Another effect, which has not yet been further investigated, concerns the influence of erythorbic acid on L-ascorbic acid uptake by the tissues. Preliminary results obtained in a study on male guinea-pigs (220–250 g, 7 animals per group), administered orally erythorbic acid (0, 20, 50, 100 or 400 mg per day) in addition to L-ascorbic acid (20 mg per day, vitamin C-deficient diet) for three days and a single oral ^{14}C-labelled dose of L-ascorbic acid (10 µCi, specific activity 4.78 mCi/mmole) on the last day of the experiment, together with erythorbic acid, indicate a significant impairment in the uptake of the labelled vitamin C, already following administration of 50 mg of erythorbic acid per day, in the adrenal glands, the lungs, the kidneys, the testes, the eyes and in the pancreas (Student's t-test, $p < 0.01$). Administration of 100 or 400 mg of erythorbic acid caused an even further decrease in the uptake of the labelled ascorbic acid by these tissues. In addition, uptake of ascorbic acid was also significantly reduced in cerebrum, cerebellum, liver and spleen ($p < 0.01$). The percentage reduction in accumulation of the vitamin caused by administration of erythorbic acid was approximately 50% in the adrenal glands, testes, kidneys, lungs and eyes (Table).

From these preliminary results it is to be concluded that the availability of L-ascorbic acid (vitamin C) is diminished, if erythorbic acid is administered together with L-ascorbic acid.

Zusammenfassung. Eine Verabreichung von Erythorbinsäure (D-Isoascorbinsäure, D-Araboascorbinsäure) vermindert die Aufnahme von Ascorbinsäure (Vitamin C) in verschiedenen Organen des Meerschweinchens. Da Erythorbinsäure nur eine sehr geringe Vitamin-C-Aktivität besitzt, wird bei einer gleichzeitigen Einnahme von Erythorbinsäure und Ascorbinsäure die Verfügbarkeit des Vitamin C für den Tierorganismus signifikant reduziert.

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Synthesis of Ochratoxins T_A and T_C, Analogs of Ochratoxins A and C

Ochratoxins are toxic metabolites produced by several species of *Aspergillus* and *Penicillium*[1–4]. Ochratoxin A (OA), 7-carboxy-5-chloro-3,4-dihydro-8-hydroxy-3-methyl isocoumarin moiety (ochratoxin α) linked by an amide bond to L-β-phenylalanine through the 7-carboxyl group, and ochratoxin C (OC), the ethyl ester of OA, are the most toxic metabolites within this series. In contrast, ochratoxin α (Oα) has not proven toxic to test animals[5,6]. In order to pinpoint the influence of the side chain in the Oα moiety on the toxicity of OA, we have investigated the effect of substituting tyrosine for phenylalanine. This paper presents a method for the synthesis of ochratoxin T_A (OT_A) and T_C (OT_C), and describes the physicochemical properties of these analogs.

Materials and method. Ochratoxin A was produced in rice by *Aspergillus ochraceus* 3174 as described[7,8]. Ochratoxin α was prepared by acid hydrolysis of natural crystalline OA[5], and was purified by Adsorbosil chromatography[5].

Thin layer chromatography (TLC). Silica gel G and silica gel H (both from Brinkmann Instruments Co.), coated to the glass plate to a thickness of 0.25 mm and 0.5 mm respectively, were used for analytical and preparative TLC. Benzene: acetic acid (3:1) was used as a developing reagent. The fluorescent spots or bands were detected under a longwave u.v. light or charred with H_2SO_4.

Preparation of OT_C and OT_A. 25 mg tyrosine ethyl ester and 27 mg of ethoxy-1-ethoxy carbonyl-1,2-dihydroquinoline (EEDQ) were added to a tetrahydrofuan solution containing 27 mg of Oα. The mixture was then stirred at room temperature overnight, filtered, and evaporated. Crystals were formed after addition of Skellysolve B to the oily residue, to which a small amount of ethyl acetate was added. The crystals were redissolved in EtOH,

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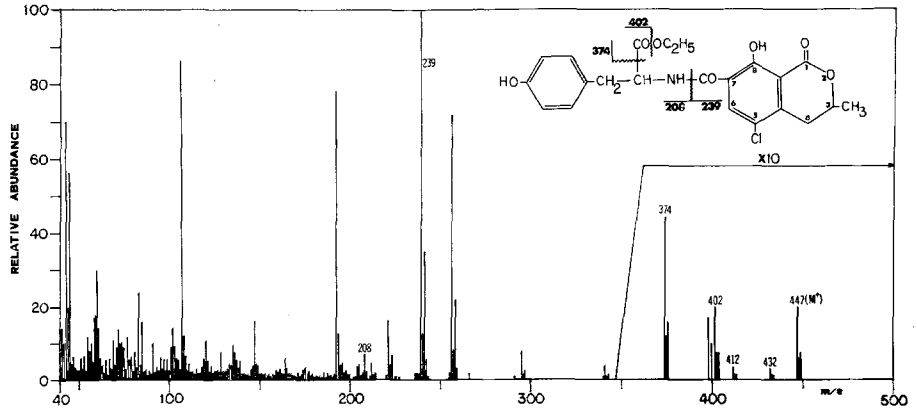

Mass spectrum and structure of ochratoxin T_C.

and applied to 4 preparative plates for TLC. The green fluorescent band (Rf 0.47) containing purified OT_C was scraped from the developed plates and eluted with methanol. 50 mg of OT_C was recovered after the methanol was evaporated.

