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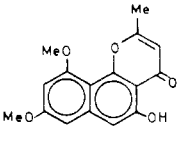
Toxic Naphtho-γ-pyrones from *Aspergillus niger*

Shibnath Ghosal,* Kanika Biswas, and Dilip K. Chakrabarti

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.



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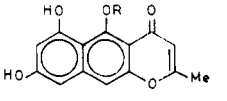

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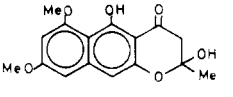

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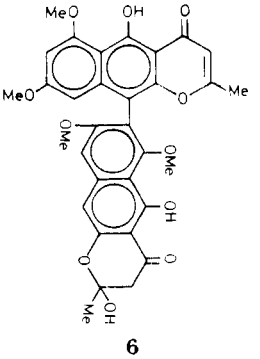

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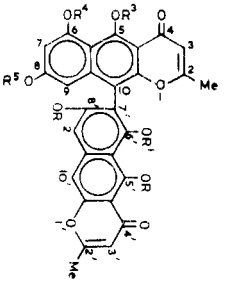

3, R = H, R′ = Me, R² = Me, R³ = H, R⁴ = Me, R⁵ = Me
4, R = Me, R′ = H, R² = Me, R³ = Me, R⁴ = H, R⁵ = Me
5, R = H, R′ = Me, R² = Me, R³ = H, R⁴ = H, R⁵ = Me
8, R = H, R′ = Me, R² = Me, R³ = Me, R⁴ = H, R⁵ = Me

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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Table I. Naphtho-γ-pyrones[a] Obtained from Three Sources

source	mango skin + pulp, g	mycelium, g	naphtho-γ-pyrones, mg 1	2	3	4	5	6
acetone extract of infected mango	1000		2.3	4.2	16	3.7	17.5	6
flask culture in Richard's medium		27/10 flasks	5.5	3.8	12	7.3	12.5	3.3
flask culture in Richard's medium in presence of autoclaved mango pulp (30 g/flask)		60/10 flasks	18	8.4	81	36	87	18.5

[a] The mean of three determinations (see Experimental Section) is recorded.

characterization of 1–6 and the pharmacological profile of activity of the total mixture and two individual naphtho-γ-pyrones (2 and 5).

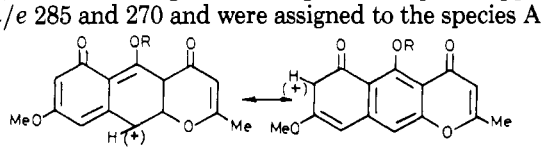
The nature and abundance of the naphtho-γ-pyrones obtained from the three different sources are recorded in Table I.

The apportionment of the naturally occurring minor entities and the residual amounts present in the crystallizing mother liquors was accomplished by means of preparative layer chromatography of aliquots of the mixture extractives and absorptiometry of the eluates of TLC scrapings at several R_f values after the components present in these layers were located by comparison with markers. For quantitative analysis of the naphtho-γ-pyrones by absorptiometry, the maximum near λ 280 nm was found to be most suitable. There was a linear relationship between absorbance and concentration between 5×10^{-3} mg and 12×10^{-3} mg.

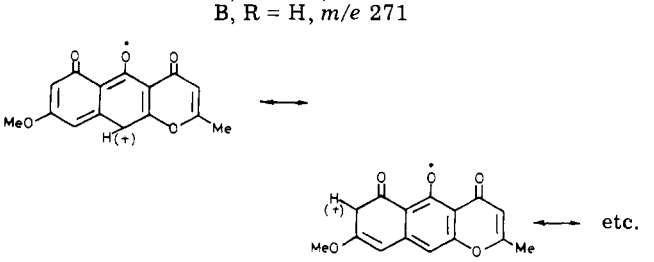
The data recorded in Table I indicate that this strain of *A. niger* is a producer of naphtho-γ-pyrones both in vivo and in vitro and that mango pulp acts as a promoter for the production of these compounds.

