Toxicity and Mutagenicity of Molds of the *Aspergillus glaucus* Group. Identification of Physcion and Three Related Anthraquinones as Main Toxic Constituents from *Aspergillus chevalieri*

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Several strains of the *Aspergillus glaucus* group were screened for their production of known mycotoxins, and the toxicity of organic extracts of the cultures was tested. One strain of *A. chevalieri*, ZT 8268, was selected for further investigation. Four anthraquinone derivatives were isolated and identified by UV, IR, NMR, and mass spectra. These compounds, physcion, physcionanthrone (described as physciondianthranol; J. N. Ashley, H. Raistrick, T. Richards *Biochem. J.* **1939**, *33*, 1291), physcionanthrone B, and erythroglaucin, were tested for toxicity in the mouse and chicken embryo (except dianthrone), 1-day old cockerels and for mutagenicity (except dianthrone) in the "*Salmonella*/mammalian microsome" test (strains TA 1535, TA 100, TA 1537, and TA 98). The isolated compounds were found to be toxic by intraperitoneal injection in the mouse and in the chicken embryo test and were positive in the mutagenicity test (strain TA 1537). No toxic effects could be demonstrated after oral application in the mouse and in 1-day-old cockerels.

Toxicity of *Aspergillus chevalieri* to animals was first reported by Carll et al. (1954). Organic extracts of wheat inoculated with this fungus were found to cause severe lesions after dermal application. Feeding of *A. chevalieri* infected bread to calves resulted in death of the animals within a few days. Extensive hemorrhages occurred in chickens receiving ground wheat infected with the same fungus for 13 days (Schumaier et al., 1961). Scott (1965) reported toxicity of *A. chevalieri* infected corn to 1-day-old ducklings, and Kinosita and Shikata (1965) observed hepatotoxicity in mice after feeding rice infected with the same fungus. In an extensive survey for the detection of mycotoxin-producing fungi, Saito et al. (1971) found some toxicity of members of the *Aspergillus glaucus* group against HeLa cells and mice. In all these investigations only part of the selected *A. chevalieri* strains proved to be toxic. Whereas in most strains toxicity could be attributed to known mycotoxins, a few representatives of the *A. glaucus* group were described to develop oral and intraperitoneal toxicity not induced by known *Aspergillus* toxins (Reiss, 1972). In a survey of toxic fungi (Umeda et al., 1974), culture filtrates but not mycelial extracts of *A. chevalieri* were shown to be toxic to HeLa cells. The toxic metabolites were not identified.

The present investigation was undertaken to study the toxicity of several strains of the *Aspergillus glaucus* group and to identify some of the biologically active compounds. In extracts of *A. chevalieri* cultures, none of the known *Aspergillus* mycotoxins such as aflatoxins, sterigmatocystin, ochratoxin A, rubratoxin B, and patulin could be detected. Nevertheless, intraperitoneally administered culture extracts were highly toxic. It was therefore suspected that anthraquinones which represent a considerable amount in organic extracts (Ashley et al., 1939) could be responsible for the toxic action.

Out of several hundreds of naturally occurring anthraquinones (Thomson, 1971), a small part has been subjected to limited toxicological testing (e.g., Enomoto and Ueno, 1974; Tatsuno et al., 1975; Wells et al., 1975). Only recently results of three mutagenicity studies on approximately 190 naturally occurring and synthetic anthraquinones showed about one-third of them to cause frameshift mutations (Brown and Brown, 1976; Brown et al., 1977; Brown and Dietrich, 1979). "DNA attacking ability" has been demonstrated for a few anthraquinones (Swanbeck, 1966; Ueno and Kubota, 1976), and carcinogenicity was found for the 2-methyl-1-nitroanthraquinone (U.S. DHEW, 1978) and for the dimeric anthraquinone derivative luteoskyrine (Uraguchi et al., 1972).

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EXPERIMENTAL SECTION

Microorganisms. Several strains from the *Aspergillus glaucus* group isolated and described by Blaser (1974/75) were used for screening tests for known mycotoxins. Most of the chemical and toxicological studies were performed on culture extracts of *Aspergillus chevalieri* ZT 8268 previously isolated by Blaser (1974/75). The fungi were grown on a liquid medium containing 3% malt extract (Oxoid, L 39), 0.05% yeast extract (Merck, No. 3753), and 5.2% NaCl at 28 °C for 11 days. Detailed culture techniques and conditions will be described by Blaser (unpublished data). Cultures were kindly provided by P. Blaser, Institute of Food Science, ETH Zürich.

