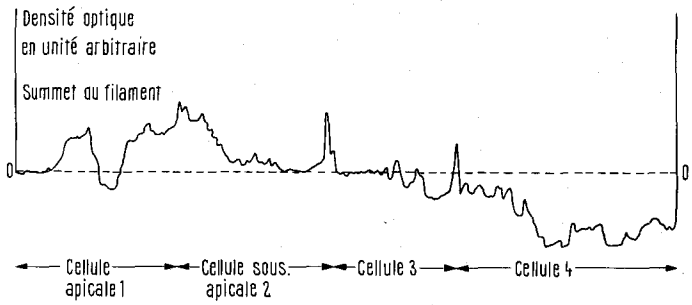

Enregistrement au Chromoscan, à partir d'un film en négatif, d'un filament de protonéma: localisation de la phénylalanine tritié dans la cellule apicale.

s'étalent. Au 20e jour de culture, sous les spores et sous la cellophane, 0,02 ml d'une solution de phénylalanine tritiée (25 µC/ml) est alors déposé à l'aide d'une microseringue.

48 h après l'application de l'acide aminé marqué, les différentes parties des filaments sont autoradiographiées selon la technique adaptée de KNOPP, HAHN et BOPP[5].

Les cellules apicales des filaments sont un peu plus fortement marquées que les sous-jacentes (Figure); la phénylalanine est incorporée dans les protéines cytoplasmiques. Les apex seraient donc bien des centres d'appel pour les métabolites. Aucun phénomène de diffusion n'est intervenu, car le marquage observé au niveau des filaments aériens est uniquement intracellulaire.

En attendant de pouvoir expliquer le mécanisme responsable de cette orientation géotropique négative du protonéma du *Ceratodon purpureus*, il est possible d'utiliser cette propriété pour entreprendre l'étude des transports et échanges de divers métabolites au sein du protonema. Des expériences sont en cours avec des acides aminés et du glucose marqués, métabolites dont on connaît le rôle dans la ramification des axes protonématiques en relation avec les hormones de type kinétine et auxine, qui interviennent lors de la caulogenèse.

Les premiers résultats obtenus démontrent déjà que certaines molécules circulent dans les deux sens, d'autres au contraire sont transportées en direction acropète. Cette technique doit donc nous permettre d'approfondir la physiologie du protonéma, matériel très favorable à l'étude de la morphogenèse sur des végétaux chlorophylliens.

Summary. A method of detecting translocation of metabolites in protonema of *Ceratodon purpureus* (Hedw.) has been investigated.

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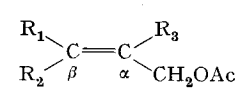
A Simple Method for Conformational Assignment of Trisubstituted Allylic Alcohols

Although there are an increasing number of natural products containing a trisubstituted allylic alcohol grouping in their gross structures, only limited methods[1-3] have been proposed for determining the stereochemistry of these compounds. Recent study on Z-E conformational isomerism of nerol and geraniol[4] prompts us to present our results.

Our method is composed of an acetylation of an alcohol sample and an estimation of relative shift value(s) (ν_{rel}) of appropriate proton signal(s)[5] with or without an addition of a NMR shift reagent Eu(DPM)$_3$. As far as an approximate linear relationship is confirmed between shift of the signal and amount of the reagent, the observed ν_{rel} can be easily correlated to a pronounced, structural feature of one isomer and a pure specimen of the other isomer is not necessary for comparison. Thus, the tabulated result clearly indicates that the stereochemistry of the

Relative shift values of trisubstituted allylic alcohols.



A: R_3 = H, R_1 or R_2 = CH_3
B: R_3 = CH_3, R_1 or R_2 = H

Relative position against $-CH_2OAc$ group	ν_{rel}[a]	$-CH_2-O-$	Examples (acetates)[b]
C_α-CH_3 (B)	27–35	singlet	11, 12, 13, 14, 15
C_β-*cis*-CH_3 (A and B, R_2 = CH_3)	18–21	doublet	1, 3, 5, 6, 7, 8, 13[c]
C_β-*trans*-CH_3 (A and B, R_1 = CH_3)	8–11	doublet	2, 4, 9, 10, 12[c]
C_α-H (A)	48–53	doublet	1, 2, 3, 6, 7
C_β-*cis*-H (B, R_2 = H)	30–35	singlet	11, 12
C_β-*trans*-H (B, R_1 = H)	17–22	singlet	11, 13, 14, 15