The identity of the known compounds was established by correspondence of physical and spectral properties of the compounds and their methyl ether derivatives with those reported in the literature and also by direct comparison with authentic samples. The characterization of only the new naphtho-γ-pyrones is described here.

Isoaurasperone (4). This compound, $C_{32}H_{26}O_{10}$ (M^+, 570), exhibited IR spectrum closely similar to that of aurasperone A (3) (Tanaka et al., 1966) but chromatographically was more polar than the latter. The UV maxima positions of the compound, in neutral ethyl alcohol, were similar to those of rubrofusarin monomethyl ether (7). The UV maxima remained unaltered on addition of $AlCl_3$. The C(5) and C(5′) oxygen functions must therefore be substituted. In the mass spectrum, the loss of 17 mass units from the molecular ion indicated the presence of a methoxyl function peri to the pyrone carbonyl (Arends et al., 1973). Two other significant fragment-ion peaks appeared at *m/e* 285 and 270 and were assigned to the species A and



A, R = Me, *m/e* 285
B, R = H, *m/e* 271



C, *m/e* 270

C, respectively. The mass of the fragment ion peaks were accurately measured. The ^1H NMR spectrum showed six protons associated with the aromatic and the pyrone rings. In the corresponding acetate derivative, the meta-split doublets, associated with C(7)–H and C(9)–H, and the upfield singlet, associated with C(9′)–H, were shifted downfield by 0.12–0.22 ppm (see Experimental Section), indicating the presence of ortho/para acetoxy function with respect to these protons (Ghosal et al., 1977). Thus structure 4 was assigned to isoaurasperone. Although this compound was not reported before in nature or prepared synthetically, it was found to be present, as a minor entity, in the sample of aurasperone A kindly provided by Dr. Tanaka.

Aurasperone D (5). This compound, $C_{31}H_{24}O_{10}$ (M^+, 556), showed UV absorption spectrum, in ethyl alchohol, characteristic of linear naphtho-γ-pyrones. The longer wavelength maximum at λ 380 nm was shifted bathochromically, by about 40 nm, in the presence of $AlCl_3$, and thereafter remained unaltered on addition of HCl. These observations suggested the presence of chelated hydroxyl group(s) (at C(5) and/or C(5′)). On methylation with dimethyl sulfate and alkali, it afforded aurasperone A dimethyl ether. In the mass spectrum of the parent compound, aside from the molecular ion peak, a significant fragment ion peak appeared at *m/e* 271 which was ascribed to the species B. The ^1H NMR spectrum of the compound was very similar to that of aurasperone A in respect of the aromatic and pyrone ring protons (Wang and Tanaka, 1966), the only exception being in the meta-split doublet due to C(9)–H which was shifted downfield by about 0.2 ppm. This contention was further supported from the fact that in the corresponding acetate derivative, the C(9)–H experienced a further downfield shift by about 0.25 ppm which was caused by a *p*-acetoxy function (Ghosal et al., 1977). On treatment with ethereal diazomethane, aurasperone D afforded a monomethyl ether (M^+, 570) which was different from aurasperone A. In its mass spectrum, the monomethyl ether exhibited prominent loss of 17 mass units from the molecular ion. The C(5) must now contain a methoxyl function. Further, it showed a significant fragment ion peak at *m/e* 270 due to the species C. On the basis of the above data, aurasperone D and its monomethyl ether were assigned structures 5 and 8, respectively. Although methylation of a chelated hydroxyl (C(5)–OH) in preference to a nonchelated one (C(6)–OH) is rather unusual, but it had precedence in rubrofusarin. Rubrofusarin on treatment with ethereal diazomethane afforded 7. The difference of aurasperone-D monomethyl ether with aurasperone A lends further support to this conclusion.