Extraction of Fungal Metabolites. Culture medium and mycelium were homogenized together in a Waring blender for 5 min and extracted three times with equal volumes of dichloromethane. The dark-red extract was concentrated in a Rotavapor, dried under vacuum for 24 h, and stored at 4 °C. The yield was approximately 1 g of extract/L of culture medium for strain ZT 8268. In order to isolate the toxic principle of strain ZT 8268, dried crude dichloromethane extract was dissolved in methanol and fractionated on a Sephadex LH-20 column. The different fractions were dried, subjected to TLC, and tested for toxicity to white mice.

Screening for Known Mycotoxins. A number of strains of the *A. glaucus* group were screened for the presence of known mycotoxins following the TLC methods of Reiss (1973) and Stoloff et al. (1971). Reference mycotoxins were purchased from Fluka, Switzerland (aflatoxins B_1, B_2, G_1, G_2, sterigmatocystin, rubratoxin B, ochratoxin A), and Serva, FRG (Patulin). A sample of gliotoxin was kindly provided by Y. Kishi, Harvard University, Cambridge, MA.

Toxicity Tests. (*a*) *Chicken Embryo Inoculations.* The method of Verrett et al. (1964) for chicken embryo inoculation was modified in the following points. (1) Test solutions were injected into the air cell after 4 days of incubation. (2) The vehicle used for injections was acetone or propylene glycol (1,2-propanediol). (3) The drilled hole for injection was not larger than 1 mm and was sealed with

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Table I. Toxicity of Dichloromethane Extracts from 11-Day Cultures of Some Selected Strains of the *A. glaucus* Group, to Male Mice (18-20-g Vehicle Used Was Me_2SO If Not Otherwise Stated)

strain/culture no.	classification	LD_{50} ip, mg/kg	LD_{50} po[a] mg/kg
ZT 2868/I	*A. chevalieri* (Mangin)	65	>1800
ZT 8268/II	*A. chevalieri* (Mangin)	<30 (PEG 400)	>2000 (PEG 400)
ZT 8268/III	*A. chevalieri* (Mangin)	<10 (corn oil)	>2000 (corn oil)
ZT 8268/IV	*A. chevalieri* (Mangin)	20	>2000
ZT 8202	*A. repens* (De Bary)	20	>1800
ZT 8203	*A. repens* (De Bary)	30	>1600
ZT 8218	*A. repens* (De Bary)	<30	>1800
ZT 8233	*A. herbariorum* (Link ex Fries)	<30	>1800
ZT 8298	*A. heterocarioticus* (= CBS 410.65)	940	>1800
ZT 8305	*A. pseudoglaucus* (= CBS 123.28)	<30	>2000
ZT 8306	*A. repens* (De Bary)	50	>1900

[a] Highest dose treated.

warm paraffin after inoculation. Groups of ten fertile white Leghorn eggs were inoculated with 0.015–0.025 mL of the test solution, sealed, and incubated at 38.2 °C and 60% humidity. A control group of 15 eggs received the same volume of vehicle.

The eggs were candled three times a week and all nonviable removed. Relative toxicity of a substance was based on the number of eggs that did not survive through the hatching period compared to control eggs.

(*b*) *Single-Dose LD_{50} in Mice.* Male Swiss albino ICR mice, weighing 20–22 g, were used in groups of 4–6 animals for oral and ip administration. Test substances were given in 0.1- or 0.2-mL vehicles. To eliminate possible solvent effects, different vehicles were used. Single-dose LD_{50} values were determined for crude fungal extract, culture filtrate, methanol-soluble, and insoluble fraction, the purified metabolites D1, D2, D3, D4, and emodin as a reference anthraquinone. LD_{50} values were calculated using the method of Litchfield and Wilcoxon (1949).

(*c*) *Single-Dose Toxicity in 1-Day-Old Cockerels.* Groups of four 1-day-old Lohmann selected Leghorn cockerels were used, receiving 0.1 mL of test solution (Me_2SO or polyethylene glycol 400 as vehicle) via crop intubation (Kirksey and Cole, 1974). Water and feed were supplied ad libitum, and weight was controlled on days 1, 4, 8, and 10. Controls received 0.1 and 0.2 mL of Me_2SO, polyethylene glycol 400 (PEG 400), emodin in Me_2SO, and emodin in PEG 400, respectively. Me_2SO and PEG 400 were analytical grade (Merck, FRG), and emodin was technical grade (Fluka, Switzerland).

