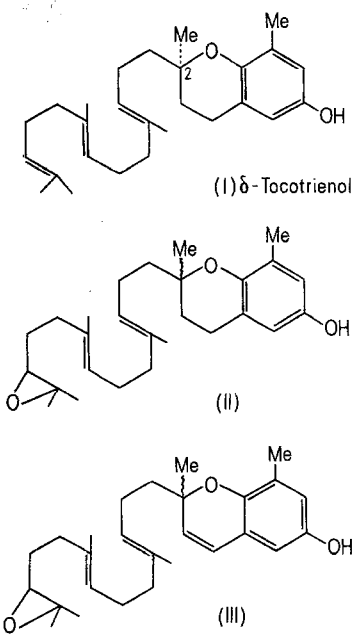

name: Yoremoku), a kind of alga to which the larvae settle specifically[1]. This finding has forced us to elucidate the active substance in the algae.

The neutral part of *n*-hexane extracts of the dried alga (3.5 kg) was fractionated by the aid of column chromatography of silica gel for bioassay, and a portion (302 mg) of the fractions showed a specific and powerful activity in favour of the settling and subsequent metamorphosis of the swimming larvae of *Coryne Uchidai*. By further purification with preparative silica gel thin layer chromatographies, the active portion was separated into 6 compounds, i.e., A (22 mg, $C_{27}H_{40}O_3$), B (89 mg, $C_{27}H_{40}O_2$), C (24 mg, $C_{27}H_{40}O_3$), D (11 mg, $C_{27}H_{38}O_3$), E (6 mg, $C_{27}H_{40}O_2$) and F (18 mg, $C_{30}H_{42}O_2$).

On the basis of chemical and physical evidence[2], structure of B and A was deduced as δ-tocotrienol (I) and its epoxide (II). The deduction was unequivocally confirmed by the synthesis[3], in which DL-epoxide (II) was obtained by the reduction of dehydro-epoxide (III). Since both epoxides have the same Rf on TLC and (II) shows M^+ and $(M-2)^+$ in its mass spectrum, the contamination of III in A is not ruled out at present. The remaining compounds have the similar physical properties and the structural elucidation is the subject of future investigation.

It was found that our synthetic materials, especially both epoxides (II and III), are effective in the assay using swimming larvae of *Coryne Uchidai* as shown in the Table[4].

Résumé. Le δ-tocotriénol (I) et ses dérivés (II), tirés de l'algue *Saragassum tortile*, ont été identifiés comme étant les substances favorisant la fixation spécifique sur l'algue des larves mobiles de *Coryne Uchidai*. Les produits de synthèse (I et II) possèdent la même activité.

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[3] Details of the syntheses will be described elsewhere.

[4] Acknowledgment. We are indebted to Hoffman-La Roche for the financial support, and thanks are due to Mr. Y. KATO of Hitachi Co., Ltd., for his measurement of high resolution mass spectra.

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The Influence of Proteolytic Enzymes on the Phosphorylation of Rat Liver Histones

There is increasing evidence that cells from tissue culture or isolated cell nuclei respond to the addition of proteolytic enzymes with stimulated synthesis of DNA[1], RNA[2,3] or cell multiplication[4,5]. Effective agents were trypsin[1-3], papain[6,7] and lysosomal preparations[4]. In addition, activated cell proliferation was observed in vivo, following i.v. or i.p. administration of proteases[6-8]. This paper describes an enhanced phosphorylation of certain histone fractions, caused by i.p. injections of low doses of trypsin and papain. The investigations were suggested by the observation that cellular proliferation is dependent on regulatory influences of nuclear proteins, and that the amount of phosphate or acetate incorporated into specific histone fractions, varies with the stage of the cell cycle[9-11].

