When RNA was hydrolyzed by Sepharose RNase, optical density at 260 nm of the reaction mixture reached to the same lebel as the case of the corresponding native enzymes (not shown here). This may indicate that the specificity of Sepharose RNases were the same as those of the native enzymes. Therefore, it could safely concluded that these enzymes could serve as very useful tools for the structural studies of RNA, especially for base analysis of RNA, because of its stability and durability.

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Reconfirmation of the Specific Nature of Reticulocytes Bioassay System to Trichothec Mycotoxins of *Fusarium* spp.

Yoshio Ueno and Noriko Shimada

Laboratory of Microbial Chemistry Faculty of Pharmaceutical Sciences, Tokyo University of Science[1)]

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In the previous communications,[2,3)] the authors reported that the incorporation of ^{14}C-labelled leucine into protein by rabbit reticulocytes was inhibited by toxic trichothecenes such as fusarenon-X and T-2 toxin, and that this inhibition was highly specific to the 12—13 epoxy trichothec compounds among more than ten kinds of mycotoxins tested. The specificity of the reticulocytes bioassay system was applied for chemical fractionation of trichothecenes from fungal metabolites[4,5)] and screening of trichothecenes-producing fungi from moldy cereals and feed.[6–8)]

In order to confirm the specificity of reticulocytes bioassay, the authors reexamined our method with toxic fungal metabolites which were recently isolated in our laboratory or supplied from several Institutes: 7-hydroxydiacetoxyscirpenol and 7,8-dihydroxydiacetoxyscirpenol were newly isolated from the culture filtrate of *Fusarium* sp. K 5036,[9)] calonectrin and deacetylcalonectrin, verrucarin A and roridin A, fusaric acid, moniliformin, chaetoglobosin A and ascradiol were kindly gifted by W.B. Turner (Imperial Chemical Industries, England), C. Tamm (Basel University, Swiss), S. Nishimura (Tottori University, Tottori-shi), R.J. Cole (National Peanut Research Laboratory, U.S.A.), S. Natori and M. Tanabe (National Institute of Hygienic Sciences, Tokyo), respectively. Cytochalasin A and B were purchased from Aldrich Chemical Comp. Inc., U.S.A.

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1) Location: *12, Ichigaya-Funagawara-machi, Shinjuku-ku, Tokyo, 162, Japan.*
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The bioassay was carried out with the method described elsewhere,[2] and the water-insoluble compounds such as verrucarin A, roridin A and cytochalasins were dissolved in ethanol. The final concentration of ethanol in the incubation was adjusted to 1%.

As summarized in Table I, the incorporation of ^{14}C-leucine into protein was markedly inhibited by a small dose of all the trichothecenes such as two calonectrins, two hydroxyldiacetoxyscirpenols, verrucarin A and roridin A. On the other hand, no severe inhibition was detected with nontrichothec mycotoxins such as fusaric acid, moniliformin, ascradiol and cytochalasins. These findings reconfirmed the specificity of reticulocytes bioassay system to trichothec mycotoxins. The fact that fusaric acid and moniliformin, both being established as fusarial mycotoxins,[10,11] exhibited no inhibitory effect in this bioassay system shows that this bioassay system is useful for screening of only the trichothec mycotoxins among toxic fungal metabolites originated from *Fusarium* spp.

Table I. Inhibitory Effects of Mycotoxins on the Incorporation of ^{14}C-Leucine in Rabbit Reticulocytes

Mycotoxins	Concentration (μg/ml)	Percent activity of control (%)
—	—	100
Calonectrin	1	72
	10	18
Deacetylcalonectrin	1	86
	10	75
7-Hydroxy-DS[a]	1	16
7,8-Dihydroxy-DS	1	30
Verrucarin A	0.01	62
Roridin A	0.01	36
Fusaric acid	100	100
Moniliformin	10	91
	20	56
Cytochalasin A	10	102
	20	70
Cytochalasin B	10	99
	50	71
Chaetoglobosin A	10	95
Ascradiol	100	95

a) DS: diacetoxyscirpenol

The dose-response curves of the above six trichothecenes were summarized in Fig. 1. The inhibitory effect on the incorporation of ^{14}C-leucine was proportional to the increasing concentration of the toxins. The concentration of drugs required for 50% inhibition (ID 50) was estimated to be 0.006 μg/ml for roridin A, 0.015 μg/ml for verrucarin A, 0.3 μg/ml for 7-hydroxy-DS, 0.6 μg/ml for 7,8-dihydro-DS, 2 μg/ml for calonectrin and about 20 μg/ml for deacetylcalonectrin. It should be noticed that roridin A and verrucarin A both having a