(*d*) *Short-Time Feeding Experiment in Mice.* The dried dichloromethane extract of an 11-day culture was used in a feeding experiment. Three groups of six mice, weighing 18–20 g, were dosed daily by gavage for 12, 24, and 35 days, respectively. The daily dose was 6 mg/kg. The fungal extract was dissolved in Me_2SO (20%) and mixed with corn oil (80%) to give 0.1 mL of solution/animal. A control group of six animals received 0.1 mL vehicle/day. Weight was recorded every 2 days. The animals were killed after 13, 25, and 36 days, respectively, and liver and kidneys were removed and fixed for histological examination.

Histological Preparation. Tissue specimens were removed from the animals at the end of the experiment, fixed in 4% buffered formaline, put in paraffin blocks, and stained with hematoxylin and eosin.

Gel Filtration Chromatography. Fractionation of crude extracts from strain ZT 8268 was achieved on Sephadex LH-20 columns (2 × 60 cm) using methanol as eluant. Fractions were concentrated on a Rotavapor and tested for toxicity. The problem of separating physcion and erythroglaucin (Steglich and Reininger, 1972) could be solved by gel filtration on the same system using 5 × 40 cm columns and methanol at a flow rate of 60–80 mL/h. Corresponding fractions of several runs were combined and checked for purity by TLC.

Identification of Isolated Metabolites. TLC was carried out using silica gel plates (Merck, No. 5553, 5554) developed in benzene–methanol–acetic acid (BMA, 18:1:1) and hexane–acetone–acetic acid (HAA, 18:2:1) according to the method of Stoloff et al. (1971). Compounds were located under visible and UV light (254 and 366 nm).

Melting point determinations were carried out using a Büchi melting point apparatus.

Mass spectra were determined on a Hitachi/Perkin-Elmer RMU-6M double-focus, high-resolution spectrometer with an ion source of 70 eV. A field desorption mass spectrum was taken on a Varian MAT 311A. NMR spectra (90-MHz) were taken with a Brooker WH-90 and chemical shifts were expressed in ppm from $(CH_3)_4Si$ as internal reference and coupling constants in hertz. Abbreviations used are: s = singlet, d = doublet, m = multiplet.

Tests for Mutagenicity. The mutagenic effects of the isolated anthraquinones were tested on *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537, kindly provided by Dr. B. N. Ames (University of California, Berkeley). Tests were carried out following the method described by Ames et al. (1975). Mutagenic activity was tested directly and in the presence of the hepatic S-9 enzyme preparation from Aroclor 1254 induced albino rats ZUR-SIV/Z. The compounds were tested in concentrations of 200 μg/plate with strains TA 98 and TA 100 and 100 μg/plate with strains TA 1535 and TA 1537. All experiments were performed in duplicate. Negative controls for spontaneous reversion were run with all strains. Positive controls for induced reversion employed β-naphthylamine. 2-Aminofluoren was used as a control to confirm activity of the S-9 enzyme preparation. Since pilot studies with physcion and erythroglaucin (5 and 1.25 mg/kg, ip) in the micronucleus test (Schmid, 1976) on mice gave negative results, the test was not run with other extracts or substances.

RESULTS AND DISCUSSION

Screening Tests for Known Mycotoxins and Mammalian Toxicity. All strains tested for the production of the mycotoxins, aflatoxins B_1 and B_2 and G_1 and G_2, sterigmatocystin, ochratoxin A, rubratoxin B, and patulin were negative. Gliotoxin was not detectable in the tested strain ZT 8268.

Dichloromethane extracts from a number of selected strains were tested for toxicity in white mice. No strain tested produced any toxic effects after oral administration but all except one (strain ZT 8298) showed considerable toxicity after ip injection (Table I). The animals showed abdominal bloating and impaired locomotion. Severe ob-

Table II. Yield and Toxicity of Fractions after Chromatography of Crude Dichloromethane Extract of Strain ZT 8268 on Sephadex LH-20 to Mice (18-20 g)

fraction	%	approx. LD_{50}, ip, mg/kg
9-11	28	300
12-17	36	>1080[a]
18-20	10	200
21-28	10	30
29-34	4	>135[a]
35-45	4	>120[a]
46 ff	7	>200[a]
crude extr.	100	30

[a] Highest dose tested.

stipation and extensive peritoneal adhesions as a result of strong local irritation were found at postmortem examination. Strain ZT 8298 in contrast to the other strains tested showed only weak ip toxicity and on TLC absence of the toxic anthraquinones described later.

Studies on *A. chevalieri* ZT 8268. (*a*) *Fractionation of ZT 8268 Extract.* Results of toxicity testing of the fractions from Sephadex chromatography are listed in Table II. Fractions 21–28 were found most toxic. A similar high toxicity was observed for the residue obtained after dissolving crude extract in methanol. The poor solubility in methanol was therefore chosen as a first purification step in isolating the toxic components.

