[Chem. Pharm. Bull. 24(10)2317—2321(1976)]

UDC 547.638.4.02 : 581.192 : 582.282.123

The Structures of Toxic Metabolites of *Aspergillus candidus*. II.[1] The Compound B (Xanthoascin), a Hepato- and Cardio-toxic Xanthocillin Analog

Chikako Takahashi,[2a] Setsuko Sekita, Kunitoshi Yoshihira, and Shinsaku Natori[2]

National Institute of Hygienic Sciences[2]

(Received December 6, 1975)

The structure of the compound B (xanthoascin) was proved to be 1-(4-hydroxyphenyl)-4-(2,2-dimethyl-6-chromanyl)-2,3-diisocyano-1,3-butadiene (**3**) by chemical and physical methods.

The mold, *Aspergillus candidus* Link, produces several phenolic compounds, in which the compound A (terphenyllin) has been shown to be the causative agent for the characteristic cytotoxicity while the compound B for the hepato- and cardio-toxicity to experimental animals.[1,3] The structures (**1**, **2**) of terphenyllin and the compound E (deoxyterphenyllin) were reported in the previous paper.[1]

Now the toxin B, designated xanthoascin, $C_{23}H_{20}O_2N_2$, yellow needles of mp 165—170° (decomp.), has been proved to be a xanthocillin analog as shown in the formula (**3**). The spectral data of xanthoascin showed a phenolic nature of the compound. It forms monoacetate (**4**) and monomethyl ether (**5**). The most characteristic feature of the infrared (IR) spectrum of xanthoascin was the absorption at 2150 cm^{-1}, which was assigned as the streching vibration of isonitrile group(s),[4-9] and the group unusal in natural products reminded us of the similarity

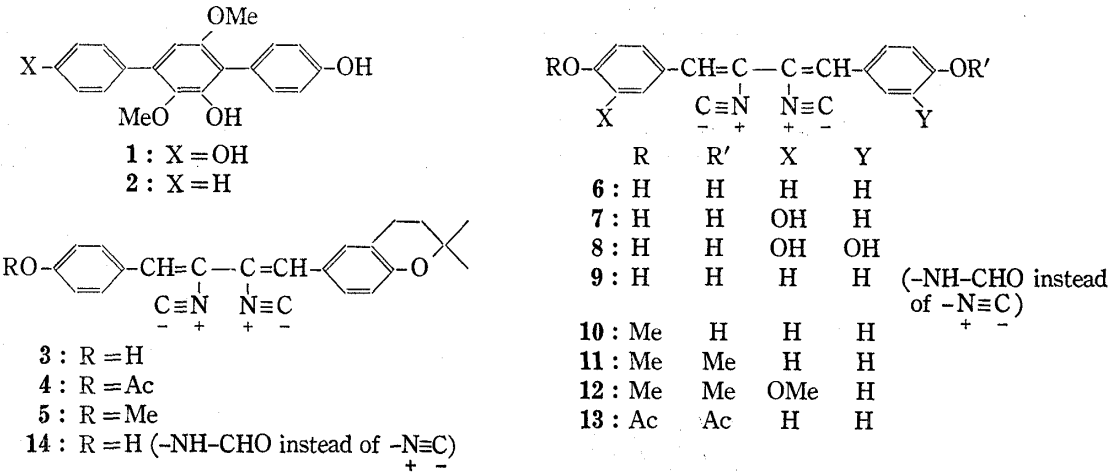

Chart 1

1) Part I: C. Takahashi, K. Yoshihira, S. Natori, and M. Umeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 613 (1976).
2) Location: *Kamiyoga-1-chome, Setagaya-ku, Tokyo*; *a*) Present address: *Kasukabe City Hospital, Kasukabe, Saitama.*
3) C. Takahashi, K. Yoshihira, S. Natori, M. Umeda, K. Ohtsubo, and M. Saito, *Experientia*, **30**, 529 (1974).
4) H. Hagedron and H. Tönjes, *Pharmazie*, **12**, 567 (1957).
5) H. Achenbach, H. Strittmatter, and W. Kohl, *Chem. Ber.*, **105**, 3061 (1972).
6) S. Pfeifer, H. Bär, and J. Zarnack, *Pharmazie*, **27**, 536 (1972).
7) K. Ando, G. Tamura, and K. Arima, *J. Antibiotics*, **21**, 587 (1968).
8) A. Takatsuki, S. Suzuki, K. Ando, G. Tamura, and K. Arima, *J. Antibiotics*, **21**, 671 (1968).
9) M. Ishida, T. Hamazaki, and Y. Hatsuda, *Agr. Biol. Chem.*, (Tokyo), **36**, 1847 (1972).

to xanthocillins and related compounds of 1,4-diphenyl-2,3-diisocyano-1,3-butadiene derivatives (**6**—**12**) from *Penicillium notatum*,[4-6] *Dichtomomyces albus*,[7] and *Aspergillus chevalieri*.[8] Indeed the ultraviolet (UV) spectrum of xanthoascin, λ_{max}^{MeOH} 244, 302, 365, 385(sh) nm, is nearly superimposable with those of the 1,4-diphenyl-1,3-butadienes (**6**—**12**).[7-10]

