Cadmium is a toxic metal which is increasingly finding its way into our environment as an industrial pollutant[8]. Since absorbed cadmium is very slowly excreted, the body burden increases with age[9]. It is this cumulative nature of cadmium that makes it potentially toxic at low environmental concentrations. The results presented here indicate that cadmium potentiates sleep time induced by hexobarbital. The peak effect following cadmium treatment starts on day 2 and extends to day 5. The short-term nature of this peak effect may be related to the fact that cadmium is known to induce the synthesis of a cadmium-binding protein, metallothionine[10,11]. Conceivably, the presence of this protein could bind cadmium, thus rendering the metal inert. The minimal effective dose of cadmium ion required to potentiate the sleep time was 840 µg/kg which corresponds to 20% of the LD_{50} dose of cadmium ion as determined in our laboratory. In any study designed to link contaminant burdens with biological response and environmental exposure, it is of utmost importance to establish a dose-response relationship between the contaminant being examined and the specific changes induced in the biological system[12].

Zusammenfassung. Nachweis, dass Cadmium bei Ratten die Hexobarbital-Schlafdauer erhöht. Eine wirksame Reaktion war bereits bei einer Cadmium-Dosis von 840 µg/kg beobachtet, und die Schlafdauer wurde bereits nach einer einzigen Dosis vom 1. bis zum 10. Tage erhöht, wobei zwischen dem 2. und 5. Tage die Maximalwirkung beobachtet wurde.

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Toxic Metabolites of *Aspergillus candidus*

In our course of chemical and biological surveys on toxic mold metabolites, a strain (69-SA-156 = NHL 5107) of *Aspergillus candidus* Link was noticed by the production of new toxic metabolites[1,2]. The extracts of the mycelium and the filtrate on potato-dextrose medium, or of the culture on rice, were separated by chromatographic methods and monitored by cytotoxicity test using HeLa cells[1,2] and 2 new toxins, tentatively named toxin A and B, were isolated.

The toxin A, m.p. 244–245°, $C_{20}H_{18}O_5$, has apparently no effect on mice with a dose of 200 mg/kg by single oral or subcutaneous administration. It evokes, however, characteristic morphological changes on HeLa cells; that is, slightly enlarged cells with faintly stained cytoplasm, evenly distributed chromatin and relatively small nucleoli. 50% growth inhibitory dose is approximately 10 µg/ml. The growth is recoverable when the cells are treated for 24 h and placed in the control medium. No remarkable change is observed in the chromosome preparation of affected cells. Whereas incorporation of ^{3}H-thymidine and -uridine into the treated cells at 32 µg/ml is completely supressed, that of ^{3}H-leucine is retained as in control cells. It has been known that the similar characteristic change of cultured cells is produced by the administration of mycophenolic acid[2] and the effect is nulified by the concomitant administration of guanine[3]. In the case of the present compound, however, the normal growth cannot be restored by the simultaneous addition of any purines or pyrimidine nucleosides.

The toxin A forms the triacetate and the spectral data (especially NMR) indicated the presence of 2 methoxyls, 3 phenolic hydroxyls, 2 pairs of A_2B_2 type *o*-coupled aromatic protons, and 1 singlet aromatic proton in the toxin[4]. The methylation of toxin A with diazomethane gave a mixture of mono-, di-, and tri-methyl ethers. The oxidative degradation of toxin A and the dimethyl ether afforded *p*-hydroxylbenzoic acid and *p*-anisic acid respectively. Treatment of toxin A with boron trifloride gave an unstable polyphenol, the oxidation of which by ferric chloride or chromiun trioxide gave a quinone, $C_{18}H_{12}O_5$. The quinone forms the triacetate. The UV-spectra of the quinone ($\lambda_{max}^{dioxane}$ 255, 390 nm) and the acetate ($\lambda_{max}^{dioxane}$ 345 nm) suggested the *p*-terphenyl-quinone chromophore in the quinone[5]. The above evidence suggested the structures (**1**–**3**) for the toxin A.

The terphenyl metabolite by the fungus *A. candidus* reported recently by Marchelli and Vining[6] is assumed to be identical with the toxin A. They proposed the structure **1** for the compound but the evidence shown in

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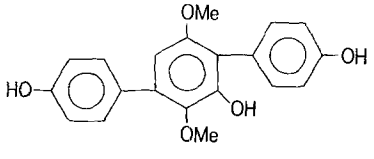

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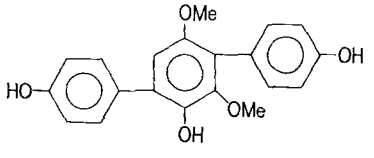

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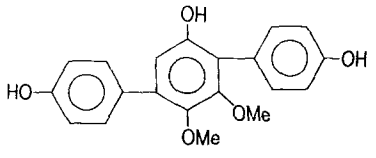

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their paper does not contradict the other isomeric structures including those having *o*- and *m*-terphenyl nucleus. The synthetic confirmation of the structure is now in progress.