(*b*) *Isolation of Toxic Metabolites* (*Figure 1*). To 1 g of crude extract 200 mL of methanol was added. One hundred milligrams of pink, insoluble material was filtered off and the methanol solution was evaporated, dissolved in benzene (50 mg/mL), and chromatographed with the same solvent in portions of 5 mL on silica gel columns (2.5 × 25 cm). The first yellow fraction was collected, and on concentration under N_2, pale-yellow needles (D3, 25 mg) were deposited. The pink residue (100 mg) was dried, dissolved in $CHCl_3$ (5 mg/mL), and fractionated on four silica gel columns (2.5 × 25 cm) using $CHCl_3$ as eluant. The yellow fractions were combined and dried under vacuum. The residue was dissolved in 500 mL of hot methanol. This solution on cooling yielded tiny white needles (D4, 10 mg). The methanol solution was chromatographed with methanol in portions of 25 mL on a Sephadex LH-20 column (5 × 50 cm), yielding a yellow and a red fraction.

(*c*) *Chemical Identification of Biologically Active Constituents in A. chevalieri ZT 8268.* The residue (5 mg) of the red colored fraction from Sephadex LH-20 was rechromatographed on a silica gel column in $CHCl_3$ and crystallized twice from ethyl acetate to dark-red leaflets and red needles (D1): mp 203 °C; UV λ max MeOH/dioxan (10:1) (log ε) 231 (4.38), 256 (4.08), 278 (3.96), 302 (3.83), 468 (3.83), 480 (3.88), 491 (3.92), 510 (3.80), 524 nm (3.74); IR ν max (KBr) 3360–3340, 2920, 1735, 1602, 1495, 1445, 1390, 1308, 1262, 1210, 1165, 1135, 1092, 1025, 950, 925, 880, 805 cm^{-1}; NMR ($CDCl_3$) δ 2.36 (s, C–CH_3), 3.95 (s, C–O–CH_3), 6.71 (d, J = 1.5 Hz, C_2–H), 7.14 (br s, C_7–H), 7.42 (d, J = 1.5 Hz, C_4–H), 12.36 (s, OH), 12.45 (s, OH), 13.37 (s, OH); MS, molecular ion peak at m/e 300, fragment ions at m/e 284, 270, 257, 243, 229, 201.

Direct comparison of D1 with an authentic sample of erythroglaucin (Figure 2) kindly provided by Professor Barton (CNRS, France) by TLC (R_f 0.61 in HAA), UV, and mass spectrometry showed no differences. The melting point was not depressed by mixture of the two samples. All spectral data were in good accordance with those published by Suemitsu et al. (1977).

The yellow fraction (50 mg) was crystallized twice from ethyl acetate to yellow needles (D2): mp 202–204 °C; UV

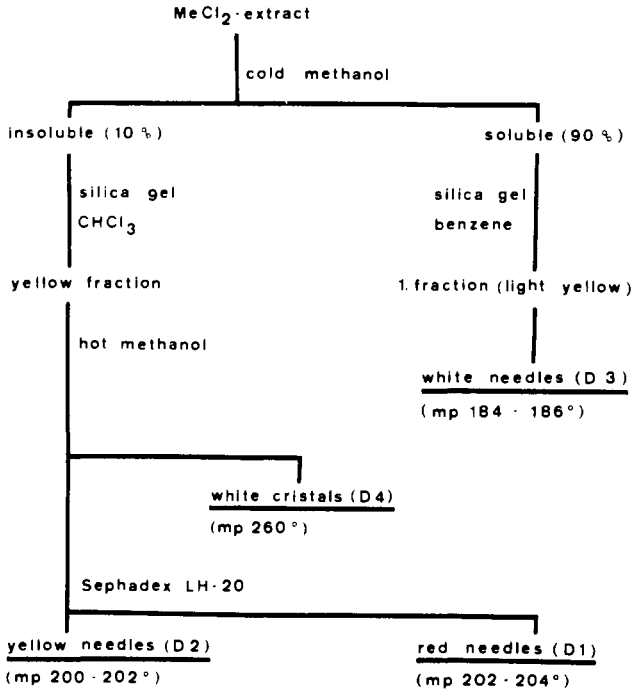

Figure 1. Scheme for the isolation of four compounds from *A. chevalieri* ZT 8268 responsible for ip toxicity in mice.

