7α-Acetoxyconessine was prepared by reacting 7α-hydroxyconessine with acetic anhydride/pyridine at room temperature overnight. The reaction product was purified by chromatography on alumina containing 6% water using ethyl ether as the eluting solvent, and crystallization from hexane/cyclohexane (2:1) and cyclohexane. Yield, 47%, m.p. 127.5–128.5°, $[\alpha]_D^{25}$ −168° (c, 0.92 in ethanol). Anal. calcd. for $C_{26}H_{42}N_2O_2$: C, 75.55; H, 10.01; N, 6.76. Found: C, 75.34; H, 10.31; N, 6.74.

7β-Acetoxyconessine was prepared from 7β-hydroxyconessine (III) in the same manner as described for 7α-acetoxyconessine. Yield, 58%, m.p. 173–174°, $[\alpha]_D^{25}$ +103° (c, 0.74 in ethanol). Anal. C, 75.46; H, 10.23; N, 7.09.

The oxidation of 7β-hydroxyconessine (III) with chromic acid in acetone[9] gave 7-oxoconessine. The reaction was quenched with aqueous methanol and the solution was made alkaline. The product was extracted into chloroform and crystallized from petroleum ether. Yield 59%, m.p. 158–159°, $[\alpha]_D^{25}$ −41° (c, 1.02 in ethanol). $\lambda_{max}^{CH_3OH}$ 236 mμ (ε = 13,200). Anal. calcd. for $C_{24}H_{38}N_2O$: C, 77.85; H, 10.26; N, 7.56. Found: C, 78.13; H, 10.08; N, 7.23.

A similar oxidation of 7α-hydroxyconessine (II) led to 7-oxoconessine, which was identified by ultraviolet and infrared absorption spectra and melting point.

Zusammenfassung. Conessin wurde mikrobiologisch durch *Cunninghamella echinulata* in zwei Produkte zerlegt, welche in reiner Form isoliert und mit 7α-Hydroxyconessin und 7β-Hydroxyconessin identifiziert werden konnten.

E. L. Patterson, W. W. Andres, and R. E. Hartman

Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River (New York USA), March 3, 1964.

[9] S. C. Pan, Anal. Chem. *34*, 766 (1962).

Metabolic Properties of Micro-Organisms in Vibrating Culture

Earlier[1] we have reported that a vibrating stirrer has some distinct technical and biological advantages for obtaining submerged homogeneous growth of filamentous fungi. Among other properties it has been shown that a high rate of multiplication of *Aspergillus oryzae* can be obtained over a wide range of growth in the exponential phase. In stationary and shaken cultures this mould gives as high a multiplication rate only in the very early stages of growth (indirect estimation) when mycelial concentrations are very small and nutrients and oxygen freely available[2]. In later stages, where the mycelial content is measurable (above approximately 5 mg dry weight mycelium/100 ml) the highest rate of multiplication was about 0.175 duplications/h in substrate A[2] with shaken cultures, and about 0.095 duplications/h in the same substrate with stationary cultures, as compared with 0.3 in vibrating cultures.

In the linear phase of growth, which is part of the late exponential phase and part of the phase of decreasing rate of multiplication, the growth rates of *Aspergillus oryzae* are also considerably higher in Vibro Mix cultures than with other methods of cultivation; values of 25, 14.5 and 6.7 mg dry weight mycelium/100 ml h being obtained for Vibro Mix, shaken and stationary cultures respectively.

The apparatus proved to be a useful tool in some physiological investigations as reported below.

(1) *Influence of mechanical stress.* It is of some interest that cultures originating from different sizes of inocula react differently to mechanical stress. A small-inoculum culture (4×10^3 conidia/100 ml) of *Aspergillus oryzae* grown with the Vibro Mixer gives a much lower yield in substrate A than does a large-inoculum culture (2×10^7 conidia/100 ml), whereas stationary or shaken cultures do not show this effect if the same substrate is used (Figure 1). For the latter type of culture, on the other hand, it is characteristic that a somewhat smaller growth rate is obtained over a larger part of the growth curve when small inocula are used (see also Meyrath[2]). The larger the amplitude of the Vibro Mixer the stronger is the influence on maximum yield by inoculum size[3].

A major metabolic characteristic, which might be connected with the lower maximum yield of mycelium, is the marked excretion of organic nitrogenous compounds of small-inoculum cultures of *Aspergillus oryzae* when grown with the Vibro Mixer. The excretion is the more pronounced the larger the amplitude[3].

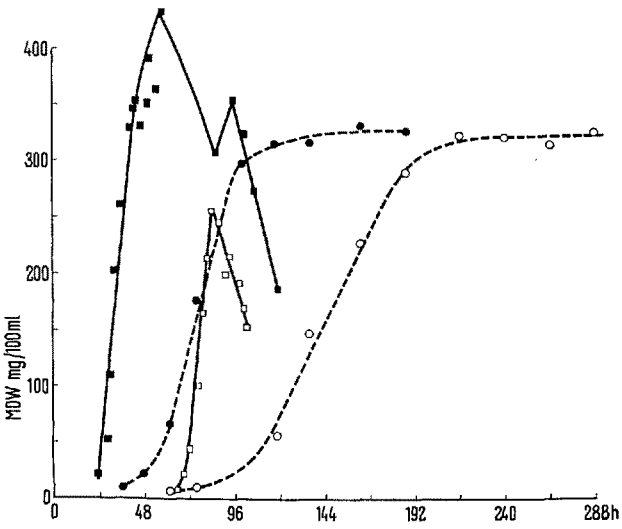

Fig. 1. Growth of *Aspergillus oryzae*, expressed in mg dry weight mycelium/100 ml, in Vibro Mix cultures (■, □) and in stationary cultures (●, ○) with large inocula (■, ●) and small inocula (□, ○).

