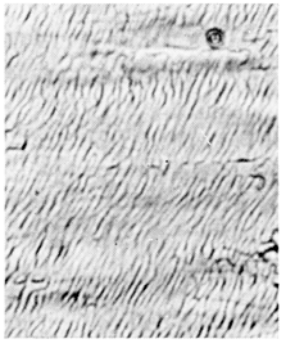
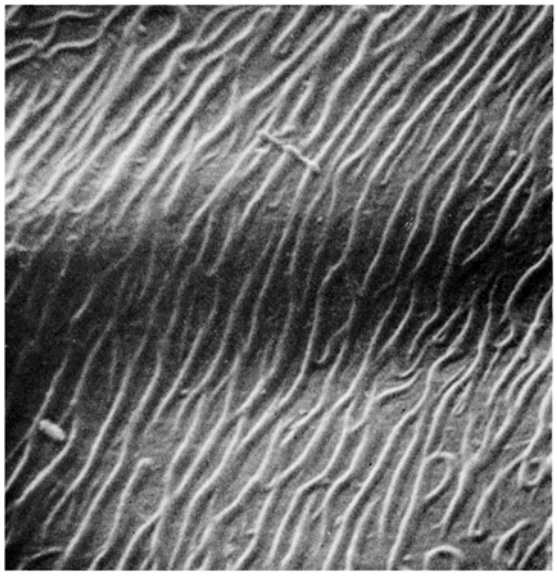
A satisfactory explanation of this epitaxy can be given on the assumption that the PBLG crystals are associated with the surface of the substrate by the same contact plane as in the epitactic overgrowth on the alkali halides.



a)



b)

Fig. 1. a) Light micrograph (×1000) and b) scanning electron micrograph (×5000) of the oriented overgrowth of the polyamide model biopolymer poly-γ-benzyl-L-glutamate on the polyamide Nylon 6.

This problem will be discussed when the results of an electron microscopy and diffraction investigation are available.

The results of cursory attempts with PBLG as a deposit on other Nylon types such as Nylon 6,6, Nylon 6,10, Nylon 8 and Nylon 11 under the same experimental conditions, point to a pronounced specific character of this epitaxy.

The arrangement of the macromolecules of the overgrowth with respect to the known arrangement of the macromolecules of the surface of the substrate can be found by electron diffraction. From this arrangement can be inferred the molecular details of the association, such as correspondence of charge distribution in substrate and deposit molecules in the contact plane as well as the kind of intermolecular forces between the associated molecules.

Thus epitaxy between non-identical polyamides opens a new line of study at the molecular level of the law of association of such polyamides, especially of biopolymers of this type.

Zusammenfassung. Die orientierte Verwachsung zwischen zwei nicht identischen Polyamiden wird als neuartiger Typ der Epitaxie am Beispiel der Aufwachsung des biopolymeren Polyamids Poly-L-Glutaminsäure-γ-benzylester auf Poly-ε-caprolactam beschrieben. Der Verwachsungstyp bietet einen neuen Weg zur Untersuchung der Voraussetzungen für die Assoziation nicht identischer hochmolekularer Polyamide.

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[4] The author thanks Dr. R. Holm, Farbenfabriken Bayer AG, Leverkusen, for the scanning electron micrograph.

A New Reagent for the Cleavage of the Tertiary Butyloxycarbonyl Protecting Group

The tertiary butyloxycarbonyl (BOC) protecting group[1] is now widely used in peptide chemistry, especially in the solid phase peptide synthesis[2]. Mostly, the splitting off is effected with 1 *N* HCl/acetic acid, 4 *N* HCl/dioxane or trifluoracetic acid/methylene chloride (1:1).

The use of mercaptoethanol in conjunction with trifluoracetic acid/methylene chloride has been proposed by Westall and Robinson[3], but in a footnote to their paper, the authors advised another more stable reducing agent.

This is the reason why we decided to publish our results obtained with mercapto ethane sulfonic acid (Mesna®)[4]. This reagent, dissolved in glacial acetic acid, splits off very rapidly and selectively the BOC group without damaging the benzyl ester (O Bzl) bonds, or the benzyloxycarbonyl (Z) group.

A 50% solution of Mesna (3.45 *M*) was used in our first experiments: the BOC amino acid (3 µmoles) was dissolved in 0.1 ml of the reagent and left for 2–5 or 30 min. After these periods, concentrated NaOH was added to stop the reaction.

High voltage electrophoresis was then effected in a pyridine/acetic acid/water buffer (pH 6.2). This experiment was made with BOC Gly, BOC Ala, BOC Val, α BOC ε Z Lys, α BOC γ O Bzl Glu and BOC Trp.

After 2 min, none of the BOC amino acid could be detected. The only products were Gly, Ala, Val, ε Z Lys, γ Bzl Glu and Trp.

Even after 1 h of reaction, we did not detect any compound migrating at the same position as free lysine

[1] F. C. McKay and N. F. Albertson, J. Am. chem. Soc. *79*, 686 (1957).

[2] R. B. Merrifield, J. Am. chem. Soc. *85*, 2149 (1963); Biochemistry *3*, 1385 (1964).

[3] F. C. Westall and A. B. Robinson, J. org. Chem. *35*, 2842 (1970).

[4] Mesna® is the trade mark of the sodium salt of the mercapto ethane sulfonic acid marketed by UCB as a mucolytic agent; it is stable and non-toxic. In this paper, we used Mesna® as mercapto ethane sulfonic acid. The free acid is obtained from the sodium salt by ion exchange with a Dowex $50H^+$ resin and lyophilisation of the aqueous solution.

or free glutamic acid. The Trp recovered after deprotection and elimination of the Mesna® has exactly the same spectrum as free Trp.