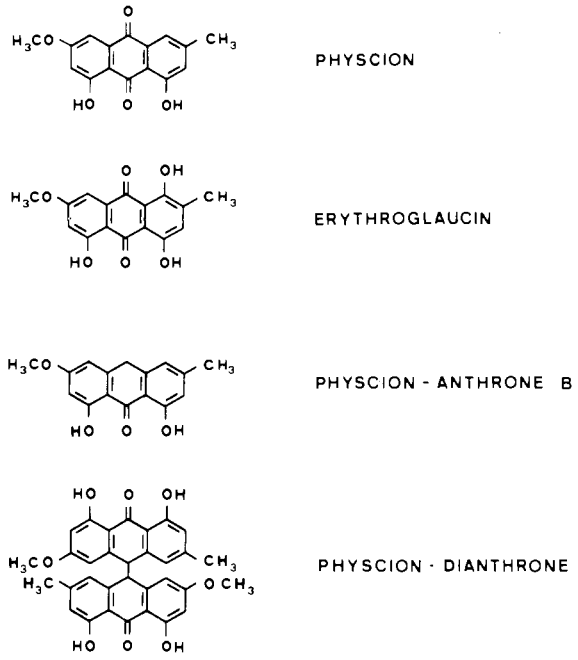

Figure 2. Chemical structures of the four compounds isolated from *A. chevalieri* ZT 8268.

λ_{max} (MeOH) (log ε) 224 (4.28), 255 (3.94), 265 (3.95), 287 (3.92), 435 nm (2.70); IR ν_{max} ($CHCl_3$) 2940–3040, 2840, 1680, 1629, 1614, 1570, 1486, 1390, 1368, 1306, 1262, 1105, 1100, 1034, 992 cm^{-1}; NMR ($CDCl_3$) δ 2.40 (s, C–CH_3), 3.90 (s, C–O–CH_3), 6.65 (d, J = 2.0 Hz, C_7–H), 7.06 (m, C_2–H), 7.35 (d, J = 2.0 Hz, C_5–H), 7.59 (m, C_4–H), 12.06 (s, OH), 12.26 (s, OH); MS, molecular ion peak at m/e 284. Fragment ions of methyl-, methoxy-, and hydroxyanthraquinones at m/e 255 (M – CO – H), 241 (M – CO – CH_3), 227 (M - 2CO - H), 213 (M – 2CO – CH_3), 198 (M – 2CO – CH_2O), 185 (M – 3CO – CH_3). Direct comparison of D2 with an authentic sample of physcion (Figure 2) kindly received from Professor M. Takido (Tokyo) by TLC (R_f 0.53 in HAA), UV, and mass spectrometry showed no differences. The melting point was not depressed by

mixture of the two samples. All spectral data were in good accordance with these published by Suemitsu et al. (1975).

The pale-yellow needles (D3) were recrystallized twice from benzene: mp 184–186 °C; UV λ_{max} ($MeCl_2$) (log ϵ) 231 (4.47), 256 (4.08), 273 (4.12), 304 (4.07), 355 nm (4.36); IR ν_{max} (KBr) 1647, 1620, 1600, 1485, 1398, 1380, 1360, 1330, 1280, 1250, 1220, 1186, 1160, 1070, 1032, 980, 946, 904, 836, 792 cm^{-1}; NMR ($CDCl_3$) δ 2.35 (s, C–CH_3), 3.85 (s, C–O–CH_3), 4.21 (br s, C–H_2), 6.39 (m, 2 H), 6.67 (br s, 2 H), 12.23 (s, OH), 12.64 (s, OH); MS, molecular ion peak at m/e 270. Fragment ions at m/e 255, 242, 241, 227, 210, 199, 184, 181, 152, 139, 128, 121, 115. The compound had an R_f value of 0.47 in HAA and gave the color reactions described by Ashley et al. (1939) for physcionanthranol B (Figure 2) and on oxidation with chromic acid yielded a substance which in TLC and mixture melting point determination proved to be identical with physcion. Although it was not possible to obtain an authentic sample of physcionanthranol B, there seems to be little doubt about the identity.

The NMR spectrum shows two singlets at δ 12.23 and 12.64, corresponding to the two phenolic OH in positions 1 and 8 and a broad singlet at δ 4.41 (2 H) for the two protons at C-9. Anthralin (1,8,9-trihydroxyanthracene) gives an adequate signal at δ 4.40 (Segal et al., 1971), indicating that these compounds are preferably in the semiquinone form. From these NMR data there is good evidence that the compound is present in the tautomeric form (Segal et al., 1971) of physcionanthrone B (physcion-9-anthrone).