Aurasperone E (6). This compound, $C_{32}H_{28}O_{11}$ (M^+, 588), showed UV absorption maxima closely similar to those of fonsecin methyl ether (9) (Galmarini et al., 1962). In its mass spectrum, the molecular ion of aurasperone E experienced a facile loss of elements of water to give a fragment ion peak at *m/e* 570. The subsequent fragment ion peaks were superimposable to those of aurasperone A. In the ^1H NMR spectrum of the compound, the upfield

Table II. Effect[a] of Naphtho-γ-pyrones on Hexobarbital Hypnosis

group	drugs (dose, mg/kg ip)	hexobarbital sleeping time, min, mean ± SEM[b]	P
1	hexobarbital (100)	24 ± 1.8	
2	**2** (10) plus hexobarbital (100)	40.7 ± 3.6	<0.01[c]
3	total naphtho-γ-pyrones (10) plus hexobarbital (100)	50.3 ± 3.3	<0.01[c]
4	**5** (10) plus hexobarbital (100)	47.2 ± 2.9	<0.001[c]
5	total naphtho-γ-pyrones (10) plus mango extractives (10) plus hexobarbital (100)	60.4 ± 3.3	<0.01[d]
6	**5** (10) plus mango extractives (10) plus hexobarbital (100)	55.8 ± 2.7	<0.01[e]
7	mango extractives (10) plus hexobarbital (100)	26.4 ± 4.1	

[a] Ten animals in each experiment. [b] Standard error of the mean. [c] Significance in relation to group 1. [d] Significance in relation to group 3. [e] Significance in relation to group 4.

pyrone ring proton was absent and the usual C–Me signal was replaced by another methyl signal associated with a hemiacetal function. On treatment with methanolic HCl, it afforded aurasperone A. A better yield of aurasperone A was obtained when the transformation was carried out with HCl in benzene. On the basis of the above data, aurasperone E was assigned structure **6**.

The three test compounds, viz., the total naphtho-γ-pyrones (mixture of yellow solid from the petroleum ether and chloroform extracts of the mycelium, cultured in Richard's medium in the presence of mango pulp), aurasperone D (**5**), and rubrofusarin (**2**), produced significant pharmacological actions on the central nervous system (CNS) of albino mice and rats.

In the primary observational tests (Turner, 1965), all the three test compounds (in dose of 10 mg/kg ip) produced a transient stimulation, followed by varying degrees of CNS depression in albino mice and rats. The depressant activity was characterized by considerably diminished spontaneous motility, grouping of animals on one side of the cage and ptosis. However, reflexes were intact and the animals responded to external stimuli. On increasing the dose (20 mg/kg ip), the total naphtho-γ-pyrones and, also, **5** produced hindlimb paralysis and catalepsy in both albino mice and rats. In the case of **2**, these symptoms appeared only on increasing the dose further (50 mg/kg ip).

The three test compounds produced, in varying degrees, hexobarbital-induced sleeping time in mice (Kuhn and Van Maanen, 1961). This observation provides further evidence for the CNS depressant activity of the naphtho-γ-pyrones. Interestingly, although healthy mango extractives had no CNS activity per se, they produced synergistic effects on the hypnotic potentiation of the first two test compounds. This could be due to the presence of a trace amount of mangiferin in the mango extractives. A similar synergistic effect of mangiferin, in combination with the secoiridoid, swertiamarin, was previously recorded (Bhattacharya et al., 1976). The results of the hypnotic potentiation of the three test compounds are recorded in Table II.

In doses of 50 mg/kg ip, both **5** and the total naphtho-γ-pyrones produced clonic convulsions in albino mice and rats, followed by severe CNS depression and ultimately death of all the 40 treated mice and rats, due to respiratory failure. On postmortem, 80% of the animals showed complete collapse of lungs and feebly beating hearts. Death occurred within 1 h of administration of the two test compounds. **2**, in doses of 50 mg/kg ip, did not produce any one of the above effects; the treated animals in this case became normal on keeping overnight.

The toxicity (Miller and Tainter, 1944) and LD_{50} after single intraperitoneal administration of the total naphtho-γ-pyrones and of **5** in albino mice were studied. The LD_{50} (in mg/kg ± SEM) of the two compounds were 44 ± 10 and 47 ± 12, respectively.