Ochratoxin T_A was prepared by alkaline hydrolysis of OT_C. 6/10 ml of 1 *N* NaOH was added to an OT_C solution (20 mg in 1.2 ml EtOH, the solution turned from a light pink to a faint yellow upon addition of NaOH). Upon dilution with distilled water, acidified with 1 *N* HCl, the mixture was extracted exhaustively with ethyl ether, evaporated, and then applied to four preparative TLC plates. After developing, the plates showed two fluorescent bands, one at the origin (blue) and the other with an Rf of 0.26 (greenish). The latter band was scraped from the plates, and then eluted with methanol. 14 mg of OT_A was obtained after the MeOH was evaporated.

Results and discussion. Characterization of OT_C and OT_A. Thin layer chromatography of the purified analogs at 10 µg per spot revealed only 1 round green fluorescent spot at an Rf value of 0.47 for OT_C and 1 of 0.26 for OT_A. The Rf value for OA standard was found to be 0.57 under the same conditions. Tyrosine was the only amino acid detected (Beckman Spinco Model 120B amino acid analyzer) after hydrolysis in 6 *N* HCl at 110 °C for 48 h. Ochratoxin T_A was readily converted back to OT_C upon esterification with EtOH in BF_3. Although mass spectral analysis (Associated Electrical Industries, Ltd. M-9 mass spectrometer) for OT_A was unsuccessful because of the low volatility of the product, excellent mass spectral results for OT_C were obtained (Figure). The presence of halide (chlorine) ion in the molecule was evident from the absorption intensities of a number of ion peak pairs, such as at *m/e* 447 and 449, 402 and 404, 374 and 376, 239 (base peak) and 241, having a ratio of approximately 3 to 1. The molecular weight of OT_C as determined by the mass spectrometrical method was found to be 447 (requires 447). The absorption maxima of OT_C above 300 nm resembled those of OA (λ maxima at 330 nm and 380 nm), and were further found to be dependent upon pH and environmental conditions[5]. Molar absorptivities ε (calculated on the basis of tyrosine content) for OT_C at 330 nm and 380 nm were found to be 2610 and 9660 in EtOH, and 7570 and 200 in acidic EtOH (2 ml EtOH and 1 ml 0.1 *N* HCl), while those for OT_A were found to be 9770 in EtOH (380 nm) and 7770 in acidic EtOH (330 nm). Spectrophotometrical titration[5] revealed that the dissociation constants for the phenolic hydroxyl group in the isocoumarin ring of OT_C and OT_A were 6.41 and 7.09 (compared to values of 7.14 for OC nad 7.05 for OA; ref. [5]) respectively. $[\alpha]_D^{25}$ for OT_C and OT_A in EtOH were found to be −41.2 (c. 0.28) and −141 (c. 1.3).

Chicken embryo assay. The toxicities of OT_C and OT_A were tested by chicken embryo assay as previously described, and the results are shown in the Table. Ochratoxin T_C was found to be slightly more toxic than OA, but OT_A is slightly less toxic than either of these[5]. Since OT_C has a lower pK for the phenolic hydroxyl group than does OA, whereas OT_A has a higher pK value than OA, these results support our previous postulation regarding the importance of the dissociation of the phenolic hydroxyl group in ochratoxin for intoxication. Nevertheless, studies on other animal systems will have to be carried out to pinpoint the lesions on target organs or tissues before any conclusions can be made concerning the mode of action of these new analogs[9].

Testing of culture filtrates of *Aspergillus ochraceus* and *Penicillium viridicatum*. In an attempt to find whether some ochratoxin-producing fungi might produce OT_C and OT_A, crude extracts obtained from rice in which *A. ochraceus* 3174 and *P. viridicatum* were grown for 1 week at room temperature were analyzed by TLC method. No fluorescence spots having Rf values resembling those of OT_C and OT_A were detected. It was therefore concluded that these 2 cultures, at least, do not produce these analogs in nature under the conditions described. Whether other ochratoxin producers synthesize these analogs, and whether the same organisms we tested produce these analogs when incubated under other conditions, are two questions which remain to be investigated[10].

Zusammenfassung. Zwei Ochratoxinanaloge, Ochratoxin T_A (OT_A) und T_C (OT_C) wurden aus Ochratoxin-α chemisch synthetisiert indem Phenylalanin und sein Äthylester im Molekül durch Tyrosin und Tyrosinäthylester substituiert wurden. Der Hühnerembryonentest ergab, dass OT_C etwas toxischer und OT_A etwas weniger toxisch ist als Ochratoxin A (OA).

Toxicity of OT_A and OT_C to 8-day-old chicken embryos

Analogs	Amount injected (µg/egg)	Toxicity (No. dead/No. surviving embryos, 2 weeks after injections)
OT_C	28	3/3
	14	9/10
	7	7/10
	3.5	1/10
OT_A	21	6/10
	10	1/10
	5	1/10

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Blockade of the Hydrosmotic Effect of Vasopressin by Cytochalasin B

Cytochalasin B (CB) is a macrolide antibiotic independently isolated by Turner in England[1] and by Rothweiler and Tamm[2] in Switzerland, under the name of phomin. Since the report by Carter in 1967[3], CB has been intensively investigated in many cell systems. It has been shown that this substance interacts with microfilaments, disorganizing the ectoplasmic cell web[4,5] and alters several cell functions, such as cytokinesis, motility,