The white needles (D4), mp 260 °C (decomposition), were recrystallized from hot methanol: UV λ_{max} (MeOH) (log ϵ) 279 (3.85), 360 (3.95); IR ν_{max} (KBr) 3360–3480, 2920, 2850, 1730, 1640, 1620, 1600, 1488, 1370, 1329, 1312, 1282, 1260, 1220, 1190, 1165, 1070, 902 cm^{-1}; NMR (Me_2SO) δ 2.21 (s, C–CH_3), 2.27 (s, C–CH_3'), 3.81 (s, C–O–CH_3), 3.86 (s, C–O–CH_3'), 4.63 (s, C_9–H, C_9–H), 6.09 (m, H), 6.21 (m, H), 6.30 (m, H), 6.42 (d, H), 6.49 (s, H,H'), 6.70 (m of d, H,H'), 11.63 (s, OH), 11.69 (s, OH'), 11.89 (s, OH), 11.95 (s, OH'); MS, molecular ion peak at m/e 538 (4.0), fragment ions at m/e 534 (4.2), 270 (100).

The compound D4 (R_f 0.28 in HAA) on oxidation with chromic acid yielded a substance which on TLC proved to be identical with physcion. Although physicochemical data, i.e., melting point, solubility, color tests, and C, H analysis, agree with data published by Ashley et al. (1939) for physcionanthranol A (Figure 2), it was not possible to confirm this structure by NMR data. In contrast to the NMR spectrum of anthrone B, showing a singlet at δ 4.21 (in Me_2SO) for the two protons at C_9, D4 gave a singlet at δ 4.63 for one proton at C_9. The methyl and methoxy groups appeared as two singlets with a difference of 5.5 and 6.0 Hz, respectively, in chemical shift. These NMR data and the results of an additional field desorption mass spectrum led to the conclusion that the compound described by Ashley et al. (1939) as physcionanthranol A in fact is the physcion-9,9'-dianthrone (Labadie, 1970).

(d) Biological Properties of Extracts and Metabolites of A. chevalieri ZT 8268. Toxicity to White Mice. Results of toxicity tests in white mice of different extracts and purified metabolites are listed in Table III. None of the orally administered compounds produced any symptoms except for a weak laxative action. Local irritations after intraperitoneal administration were less pronounced with purified metabolites than with the crude fungal extract. Emodin was included in the test as a commercially available reference anthraquinone, widespread in vegetable laxatives and probably the most ubiquitous anthraquinone

Table III. Toxicity of Different Extracts and Purified Metabolites from *A. chevalieri* ZT 8268 to White Mice (Compounds Were Administered in 0.1–0.2 mL of Me_2SO/20-g Animal

test agent	LD_{50}, ip, mg/kg	LD_{50}, po,[a] mg/kg
ZT 8268 crude $MeCl_2$ extract	10–60	>2000
MeOH soluble fraction	45	>580
MeOH insoluble fraction	10	>250
aqueous culture filtrate	1500	>2100
physcion	10	>100
physciondianthrone	>25	nd
physcionanthrone B	2	>200
erythroglaucin	>30	nd
emodin	35	>1000

[a] Highest dose tested; nd = not determined.

Table IV. Toxicity of Erythroglaucin, Physcion, Physcionanthrone B, and Emodin in the Chicken Embryo Test (Toxicity Calculation Based on 2 × 2 Contingency Tables; P = Significance Probability)

test agent	dose, μg	surviving/ dead	P, %	rel. toxicity
control		17/3		
erythroglaucin	100	6/4	13	
	10	8/2	73	
physcion	1000	4/6	1.2	+
	100	6/4	13	
	10	6/4	13	
anthrone B	1000	3/7	0.3	++
	100	2/8	0.06	+++
	10	6/4	13	
emodin	1000	1/9	0.01	+++
	100	4/6	1.2	+
	10	5/5	4.4	(+)

(Thomson, 1971). Large differences in oral and intraperitoneal toxicity were observed. Similar results have been described also for anthraquinone glycosides from plant origin (Fairbairn, 1976).

Toxicity to 1-Day-Old Cockerels. Toxicity to 1-day-old cockerels was tested by oral administration for physcion (110 mg/kg), physcionanthrone B (76 mg/kg), physciondianthrone (90 mg/kg), erythroglaucin (50 mg/kg), and emodin (125 mg/kg). None of the anthraquinones tested produced pronounced toxic effects in the chickens. Differences in weight gain were not significant except for emodin (125 mg/kg) in Me_2SO which resulted in slightly reduced weight gain during the first 4 days. Using PEG 400 or corn oil as solvents, no differences in weight gain compared to controls were recorded. These results are strongly in contrast with those of Wells et al. (1975), who demonstrated high oral toxicity (LD_{50} 3.7 mg/kg) of emodin isolated from *Aspergillus wentii* to cockerels. This result is surprising since oral toxicity of hydroxyanthraquinones has not been reported elsewhere and is unlikely to occur.