The Figure demonstrates a significant stimulation of phosphate incorporation into liver histones F1, F1″ and F2b after a single injection of papain (0.5 or 3 mg/animal) or trypsin (3 or 9 mg/animal). A similar effect is observed with fraction 'c', a protein component, not belonging to the histone group[12]. The stimulated phosphate uptake can be produced also by application of Wobe-Mugos (Mucos-Emulsionsges. mbH., Grünwald/München, 0.2 and 1.2 ampoules/animal), an enzyme preparation used in

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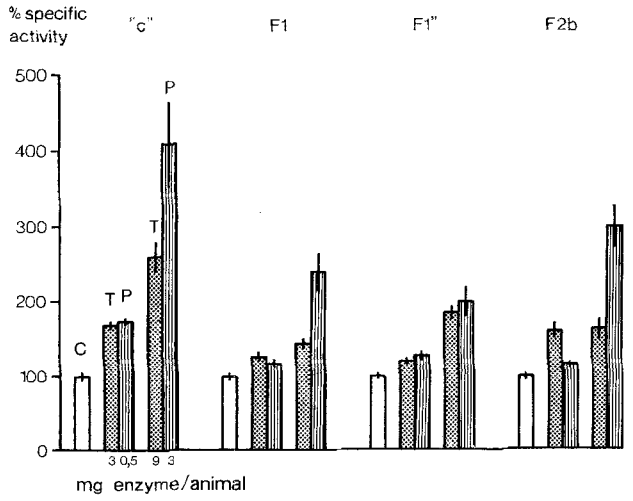

Stimulation of incorporation of ^{32}P orthophosphate into histone fractions F1, F1″ and F2b and into fraction 'c' after injection of trypsin and papain. Indicated amounts of enzymes were dissolved in 1 ml 0.9% NaCl and injected i.p. into 3-month-old male Sprague-Dawley rats (controls obtained the same amount of 0.9% NaCl). 16 h later animals were injected i.p. with 1 ml (2.5 mCi) ^{32}P, as described earlier[16]. 1 h thereafter animals were sacrificed. Livers were excized, histones extracted and separated on 15% polyacrylamide gels and specific activity was determined[16]. C, control; T, trypsin treated; P, papain treated. Values represent arithmetic means of 3 determinations ± S.D.

tumor therapy and consisting primarily of pancreatic extracts and papayotin. The phosphorylation of other nuclear protein fractions, however, is not significantly influenced by these agents.

Earlier results suggested that gene derepression induced by the action of proteolytic enzymes was caused by a degradation of nuclear proteins[13,14]. However, stimulated phosphorylation, as described in the present paper, could be sufficient to result in changes of the tertiary structure of those proteins[15], giving rise to stimulated DNA synthesis and cell proliferation.

Results. Intraperitoneal injections of proteolytic enzymes or enzyme preparations, including papain, trypsin and 'Wobe-Mugos', result in a significant stimulation of phosphate incorporation into rat liver histones F1, F1″, and F2b. The stimulation of DNA synthesis and cell multiplication by these agents, described in earlier reports, might be a consequence of stimulated phosphate uptake by nuclear proteins.

Zusammenfassung. Die i.p. Injektion der proteolytischen Enzyme Papain und Trypsin, sowie des Enzympräparates Wobe-Mugos, bewirkt eine signifikante Steigerung des Phosphateinbaues in die Histone F1, F1″ und 2Fb der Rattenleber. Die früher beobachtete Stimulierung der DNS-Synthese und Zellproliferation durch proteolytische Enzyme könnte die Folge einer Steigerung der Phosphorylierung von Kernproteinen sein.

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Cytotoxicity of New Cytochalasans from *Chaetomium globosum*

In the course of the screening tests for detecting mycotoxin-producing fungi isolated from foodstuffs, crude extracts of the mycelium and the culture filtrates from all tested isolates of *Chaetomium globosum* Kunz ex Fries were noticed to cause polynucleation and multipolar division of HeLa cells and also acute toxicity in mice[1-3]. Because of these dramatic biological effects, we conducted further chemical and biological investigations. The mycelium of fungi grown in liquid media or on rice was extracted by chloroform. The extract was separated by chromatographic methods which led the isolation of 6 metabolites showing the peculiar effect on HeLa cells; chaetoglobosin A, mp 168–170°, B, mp 186–187°, C, mp 259–261°, D, mp 216°, E, mp 279–280°, and F, mp 177–178°. These compounds showed closely related physical properties, suggesting a common molecular framework. The molecular formulae, $C_{32}H_{36}O_5N_2$ for A, B, C and D, and $C_{32}H_{38}O_5N_2$ for E and F, were established by high resolution mass spectrometry. The structures of A and B have been proposed preliminarily as I and II, chiefly from the spectroscopic data[4].

The structures suggested that chaetoglobosins belong to cytochalasans, a series of cytostatically active mold metabolites such as phomins from *Phoma* sp., cytochalasins