EXPERIMENTAL SECTION

All melting points were taken on a Köfler block in open capillaries and were uncorrected. UV spectra were recorded in Cary 14 or Spectromom 203 spectrophotometer in aldehyde-free ethyl alcohol or methanol. IR spectra were determined in KBr, and only the major bands are quoted. ^1H NMR spectra were obtained at 60 MHz using Me_4Si as an internal standard. MS spectra were determined at 70 eV, the samples were directly inserted into a probe. Separation by column chromatography was carried out using silicic acid (British Drug Houses, Poole, England) as the adsorbent, and TLC experiments were conducted with silica gel G (E. Merck, Darmstadt) using four solvent systems, viz., C_6H_6–HOAc (95:5, solvent 1), $CHCl_3$–HOAc (99:1, solvent 2), $CHCl_3$–HOAc (98:2, solvent 3), $CHCl_3$–HOAc–MeOH (15:3:1, solvent 4), as developers. I_2, $FeCl_3$, and short-wave UV lamp were used for staining and visualization purposes.

Pharmacological studies were conducted on albino mice (18–25 g) and albino rats (80–120 g). The animals were fed on standard pellet diet (Hindusthan Levers, Calcutta, India). All experiments were conducted at ambient temperature of 28 ± 2 °C. Unless stated otherwise, the three test compounds were used in doses of 10 mg/kg ip and pretreatment time was 1 h. At least ten animals were used for drug-treated and control groups, the latter receiving only the vehicle, 1% aqueous sodium carbonate solution.

Extraction of Naphtho-γ-pyrones from Mycelial Extract of *A. niger*. In a typical experiment, the fungus was grown in Richard's solution (200 mL) containing autoclaved mango pulp (30 g), in a still culture flask (1 L) at 21 °C for 21 days. The mean weight of the dry mycelial mat obtained from ten flasks was recorded (Table I). A portion of the mycelium (20 g) was successively extracted with petroleum ether (60–80 °C) and chloroform in a Soxhlet apparatus (30 h, each). The two extracts were processed separately.

Treatment of the Petroleum Ether Extract. The extract was concentrated to a small volume (about 25 mL) and kept at room temperature overnight, yielding a yellow solid (37 mg). The solid showed three major and several minor spots on analytical TLC (solvent 1). It was dissolved in benzene (10 mL) and chromatographed over a column of silicic acid (9 × 2 cm). Elution was done with benzene (2 L), and fractions (50 mL) were collected.

Flavasperone (1). The residue from fractions 2–5 crystallized from benzene–hexane as yellow needles (6 mg): mp 203–205 °C, TLC R_f 0.4 (solvent 2), UV λ_{max} nm (log ε) 241 (4.54), 282 (4.32), 370–372 (3.69); MS *m/e* 286 (M^+, relative intensity, 100%), 285 (10), 269 (5), 257 (50), 254 (5). Direct comparison (melting point, mixture melting point, co-TLC, UV) of the residue with a reference sample of flavasperone established that they were identical.

Rubrofusarin (2). Fractions 8–12 were combined, concentrated, and filtered through a short column of silica gel (60–120 mesh) to remove an intractible gummy material. The filtrate was evaporated and the residue crystal-

lized from hexane–benzene as orange needles; the mother liquor on preparative TLC, using solvent 1, afforded a further crop of **2** (total yield, 2.8 mg): mp 210 °C; TLC R_f 0.72 (solvent 1); UV λ_{max} nm (log ϵ) 224 (4.41), 280 (4.72), 325 (4.34), 395 (4.37); MS m/e 272 (M^+, 100%), 257 (20), 229 (8), 228 (8.5). Direct comparison (melting point, mixture melting point, co-TLC, UV) of the residue with a reference sample of rubrofusarin established that they were identical.