[1] J. Meyrath, Exper. *20*, 235 (1964).
[2] J. Meyrath, Antonie van Leeuwenhoek, *29*, 57 (1963).
[3] A. F. McIntosh and J. Meyrath, J. gen. Microbiol. *33*, 57 (1963).

(2) *Production of amylase.* The rate of amylase production of *Aspergillus oryzae* is considerably higher in deep-culture with the Vibro Mixer than in either stationary or shaken cultures; this property is obviously for the greater part due to the fact that amylase production is correlated with mycelium formation. A further characteristic of the Vibro Mix cultures is the increase in amylase titre during the phase of decreasing mycelium content as can be seen in Figure 2. It should be remarked that stationary or shaken cultures never show as marked an autolysis as Vibro Mix cultures.

A property which corresponds strikingly to the increased excretion of organic nitrogen is the markedly higher rate of amylase production of small-inoculum Vibro Mix cultures as compared with large inocula. This seems to indicate that the stimulated excretion of organic nitrogen during the phases of increase and decrease of mycelium content is not due simply to autolysis, i.e. dissolution of the cell contents, but represents also a change in the pattern of the synthesizing activity. To complete the picture it is necessary to indicate that stationary or shaken cultures from small inocula show neither increased rate of amylase production nor increased maximum yield of the enzyme when compared with large-inoculum cultures.

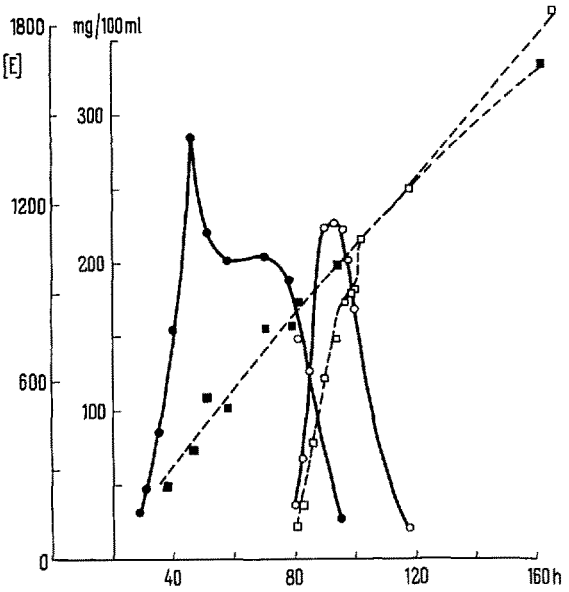

Fig. 2. Production of dextrinogenic amylase (E) (■, □) by *Aspergillus oryzae* in Vibro Mix cultures during the phases of increase and decrease of mycelium formation (●, ○) with large (■, ●) and small (□, ○) inocula.

(3) *Propagation of various micro-organisms.* Filamentous growth was obtained with various species of *Penicillium*, *Aspergillus* and *Byssochlamis*. Yeasts and acetic acid bacteria could be grown easily. Recently we have been using the apparatus advantageously for the propagation of organisms oxidizing long-chain hydrocarbons. A distinctive feature of the Vibro Mixer is the production of a fine emulsion, even with waxy components, in the three-phase system: air-water-oil.

Prospective applications. As a result of a very intimate mixing of air and water (thus allowing a low air-flow rate) the apparatus may find further applications in those cases where volatile products or substrate compounds are involved as in vinegar manufacture. In view of the homogeneous growth obtained with filamentous fungi, and as only little mycelium is attached to the vessel surface above and below the level of the substrate, the method should be suitable for continuous propagation of filamentous fungi on the laboratory or pilot plant scale[4].

Zusammenfassung. In Vibro-Mischer-Kulturen erfolgt die Amylasebildung bei *Aspergillus oryzae* in der linearen Wachstumsphase im Gegensatz zu Ruhe- und Schüttelkulturen schneller mit kleiner als mit grosser Impfung. Während der Phase der raschen Abnahme der Mycelkonzentration läuft die Amylasebildung noch weiter.

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Department of Applied Microbiology and Biology, The Royal College of Science and Technology, Glasgow (Scotland), December 19th, 1963.

[4] The experiments described in this paper were carried out at the Department of Agricultural Bacteriology and Fermentation, Swiss Federal Institute of Technology, Zürich, where careful technical assistance has been provided by Miss A. MOHLER.

Reticulol, a New Metabolic Isocoumarin

While examining the metabolic products of a strain of *Streptomyces rubrireticulae* a previously unknown, colorless, crystalline phenol, $C_{11}H_{10}O_5$, was isolated. The compound, m.p. 193–193.5°, λ^{max}_{MeOH} 245, 278, and 330 mμ (log ε 4.68, 3.86 and 3.76 respectively), was named reticulol for convenience and its structure was established as Ia by degradative and physical studies.

Microanalysis detected the presence of an O-methyl group and the formation of a diacetate (ν_{max} 1795, sh. 1782, 1200 cm^{-1}) established that two of the other oxygens were phenolic hydroxyls. The remaining oxygens were shown to be present in a hydrogen bonded aromatic ester grouping (ν_{max} 1680 cm^{-1} shifting to 1740 cm^{-1} on acetylation and 1710 cm^{-1} on methylation). No reaction was obtained between reticulol and dichlorodiphenylmethane thus eliminating the possibility that the two phenol groups are *ortho* to one another[1]. The ester function in dimethylreticulol (Ib) was shown to be cyclic by virtue of its acid stability and the ease with which it was hydrolyzed by aqueous base to the corresponding carboxylic acid (IIa) without loss of carbon. The same acid

[1] L. JURD, J. org. Chem. *27*, 872 (1962).