ZT 8268 Crude Extract in a Short-Time Feeding Experiment. In a feeding experiment in white mice over a period of 35 days with daily doses of 6 mg/kg, no toxic effects could be observed. Weight gain did not differ significantly between test animals and controls. Histological examination of liver and kidneys showed no pathological changes. By this preliminary experiment at least a highly cumulative oral toxicity of the extract can be excluded.

Toxicity in the Chicken Embryo Test. Physcion, physcionanthrone B, erythroglaucin, and emodin were subjected to the chicken embryo test. Surprisingly physcion in doses lethal to mice (50 mg/kg) revealed little toxicity in chicken embryos (1 mg/egg), whereas the toxicity of physcionanthrone B and emodin in eggs and mice was

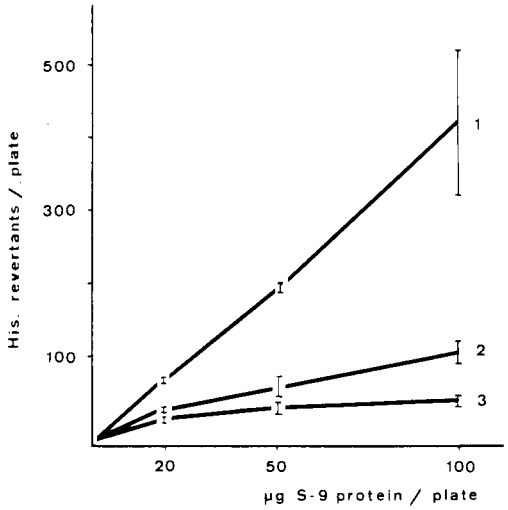

Figure 3. Comparison of the induction of revertants in *S. typhimurium* strain TA 1537. The compounds were tested in amounts of 100 µg/plate: (1) erythroglaucin, (2) physcion, (3) physcionanthrone B.

comparable. Results are summarized in Table IV. The strong local irritation of anthraquinones parenterally administered in mammals seems not to occur to the same extent in chicken embryos. The weak correlation between mammalian and chicken embryo toxicity was also observed with other mycotoxins (Gedek, 1972). The value of this method in mycotoxin testing therefore is limited and questionable.

Mutagenicity in the "Salmonella/Mammalian Microsome" Test. The tester strains TA 98 (frameshift) and TA 100 and TA 1535 (base substitution) did not respond to physcion, physcionanthrone B, and erythroglaucin. This was true in the presence and absence of S-9 microsomal preparations. In strain TA 1537 (frameshift) positive responses to the three compounds were found in the presence of S-9 only. The effect of different amounts of S-9 preparation was weak in physcion and physcionanthranol B but pronounced in erythroglaucin (Figure 3).

Positive mutagenic results of a considerable number of anthraquinones have also been reported by Brown and Brown (1976), Brown et al. (1977), and Brown and Dietrich (1979). They observed both activation and inactivation by S-9 microsomal preparations. The major part of the mutagenic anthraquinones and especially the hydroxy and nitro derivatives were active particularly in strain TA 1537. Only few compounds exhibited mutagenicity in strains TA 98, TA 100, TA 1535, and TA 1538. A similar strain-specific action for TA 1537 has been found only for a few other compounds, e.g., 9-aminoacridin, ICR-10, ICR-170, and dibenz[*a*,*h*]anthracene-5,6-oxide (McCann et al., 1975).

CONCLUSION

Our results and the additional investigation of Blaser (unpublished data, 1979) indicate that the *Aspergillus glaucus* group most probably does not represent a source of intoxication in man and animals. None of the six most frequently found mycotoxins could be detected in more than 100 strains of the *Aspergillus glaucus* group. Although the fungal extracts exhibit severe cytotoxic action when injected intraperitoneally, they seem to be harmless when ingested, at least at the minute dose levels these compounds are likely to be present in molded foodstuffs. In higher amounts, anthraquinones occur in foodstuff such as rhubarb and in a number of laxative drugs.

The effect which causes major concern is the mutagenic action of many anthraquinones. Here again, the minute amounts possibly coming from contamination with *A. glaucus* do not represent a risk to human health. The anthraquinones in vegetable foodstuffs and laxatives, however, have to be studied more extensively. Although free anthraquinones (aglycons) are not desired in laxative drugs, they regularly occur in amounts of 2–4% (Fairbairn, 1976). These aglycons are likely to be absorbed in the small intestine, glucuronated in the liver, and excreted via bile and kidneys. In addition the anthraquinone glycosides and glucuronides undergo bacterial hydrolysis in the colon. An indication of strong interactions with intestinal tissues is the pronounced staining of the colon in patients taking regularly vegetable laxatives. Further studies are particularly important since these drugs are chronically used and even misused by a considerable number of people. Especially studies on distribution and metabolism must be undertaken in order to assess the possible mutagenic effects of naturally occurring anthraquinones in man.