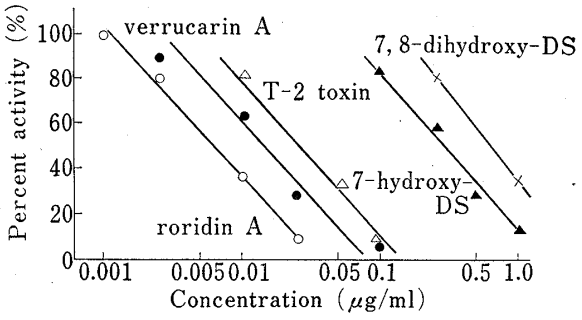

Fig. 1. Inhibitory Effects of Trichothecenes on the Uptake of ^{14}C-Leucine in Rabbit Reticulocytes

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macrocyclic ring show a lower ID 50 (1×10^{-8}M and 3×10^{-8}M, respectively) than T-2 toxin (6×10^{-8}M). These findings clearly demonstrated, as already reported,[3] that the inhibitory potency depends on a chemical nature, especially, on lipophylicity of side chains in the trichothecenes.

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Über die Cumarine der *Boenninghausenia japonica* (Sieb.) Nakai

Mitsugi Kozawa, Kimiye Baba, Masafumi Minami, Harumi Nitta und Kiyoshi Hata

Osaka Pharmazeutische Hochschule[1]

(Eingegangen am 29, Mai 1974)

Aus Rutazeen sind bisher zahlreiche Cumarinderivate isoliert worden, und für diese Familie sind die Cumarine mit der 1,1-Dimethylallyl-Seitenkette typisch. Die *Ruta*-Arten sind für uns unter anderem deshalb chemotaxonomisch interessant, weil sie Furocumarine sowie die an der Stelle 3 substituierte Cumarine enthalten, die zum großen Teil die 3-(1′,1′-Dimethylallyl)-Cumarine sind. In Japan sind diese Arten nicht wild, nur *Ruta graveolens* L. und *Ruta chalepensis* L. werden als Alzneipflanze gezüchtet, aber die ähnlichen Pflanze, *Boenninghausenia japonica* (Sieb.) Nakai (japanischer Name Matsukazeso), die früher *Ruta japonica* Sieb. genannt worden war, wächst in Japan wild, und sie interessiert uns für die Bestandteilsbeziehung mit den *Ruta*-Arten. Als Cumarine von *B. japonica* sind Bergapten (I) und Matsukaze-lacton (II)[2] isoliert worden.

In Rahmen unserer Untersuchungen über die Cumarine sowie die Chemotaxonomie von Rutazeen haben wir auch dieser Pflanze untersucht.

Die Kräuter, die auf dem Gebirge des Großbezirk Osaka gesammelt worden waren, wurden getrocknet und mit Hexan sowie Äthylacetat extrahiert und auf die in dem experimentellen Teil beschriebene Weise aufgearbeitet. Dabei erhielt man aus dem Hexan-Extrakt Xanthotoxin (III), ein weißes Blättchen (IV) vom Schmp. 87—87.5° sowie ein schwach gelbes Nadelchen (V) vom Schmp. 111—112° und aus dem Äthylacetat-Extrakt I, II, III, Umbelliferon (VI) sowie ein schwach gelbes Nadelchen (VII) vom Schmp. 249—250°.

IV ist weißes Blättchen vom Schmp. 87—87.5°, $C_{16}H_{14}O_3$ (M^+ 254). Das Ultraviolett-(UV)-Spektrum entspricht dem Substitutionstyp des Psoralens. Auf dem Chromatogramm fluoresziert es im UV-Licht blaue. Das Infrarot(IR)-Spektrum zeigt das Vorhandensein eines ungesattigten Lactons, eines aromatisierten Ringes, Furanringes und der Doppelbindung. Das Kernmagnetischresonanz(KMR)-Spektrum[3] zeigt die für die an der Stelle 3 substituierte 6,7-Furo-Cumarine typischen Signale: Zwei Singletten bei τ 2.33 (1H), τ 2.37 (1H) und ein breite Singlett bei τ 2.59 (1H) entsprechen den drei Protonen der 4-, 5- und 8-Stelle des Cumarinkomplexes, und ein Dublett bei τ 2.34 (1H, J=2) und ein Dublett-Dublett bei τ 3.20 (1H, J=2

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3) Dieses Spektrum wurde in $CDCl_3$ gemessen.