Aurasperone A (3). The residue from fractions 15–17 was dissolved in chloroform (5 mL) and was subjected to preparative layer chromatography on oxalic acid (0.4%) impregnated chromatoplates (20 × 20 cm, 2-mm thickness). The R_f zone ~0.6 (solvent 2) was eluted with chloroform, the solvent was evaporated, and the residue was crystallized from hexane–benzene as yellow crystals, mp 206 °C; the residual amount of **3** left in the hexane–benzene mother liquor was isolated by a second preparative layer chromatography and estimated by absorptiometry (total yield, 16.5 mg): UV λ_{max} nm (log ϵ) 225 (4.52), 258 (4.42), 280 (4.53), 325 (3.90), 400 (3.68), λ_{max} (EtOH–$AlCl_3$) nm 290, 332, 450, λ_{max} (EtOH–$AlCl_3$–HCl) nm 288, 330, 450; MS m/e 570 (M^+, 100%), 541 (9.8), 540 (15), 539 (25), 285 (fragment-ion A, 2), 271 (fragment-ion B, 8). The dimethyl ether, prepared by treatment of **3** with dimethyl sulfate and potassium carbonate, in anhydrous acetone, under reflux (40 h), crystallized from hexane–benzene as yellow crystals, mp 173–175 °C; TLC R_f 0.7 (solvent 1), blue fluorescence under short-wave UV light; UV λ_{max} nm 225, 275, 330, 345, 380. Direct comparison of the residue with an authentic sample of aurasperone A established that they were identical.

Treatment of the Chloroform Extract. The concentrate of the chloroform extract showed three major and one minor spot on TLC. It was chromatographed over silicic acid in a column (12 × 2 cm). Elution was carried out with benzene (300 mL), diethyl ether (600 mL), and diethyl ether–methanol (99:1, 500 mL). Fractions (100 mL) were collected and monitored by TLC. Fractions 1–3 afforded a mixture of steroidal compounds: MS m/e 442, 426, 410, 394. The nature of these constituents is currently being investigated. The residue from fractions 6–9 was obtained as a yellow amorphous solid. TLC of this compound showed the presence of three major and two minor yellow pigments: TLC R_f 0.2–0.75 (solvent 1). The components were separated by preparative layer chromatography over oxalic acid impregnated silica gel plates. The upper yellow layer, R_f 0.7, afforded a further crop of aurasperone A (10.4 mg).

Isoaurasperone A (4). The second yellow preparative layer zone, R_f 0.55, afforded a pale-yellow solid (12 mg): mp 198–203 °C, UV λ_{max} nm (log ϵ) 255 (4.53), 275 (4.59), 385–390 (3.54), no shift with $AlCl_3$; IR ν_{max} 3450 (broad), 1655, 1608, 1595, 1492, 1455, 1412, 1245, 1148, 1095, 1078, 825 cm^{-1}; ^1H NMR δ ($CDCl_3$) 7.20 (1 H, s, H-10′), 7.15 (1 H, s, H-8′), 6.50 (1 H, d, J = 2.5 Hz, H-9), 6.48 (1 H, d, J = 2.5 Hz, H-8), 6.33 (1 H, s, H-3′), 6.06 (1 H, s, H-3), 4.03 (3 H, s, OMe), 3.83 (3 H, s, OMe), 3.50 (3 H, s, OMe), 3.46 (3 H, s, OMe), 2.56 (3 H, s, C_2-Me), 2.21 (3 H, s, C_2-Me); MS m/e 570 (M^+, 100%), 553 (M – OH, 10), 538 M – OH – Me, 60), 285 (fragment-ion A, 8), 270 (fragment-ion C, 10), 268 (fragment-ion A – OH, 15). On methylation with dimethyl sulfate and potassium carbonate, in anhydrous acetone, under reflux (40 h), **4** afforded a light-yellow solid, mp 163–165 °C, which was identical with aurasperone A dimethyl ether in all respects (melting point, mixture melting point, co-TLC, UV). Acetylation of **4** with acetic anhydride and pyridine, at ordinary temperature, gave the diacetate: mp 148–151 °C; MS m/e 654 (M^+, 100%), 612 (18), 570 (14); ^1H NMR δ ($CDCl_3$) 7.27 (1 H, s), 7.20 (1 H, s), 6.72 (1 H, d, J = 2.5 Hz), 6.62 (1 H, d, J = 2.5 Hz).