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Toxic Naphtho-γ-pyrones from *Aspergillus niger*

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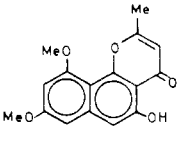
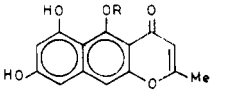
Three known and three new naphtho-γ-pyrones were isolated and characterized from the mycelial extracts of *Aspergillus niger* van. Tiegh and from mango fruits naturally infected with the same strain of the fungus. The total naphtho-γ-pyrones and one of its major components, aurasperone D, in doses of 50 mg/kg intraperitoneal (ip), produced marked central nervous system (CNS) depressant effects in albino mice and rats leading to death by respiratory failure. The amount of naphtho-γ-pyrones in the infected mango skin and pulp was about 60 mg/kg. This observation is a cause for alarm from a public health viewpoint since prolonged ingestion of infected mango preparations may cause mental deficiencies or predispose man to other ailments.

In connection with our work (Ghosal et al., 1978a) on the postinfectional changes in the chemical constituents of *Mangifera indica* L. (cv. Banarasi Langra), infected with *Aspergillus niger* van. Tiegh (CMI-IMI 205879), the presence of about a dozen naphtho-γ-pyrones in the extractives of the infected fruits was detected. Subsequently, from the petroleum ether and chloroform extractives of mycelium of the fungus, grown in Richard's medium in the presence of autoclaved mango pulp, three previously known naphtho-γ-pyrones, viz., flavasperone (**1**) (Lund et al., 1953), rubrofusarin (**2**) (Ashley et al., 1937), and aurasperone A (**3**) (Tanaka et al., 1966), and three new ones, viz., isoaurasperone (**4**), aurasperone D (**5**), and aurasperone E (**6**), were isolated in quantities sufficient for their complete characterization. Mango not only serves as a staple food for many people, its extracts are used for various therapeutic purposes, e.g., in the treatment of colon cancer, in jaundice, and in hemorrhoids. Mango fruits infected with *A. niger* are not rejected but are often used by unscrupulous traders for the preparation of processed mangoes, e.g., pickles, jams, etc. Such food materials may provide high toxin risk in man. It was therefore thought worthwhile to evaluate the pharmacological profile of activity of the naphtho-γ-pyrones. Significant activity was observed on the CNS of albino mice and rats. The details of the chemical and pharmacological evaluations are described in this paper.



1



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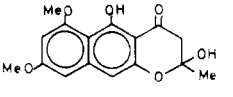
7, R = Me



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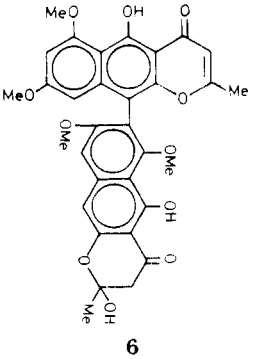

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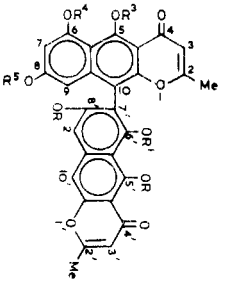

3, R = H, R′ = Me, R² = Me, R³ = H, R⁴ = Me, R⁵ = Me
4, R = Me, R′ = H, R² = Me, R³ = Me, R⁴ = H, R⁵ = Me
5, R = H, R′ = Me, R² = Me, R³ = H, R⁴ = H, R⁵ = Me
8, R = H, R′ = Me, R² = Me, R³ = Me, R⁴ = H, R⁵ = Me

RESULTS AND DISCUSSION

From the petroleum ether and chloroform extractives of the mycelium of *A. niger* van. Tiegh (CMI-IMI 205879), grown in Richard's medium in the presence of autoclaved mango pulp, six naphtho-γ-pyrones (**1**-**6**) were isolated in substantial amounts. These were separated into homogeneous entities by extensive column and preparative layer chromatography and by fractional crystallization of the compounds, their methyl ether, and acetate derivatives. When the fungus was grown in Richard's medium in the absence of mango pulp, the yields of the naphtho-γ-pyrones were greatly diminished. Earlier, the presence of about a dozen naphtho-γ-pyrones, including the mentioned six compounds (**1**–**6**), was detected (Ghosal et al., 1978a) in acetone extractives of mature skin and pulp of mango infected with the same strain of the fungus in natural condition. This paper describes the details of isolation and

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