isomeric trisubstituted allylic alcohols (types A and B) can be definitely assigned by this method. A wide application of this technique is foreseen not only for conformational assignment of natural products remaining unsolved, but also for simultaneous determinataion of *cis-trans* isomers in a mixture.

Zusammenfassung. Methode zur Identifizierung von *cis-trans* trisubstitutierten Allylalkoholen durch NMR mit dem Shift-Reagenz Eu(DPM)$_3$ und gleichzeitige Gehaltsbestimmung der Isomeren in der Mischung.

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Mycelial Suspensions Prepared Ultrasonically

In experimental work with dermatophytes, the fragmentation of the mycelial mass is still a problem, because, for most purposes, the spore suspensions should be avoided[1]. Methods proposed for disintegration, such as hand or mechanical grinding, shaking with glass beads[2], high speed blendors[3], do not give satisfactory results and often involve difficulties with contamination control.

Killed mycelial suspensions of *Trichophyton mentagrophytes*, employed as vaccines[4], have been prepared by exposure to ultrasonic energy for 2–6 h. Electronmicroscopical studies on such suspensions have demonstrated that the sound vibration does not alter the fungal structures. It acts mainly in a purely mechanical way, in a manner similar to that observed on higher plants or animal organism[5].

For the routine preparations of standard mycelial suspensions, utilized as inoculum for metabolic studies on dermatophytes, an ultrasonic cleaner (Millipore, model XX-6600850) was employed.

A monosporically selected[6] strain of *Epidermophyton floccosum* (Harz) Langeron and Milochevitsch (1930) was incubated 10 days at 30 °C on Sabouraud dextrose agar (B109 Difco). The mycelium was carefully scraped from the surface of the Petri dish (to minimize introduction of nutrient medium) and placed in a flask containing 10 ml of sterile distilled water. The flask, closed with a glass

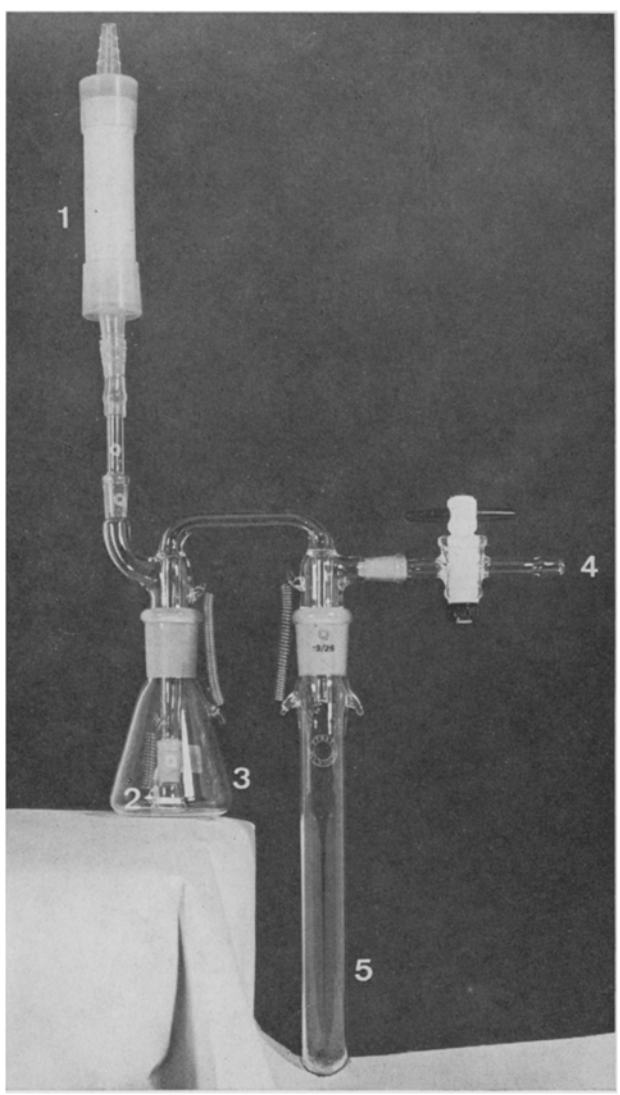

Fig. 1. Filtration system: 1. cotton filter, 2. sintered glass filter, 3. sonicated mycelial fragments, 4. connection to water pump, 5. preservable standard mycelial suspension.

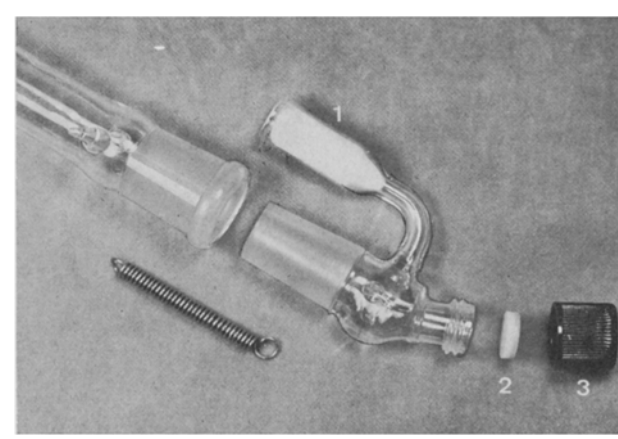

Fig. 2. Septum assembly: 1. cotton filter, 2. silicone septum, 3. perforated screw cap.