Aurasperone D (5). The yellow streak in the R_f zone 0.4, in the preparative layer chromatoplate, was eluted with chloroform. Evaporation of the solvent and crystallization of the residue from hexane–benzene afforded a microcrystalline yellow solid (29 mg): mp 115 °C; UV λ_{max} (EtOH) nm (log ϵ) 235–240 (4.70), 280 (4.71), 320–325 (4.18), 380 (3.85), λ_{max} (EtOH–$AlCl_3$) nm (log ϵ) 240–245 sh (4.67), 285 (4.58), 415–422 (3.56); IR ν_{max} 3400 (broad), 1652, 1600, 1580, 1495, 1404, 1018 cm^{-1}; ^1H NMR δ ($CDCl_3$) 7.23 (1 H, s), 7.05 (1 H, s), 6.51 (1 H, d, J = 2.5 Hz), 6.44 (1 H, d, J = 2.5 Hz), 6.12 (1 H, s), 6.08 (1 H, s) (aromatic and γ-pyrone ring protons), 3.83 (3 H, s, OMe), 3.66 (3 H, s, OMe), 3.50 (3 H, s, OMe), 2.43 (3 H, s, C-Me), 2.12 (3 H, s, C-Me); MS m/e 556 (M^+, 100%), 541 (10), 538 (42), 527 (50), 525 (38), 513 (78), 512 (45), 285 (4), 271 (32), 256 (38), 240 (59). It gave a strong green color with methanolic $FeCl_3$. On methylation with ethereal diazomethane, it afforded an amorphous solid: TLC R_f 0.72 (solvent 1); MS m/e 570 (M^+, 100), 553 (18), 539 (61), 285 (10), 270 (20). Permethylation of aurasperone D with dimethyl sulfate and potassium carbonate, in anhydrous acetone, under reflux (40 h) afforded aurasperone A dimethyl ether. Permethylation with methyl iodide and sodium hydride in tetrahydrofuran, at ordinary temperature, according to a published procedure (Ghosal et al., 1975), gave aurasperone A dimethyl ether in a better yield.

Aurasperone E (6). The residue from fractions 10–12, from column chromatography of the chloroform extractives, showed on analytical TLC the presence of aurasperone A, isoaurasperone, and aurasperone D as minor entities together with a polar ferric-positive compound: TLC R_f 0.3 (solvent 3). Attempts to separate the polar compound by rechromatography were unsuccessful. The compound was subsequently separated by preparative layer chromatography on polyamide powder (Riedel) using solvent 4 as the developer. The R_f zone 0.35 was eluted with chloroform–methanol. Evaporation of the solvent afforded aurasperone E as a microcrystalline solid (6 mg): mp 195–197 °C; UV λ_{max} (EtOH) nm (log ϵ) 230 (4.86), 282 (5.17), 322 (4.35), 330 (4.31), 400 (4.29); ^1H NMR δ ($CDCl_3$–Me_2SO-d_6) 7.18 (1 H, s), 7.11 (1 H, s), 6.49 (1 H, d, J = 2.5 Hz), 6.42 (1 H, d, J = 2.5 Hz), 6.11 (1 H, s) (aromatic and γ-pyrone ring protons), 3.84 (3 H, s, OMe), 3.72 (3 H, s, OMe), 3.58 (3 H, s, OMe), 2.21 (3 H, s, C-Me), 1.32 (3 H, s, CH_3-$C(O^-)OH$; MS m/e 588 (M^+, 80%), 570 (M – H_2O, 100), 541 (4), 540 (7), 539 (10), 303 (3), 285 (1). Calcd $C_{32}H_{28}O_{11}$: C, 65.3; H, 4.7. Found: C, 64.9; H, 5.0.