[a] 60 MHz, in CCl_4. The ν_{rel} refers to a relative paramagnetic shift against acetoxyl group (ν_{rel} = 100.0). [b] Compounds reported herein had correct spectral data. 1. geraniol; 2. nerol; 3. phytol; 4. *trans*-crotyl alcohol; 5. *cis*-crotyl alcohol; 6. *trans*-β-ionylidene-ethanol; 7. all-*trans*-vitamin A_1; 8. 9-*cis*-vitamin A_1; 9. 13-*cis*-vitamin A_1; 10. 9,13-di*cis*-vitamin A_1; 11. β-methallyl alcohol; 12. tiglyl alcohol; 13. angelyl alcohol; 14. α-santalol; 15. β-santalol. [c] $-CH_2-O-$: singlet.

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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

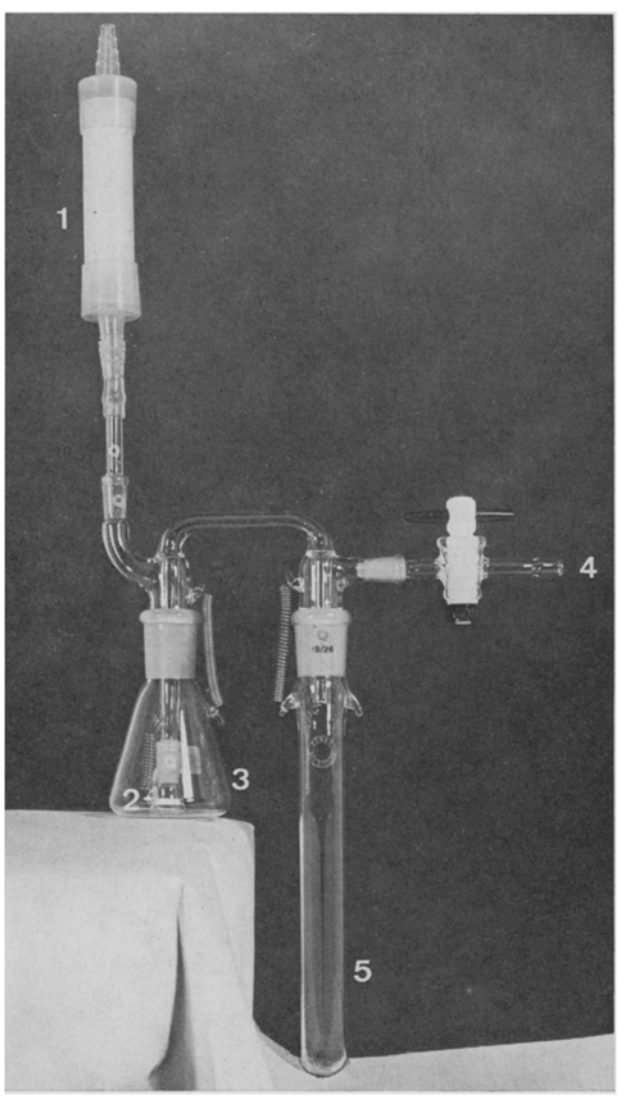

Fig. 1. Filtration system: 1. cotton filter, 2. sintered glass filter, 3. sonicated mycelial fragments, 4. connection to water pump, 5. preservable standard mycelial suspension.

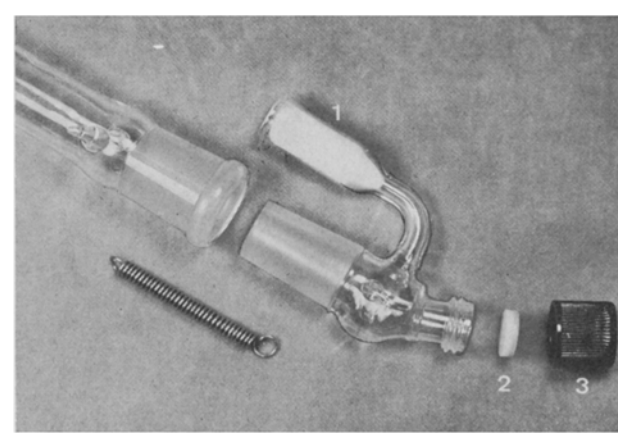

Fig. 2. Septum assembly: 1. cotton filter, 2. silicone septum, 3. perforated screw cap.

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