The toxin B, yellow needles of m.p. 165–170° (decomp.), $C_{23}H_{20}O_2N_2$, also shows cytotoxicity on cultured HeLa cells. The 50% growth inhibitory dose is about 6 µg/ml, and irregularity in cell size and round eosinophilic nucleoli are the morphological changes produced. No remarkable differential supression of ^{3}H-precursors of biopolymers is observed; that is, the incorporation of ^{3}H-thymidine, -uridine and -leucine into the cells treated at 32 µg/ml is below 10% compared to those into the control cells. It also shows an acute toxicity on mice, killing them with doses less than 100 mg/kg body weight by single s.c. injection. It acts rather slowly and caused diffuse hepatotoxic lesion with general jaundice. The chemical investigation on toxin B is also in progress.

Zusammenfassung. Zwei neue Toxine wurden aus den Mycelien und dem Zuchtmedium eines Schimmelpilzes, *Aspergillus candidus*, sowie aus dem experimentell mit demselben Pilz verschimmelten polierten Reis isoliert. Die beiden Substanzen sind sowohl chemisch als auch toxikologisch voneinander völlig verschieden.

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Adsorption to Activated Charcoal and Polarity of Cardenolides and Bufadienolides

For treatment of glycoside intoxication, adsorption of the glycosides to orally applied cholestyramin was recommended[1]. Since cardiac glycosides, except for ouabain, undergo enterohepatric circulation to varying extend, this treatment is suitable not only for oral but also for intravenous glycoside intoxication[1]. HAACKE et al.[2] recently showed adsorption to charcoal of ^{3}H-digitoxin and its metabolites in guinea-pig bile.

The aims of the present study were to establish data on the in vitro adsorption to charcoal of a series of glycosides and derivatives and to solve the question of whether or not the adsorption is influenced by the polarity of these substances.

Material and methods. Digitoxin, lanatoside C and ouabain from E. Merck, Darmstadt; digoxin, β-methyl digoxin and digoxigenin were from Boehringer, Mannheim; proscillaridin, proscillaridin-4′-methyl ether, 3-β-scillarenin and scillarenin-3-β-methyl ether were from Knoll, Ludwigshafen, Germany. Concentrations of the glycosides were determined photometrically (Zeiss PMQ III). For the bufadienolides adsorption in methanol-KOH was measured at 355 nm[3]. With the cardenolides adsorption in H_2SO_4 was measured at 235 nm[4]. Both reactions showed a linear relationship between glycoside concentration and extinction, at least in the tested range of concentrations.

Adsorption of the substances to charcoal. The assays contained 300 µg/ml of the glycoside resp. derivative in 300 µl/ml of ethanol (70%) and 0.025 to 15.0 mg/ml of activated charcoal in aqueous phase. They were incubated for 15 min at 22 °C under continuous shaking. Then the charcoal was sedimented by centrifugation at 4000 *g*. Glycoside concentration in the supernatant was determined and expressed in percent of an assay without charcoal. The results were transferred to semi-logarithmic plots and from this the charcoal concentration was determined which binds 50% of the substance.

Polarity of the substances. 0.8×10^{-5} moles of the substance were shaken mechanically for 30 min in a glass tube containing 2 ml H_2O, 3 ml isopropanol and 5 ml

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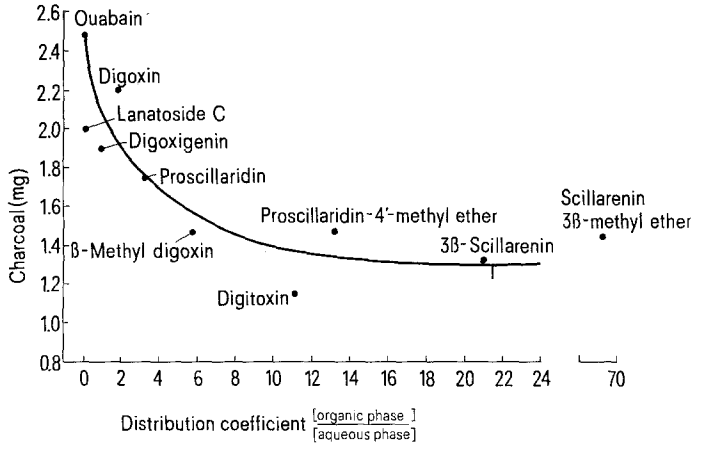

Correlation between polarity of the glycosides/derivatives and charcoal binding. Abscisse: The polarity is given as the quotient of concentrations in organic and aqueous phase of a H_2O, isopropanol, and carbon tetrachloride mixture. Ordinate: mg of activated charcoal needed to bind 50% of 300 µg of the glycosides/derivatives.