The amounts of the three minor components (**3**, **4**, and **5**) were determined, respectively, as 0.2, 0.08, and 0.15 mg, by absorptiometry of MeOH eluates of TLC scrapings of the less polar zones after they were identified by use of markers.

Transformation of Aurasperone E into Aurasperone A. Aurasperone E (2 mg) in benzene (3 mL) was treated with concentrated HCl (1 mL). The scarlet red solution that produced was diluted with water and extracted with chloroform. The chloroform extract was processed in the usual fashion to give aurasperone A (1.2 mg) (melting point, mixture melting point, co-TLC, UV).

The mycelium and culture filtrate of the fungus, grown in absence of mango pulp, were processed as described above, and the yields of the naphtho-γ-pyrones obtained are recorded in Table I.

Extraction of Naphtho-ν-pyrones from Mango Fruits Infected with *A. niger*. The skin and pulp of

mango fruits (cv. Banarasi Langra, about 1 kg), infected with *A. niger* van. Tiegh (CMI-IMI 205879), were macerated with acetone in a high-speed blender. After 4 h, the mixture was filtered and the solvent was removed under reduced pressure. The extract was poured into water (200 mL) and the suspension was successively extracted with diethyl ether, ethyl acetate, and *n*-butyl alcohol (three 100-mL portions each). The three extracts were combined since their TLC behavior was similar. The combined extract was processed for neutral, phenolic, and carboxylic fractions in the usual fashion (Ghosal et al., 1978b). The phenolic fraction was shaken with a mixture of hot hexane–benzene (1:1). Evaporation of the solvent from the hexane–benzene soluble fraction afforded a yellow solid (74 mg) from which the six naphtho-γ-pyrones were obtained as described above. The yields of the compounds are recorded in Table I. The hexane–benzene insoluble fraction contained mainly gallic acid, 4-*O*-methylgallic acid, protocatechuic acid, and catechins. The identity of these compounds was established as described before (Ghosal et al., 1978a).

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Aflatoxin Residues in the Tissues of Pigs Fed a Contaminated Diet

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Pigs fed aflatoxins for 21 days had 36% heavier livers, gained 25% less weight, and ate 18% less feed than controls, but did not differ in efficiency of feed utilization or show any gross pathological lesions on postmortem examination. Assay of liver, heart, kidney, spleen, and muscle showed that there was some carry-over of aflatoxins B_1 and B_2 to all tissues, but G_1 and G_2 were not present. Residues of B_1 metabolites, M_1 and B_{2a}, were also found in all tissues of the pigs fed aflatoxins. This is the first time that B_{2a} has been identified as a tissue residue in the pig. The average retention of the aflatoxin dosage was calculated to be 0.015 and 0.005% for B_1 and B_2, respectively.

There is strong epidemiological evidence that aflatoxins are carcinogenic (Shank et al., 1972; Campbell and Stoloff, 1974). Since aflatoxins have been found to be widely distributed in common livestock feeds and produce aflatoxicosis in farm animals (Keyl and Booth, 1971), it is possible that tissue carry-over into meat may contribute to dietary carcinogenesis in man. Ingested aflatoxins may be deposited in the tissues of animals fed contaminated rations as either the original compound or as one of its metabolites (Purchase, 1972).

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One of the problems in assessing the seriousness of aflatoxins in animal products has been the availability of suitable methodology for determining tissue carry-over. Most of the early work was based on the methodology used for assaying contamination in plant materials and failed to detect aflatoxins, even in animals showing confirmed signs of aflatoxicosis (Allcroft and Carnaghan, 1963; Platanow, 1965; Kratzer et al., 1969; Keyl and Booth, 1971). Although improvements in methodology have demonstrated aflatoxin carry-over from feed to animal tissues (Brown et al., 1973; Jemmali and Murthy, 1976), the methods still lack sensitivity and accuracy so that much of the information in the literature on aflatoxins in animal products is inconclusive. Recently, Trucksess et al. (1977) reported development of an improved method for determination of aflatoxins in eggs, which has since been mod-

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