As the cleavage rates were very high, we tried an experiment using more dilute solutions and a lower molar excess of Mesna®. BOC Ala dissolved in glacial acetic acid was cleaved with a 7 or 3.5 molar excess of Mesna®.

In the first case, splitting off of the BOC group was almost complete after a reaction time of 2 min and was fully achieved after 5 min. In the second trial, the cleavage was not complete after 5 min but was effected after 10 min.

This reagent being very effective when both products are in solution, we decided to try it in the solid phase peptide synthesis.

A first trial was made with a polymer bearing 0.4 mmoles of BOC Gly/g; we used a 7-fold molar excess of Mesna® (20% solution in glacial acetic acid) and a reaction time of 30 min.

The free amino groups were detected by a modification of the procedure of DORMAN[5,6]. The cleavage of the BOC group was quantitative, using these conditions. As this reagent is safe, easy to handle and does not destroy Trp, this procedure is routinely used in our laboratory in the solid phase synthesis of peptides up to 25 amino-acids[7].

Résumé. L'acide mercapto éthane sulfonique est utilisé pour déprotéger la fonction amine des t-butyloxycarbonyl acides aminés. La réaction, extrêmement rapide en phase homogène, a été appliquée à la synthèse de peptides en phase solide.

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UCB - Pharmaceutical Division, Rue Berkendael 68, B-1060 Bruxelles (Belgium), 18 March 1971.

[5] L. C. DORMAN, Tetrahedron Lett. *1969*, 2319.

[6] We used tribenzylamine hydroiodide instead of pyridine hydrochloride; the amount of free NH_2 groups is determined by colorimetric analysis of the iodine liberated by oxydation of the hydroiodic acid obtained in the exchange reaction.

[7] Acknowledgments. This work was supported by the grant No. 1744 of the 'Institut pour l'encouragement à la recherche scientifique dans l'industrie et l'agriculture' (I.R.S.I.A.).

Application of a Shift Reagent in Nuclear Magnetic Resonance Spectroscopy of Esters. An Approach for Simple Identification and Simultaneous Determination of Tocopherols

The use of *tris*(dipivalomethanato)europium as a dramatic shift reagent in NMR was reported by HINCKLEY[1] and SANDERS et al.[2] and several applications of this reagent in organic chemistry have been described in succession[3]. The usefulness of this reagent in the 60 MHz apparatus for structural determination of polyene esters (and ether) was also verified by the present authors for normal- and retro-vitamin A derivatives[4]. In our case, only extremely weak or no co-ordination between this reagent and conjugated carbon-carbon double bond was observed. We now report a further example in which methyl groups attached on the benzene moiety of tocopheryl acetates can be assigned confidently by using this reagent, and we propose simultaneous determination of tocopherols together with their identification by recording a NMR-spectrum of the acetates mixture.

Table I. Chemical shifts (ν)[a] and relative paramagnetic shifts ($\Delta\nu$)[b] of tocopheryl acetates

H	α		γ		δ	
	ν (cps)	$\Delta\nu$	ν	$\Delta\nu$	ν	$\Delta\nu$
6-OAc	132.8	100.0	130.6	100.0	128.9	100.0
5-Me	114.9	58.2				
7-Me	117.7	57.6	118.2	53.8		
8-Me	123.4	13.1	124.7	11.6	127.5	5.2

[a] Measured from running of the carbon tetrachloride solution of the acetate on the Varian A 60-D (60 MHz) instrument, tetramethylsilane being used as an internal standard. The second oxygen on the dihydropyran ring has little indication of co-ordination to the shift reagent under the experimental conditions (molar ratio of the reagent to the acetate = 1 — 2:3.8). [b] Shift value of the acetyl group = 100.0.

As aryl methyls in tocopherols have been known to have similar chemical shift values and practically only a single peak was observed on a conventional NMR running[5], it is for this reason that another approach for a definite characterizing of tocopherols in various binary systems was discussed[6]. Our own finding now indicates that the differentially recognizable peaks of these aryl methyls do appear simply by converting the parent phe-

Table II. Relative paramagnetic shifts ($\Delta\nu$)[a] of hydrogens and methyls on the phenol acetate moiety

H	$\Delta\nu$
o-H	70 ± 8
o-Me	56 ± 3
m-H	27 ± 5
m-Me	5–13
p-Me	ca. 5

[a] Surveyed from the results on cresyl and tocopheryl acetates using a shift reagent $Eu(DPM)_3$. Shift value of the acetyl group = 100.0.

[1] C. C. HINCKLEY, J. Am. chem. Soc. *91*, 5160 (1969).

[2] J. K. M. SANDERS and D. H. WILLIAMS, Chem. Commun. *1970*, 422.

[3] G. H. WAHL JR. and M. R. PETERSON JR., Chem. Commun. *1970*, 1167. – R. R. FRASER and Y. Y. WIGFIELD, Chem. Commun. *1970*, 1471. – P. V. DEMARCO, T. K. ELZEY, R. B. LEWIS and E. WENKERT, J. Am. chem. Soc. *92*, 5734, 5737 (1970). – D. R. CRUMP, J. K. M. SANDERS and D. H. WILLIAMS, Tetrahedron Lett. *1970*, 4949.

[4] K. TSUKIDA, M. ITO and F. IKEDA, J. Vitam. *17*, 57 (1971); Int. J.Vitam. Nutr. Res. *41*, in press (1971).

[5] M. KOFLER, P. F. SOMMER, H. R. BOLLIGER, B. SCHMIDLI and M. VECCHI, Vitams Horm. *20*, 407 (1962).

[6] H. FINEGOLD and H. T. SLOVER, J. org. Chem. *32*, 2557 (1967).