[1] D. J. GUIRDY and G. H. TRELLES, J. Bact. *83*, 53 (1962).
[2] J. KEJDA, Mykosen *11*, 589 (1968).
[3] F. W. FRIEDHOFF and S. A. ROSENTHAL, J. Invest. Derm. *23*, 155 (1954).
[4] F. REISS and L. LÉONARD, J. Invest. Derm. *26*, 449 (1956).
[5] F. REISS and L. LÉONARD, Dermatologica *117*, 401 (1958).
[6] M. HEJTMÁNEK and K. LENHART, Mykosen 7, 43 (1964).

stopper, was set in the ultrasonic cleaner for 1 min. Uniform size of the sonicated mycelial fragments was achieved by filtration through sintered glass (40–90 µm) under reduced pressure (Figure 1). The density of the resulting suspension was checked by spectrophotometer and, if neccessary, diluted with sterile distilled water, in order to obtain a standard mycelial suspension. Finally the tube containing the standard mycelial suspension was sealed with a special plug (Figure 2) consisting of a cotton filter and silicone septum (standard type used in gas-liquid chromatography) which allows sterile withdrawal by a syringe. All procedures were carried out under aseptic conditions.

The viability of dermatophytes in sterile distilled water has been evaluated for one year[7], thus we expect the septum assembly can be used for long term stocking of standard mycelial suspensions.

Résumé. Une méthode simple de préparation des suspensions mycéliales standardisées de dermatophytes a été établie. Le mycéliium fut dispersé à l'aide d'ultrason et les particules mycéliales homogénéisées par filtration sous pression réduite; la suspension mycéliale fut standardisée par spectrophotométrie. Les suspensions uniformisées ont pu être conservées dans des récipents munis de garde à coton.

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A New Culture Flask for Plant Tissue Suspension Cultures

The growth of plant tissue in vitro is generally measured by parameters, such as dry weight, fresh weight, or total nitrogen of the tissue[1]. In all such measurements the material, or a portion thereof, must be sacrificed.

A culture vessel especially designed for the measurement of growth rates of suspension cultures of plant tissue is described. The use of this flask allows the preservation of the tissue and is based on the relationship found between settling volume and dry weight of tissue. It is basically a modified 250 ml Nephelo flask with a side arm that is covered with a Morton Closure and is sufficiently high to allow shaking of the cultures (Figure 1). Because the extension tubes of these flasks are of uniform diameter (gauge material), determinations of volume may be made directly on changes in height of the settled tissue. Height determinations are made with a millimeter rule. It is feasible to add graduations to the extension tube but this would add 25% to the cost of the flask.

Flasks, containing 100 ml of modified White's 10× salts liquid medium[2] with 2% sucrose and pH adjusted to 6.3 with NaOH prior to autoclaving, were inoculated with 10 ml of a 45-day-old suspension culture of *Vinca rosea* L. VA6 crown gall tissue. Cultures were incubated at 27 °C on a gyrotary shaker at 150 rpm. Periodically, 5 randomly selected flasks were inverted for 1 h and the settling heights determined to the nearest millimeter. The tissue had settled after 20 min at which time measurements could have been made. Tissues from the individual flasks were washed on filter paper with five 100 ml portions of distilled water. Dry weights were determined after drying at 80 °C for 48 h.

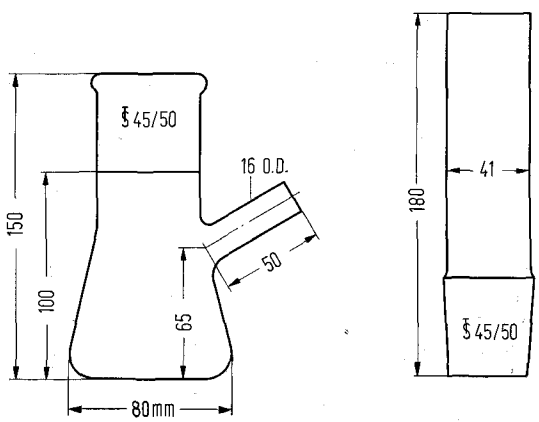

Fig. 1a. Culture flask components.

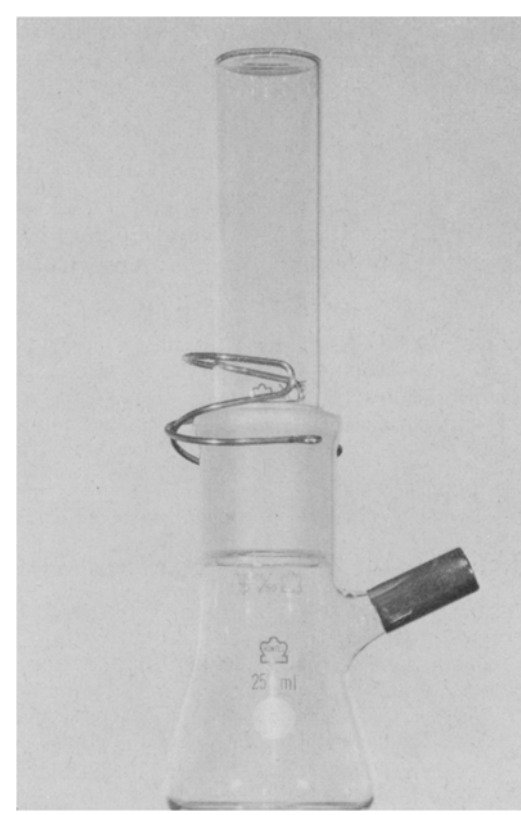

Fig. 1b. Assembled culture flask.

[1] M. C. LANCE, Rev. gen. Bot. *64*, 123 (1957).
[2] J. LIPETZ, Am. J. Bot. *49*, 460 (1962).