polycephalum*, a second crystalline (colourless) material was obtained from the neutral ether extracts of the tissue. Analyses showed that this substance was probably a C_{30} sterol not hitherto found in Nature. It is proposed that the compound be referred to as 'physarosterol'.

Physarosterol was isolated by separately homogenizing three different batches of tissue (15–20 gm. each) in an hydraulic homogenizer[1] with three volumes of 0·007 *M* potassium arsenate buffer[2] (*p*H 7·0). The homogenate was acidified with sulphuric acid and a one-third volume of alcohol was added. After addition of filter aid, the slurry was filtered by vacuum. The yellow filtrate was saturated with sodium chloride, filtered, and continuously extracted with ether until no more pigment was carried over into the solvent. Then the ether extract was concentrated to 100 ml. at 60° C., made alkaline with 4 *N* sodium hydroxide solution and again extracted five times with ether (separatory funnel). The aqueous phase which contained the yellow pigment was stored for later processing. The nearly colourless ether solutions were combined and allowed to evaporate slowly at room temperature. Gradually a layer of lipid formed on the top of the solution and crystals began to be deposited on the bottom of the vessel.

When crystal formation had ceased, the lipid and underlying solution were decanted and the crystals removed by filtration. Approximately 25 mgm. of sterol were obtained from 100 gm. of tissue. Recrystallization was effected twice from a minimum volume of warm alcohol–ether (1 : 2). The crystals were dendritic leaves. The following analysis was obtained: calculated for $C_{30}H_{52}O_3$: C, 78·21; H, 11·39. (Calculated for $C_{30}H_{50}O_3$: C, 78·54; H, 10·98); found: C, 78·17; H, 11·31 $[\alpha]_D^{28}$ −55·3° (*c.* 0·5 in chloroform), melting point 137–139° C. (Fischer Johns apparatus).

The compound gave an immediate digitonide precipitate and an immediate lavender colour in the Lieberman–Burchard test (eventually a green precipitate was formed). Concentrated sulphuric acid produced an intense orange colour. Physarosterol gave no colour either with tetranitromethane or with ferric chloride. A bromine solution was immediately decolorized by a solution of the sterol in glacial acetic acid. A test for alkoxy groups was negative. No carbonyl band was observed in the 260–290 mμ region of the ultra-violet (*c.* 23 mgm./10 ml. in chloroform).

The closest fitting empirical formula for physarosterol ($C_{30}H_{52}O_3$) would allow for the presence of five-ring structures. The *ABCD* rings of the cyclopentenophenanthrene nucleus would account for four of these rings. In the absence of a carbonyl group, a fifth carbocyclic or methylene dioxy ring could be excluded because the evidence for a double bond accounted for the fifth ring structure. Furthermore, since the tetranitromethane test was negative, the double bond may have been sterically hindered and endocyclic.

Two attempts were made to acetylate physarosterol by exposing it to acetic anhydride and pyridine at room temperature for 24 hr. In both experiments, the parent compound was recovered unchanged. This difficulty suggested that the hydroxy groups were in juxtaposition to tertiary carbon atoms.

In summary, these results indicated that physarosterol was a C_{30}, unsaturated, trihydroxy sterol with one of its hydroxyl groups in the 3β position.

Because the isolation of this substance is incidental to other aspects of slime mould chemistry at present being pursued, it is not anticipated that any further work will be carried out in this laboratory in regard to its structure.

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[1] Emanuel, C. F., and Chaikoff, I. L., *Biochim. Biophys. Acta*, **24**, 254 (1957).
[2] Emanuel, C. F., and Chaikoff, I. L., *Biochim. Biophys. Acta*, **24**, 261 (1957).

Inhibition of Pancreatic Deoxyribonuclease by Acidic Polymers

In a recent publication the inhibition of pancreatic ribonuclease by a series of polymers possessing phenolic and carboxylic or sulphonic acid groups was compared with their action against influenza and vaccinia viruses in embryonated eggs. This communication describes the action of certain of these polymers on pancreatic deoxyribonuclease (Worthington Biochemical Corporation).

Enzyme activity was determined by the increase in optical density at 260 mμ of enzymatic digests at 29° ± 0·2° C. (Kunitz's method) in the presence or absence of graded inhibitor dilutions. The rates were computed from the linear portions of density – time graphs by the method of least mean squares, and by interpolation the inhibitor concentration reducing the rate to one-half of the control value (IC_{50}) was determined. The method permitted a reproducibility of rate measurements of ± 5 per cent in duplicate samples. While the absolute rate of the deoxyribonuclease reaction varied from day to day, this had no bearing on the IC_{50} values, which are relative figures, and were reproducible to ± 15 per cent or better. Table 1 shows the experimental conditions.