The ^{1}H-nuclear magnetic resonance spectra (PMR) at 220 MHz of the compounds along with decoupling experiments showed the presence of two tertiary methyl groups (δ 1.37, 6H, s), two pairs of methylene groups (δ 1.85, 2H, t and δ 2.83, 2H, t, J=6.6 Hz), two olefinic protons (δ 6.97, 1H, s and δ 6.99, 1H, s), the aromatic protons of 1,2,4-trisubstituted benzene (δ 6.85, 1H, d, δ 7.57, 1H, d, and δ 7.60, 1H, dd; J=8.6 and 2.3 Hz) and those of 1,4-disubstituted benzene (δ 6.92 and 7.73, each 2H, d, J=8.8 Hz), and a hydroxyl (δ 5.41, 1H, s). The methylene signal at δ 2.83 couples with other methylene signal at δ 1.85 and also exhibits a long range coupling to the aromatic proton at δ 7.57. The PMR spectrum of the acetate (**4**) was nearly the same, reasonable low field shifts being observed for the ring protons in the 1,4-disubstituted benzene. These data are compared with those of xanthocillin-X (**6**) and the acetate (**13**) and are shown in Table I.

Table I. ^{1}H-NMR Spectra of Xanthoascin and Related Compounds (220 MHz)

Compound	Solvent	4-Hydroxyphenyl			2,2-Dimethyl-6-chromanyl						-CH=
		4-OH or 4-OAc	3-H and 5-H	2-H and 6-H	2-$(CH_3)_2$	3-CH_2	4-CH_2	5-H	7-H	8-H	
Xanthoascin (**3**)	$CDCl_3$	5.41	6.92 7.73 (8.8)		1.37	1.85 2.83 (6.6)		7.57	7.60 (2.3)	6.85 (8.6)	6.97 6.99
Xanthocillin-X (**6**)	CD_3OD	—	6.88 7.72 (8.8)								6.99
Xanthoascin acetate (**4**)	$CDCl_3$	2.33	7.20 7.82 (8.8)		1.37	1.85 2.83 (6.6)		7.59	7.62 (*ca.* 2)	6.85 (8.8)	7.02
Xanthocillin-X acetate (**13**)	$CDCl_3$	2.34	7.22 7.84 (8.7)								7.13

δ value in ppm from TMS (in parenthesis, coupling constants in Hz)

These observations suggested that xanthoascin might be a xanthocillin analog with 2,2-dimethyl-6-chromanyl and 4-hydroxyphenyl groups. The aryl groups were unequivocally proved by a degradation reaction. The oxidation of xanthoascin with chromium trioxide in acetic acid under controlled condition afforded two acidic products, mp 215—218° and 171—174°, which were identified respectively with 4-hydroxybenzoic acid and 2,2-dimethylchroman-6-carboxylic acid[11,12] by the direct comparison with the authentic samples. To discriminate the isonitrile groups from nitrile groups hydrolysis[4] of xanthoascin was performed and the formation of diformylamino derivative (**14**) was confirmed by the spectral data.[4,13]

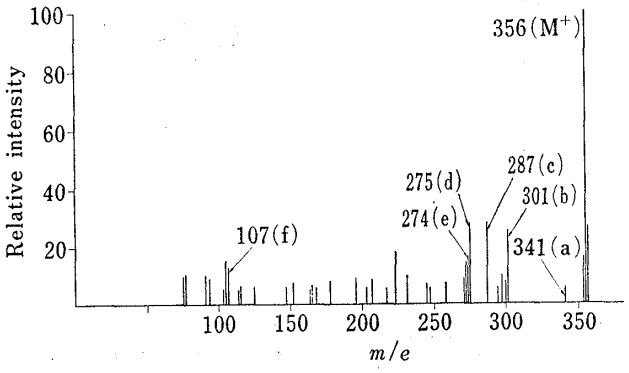

Fig. 1. Mass Spectrum of Xanthoascin (**3**)

10) H. Tönjes and H. Pötter, *Pharmazie*, **21**, 217 (1966).
11) K. Shima, S. Hisada, and I. Inagaki, *Yakugaku Zasshi*, **92**, 1410 (1972).
12) N. Ojima, S. Takenaka, and S. Seto, *Phytochem.*, **14**, 573 (1975).
13) B.J. Burreson, C. Christophersen, and P.J. Scheuer, *Tetrahedron*, **31**, 2015 (1975).

The mass spectra of xanthocillin groups have been proved to be a good guide for the determination of the substitution pattern;[5,6] *i.e.* fragments, M^+-CN, M^+-HCN, M^+-X, XC_4HN, XCH_2, XH, appear as predominant peaks where X is the aryl group. In the spectrum of xanthoascin (Fig. 1) the molecular ion appears as the base peak as those of other xanthocillins but any of the characteristic fragments was not observed except XCH_2 from 4-hydroxylphenyl group (fragment f in Chart 2). It has been reported that the mass spectrum of 2,2-dimethylchroman shows a M^+-55 ion as the base peak formed by elimination of a stable isobutenyl radical.[14] In the case of xanthoascin the fragmentation ($M^+ \rightarrow$b) shown in Chart 2 is supposed to occur preferentially to make the first glance of the spectrum obscure.

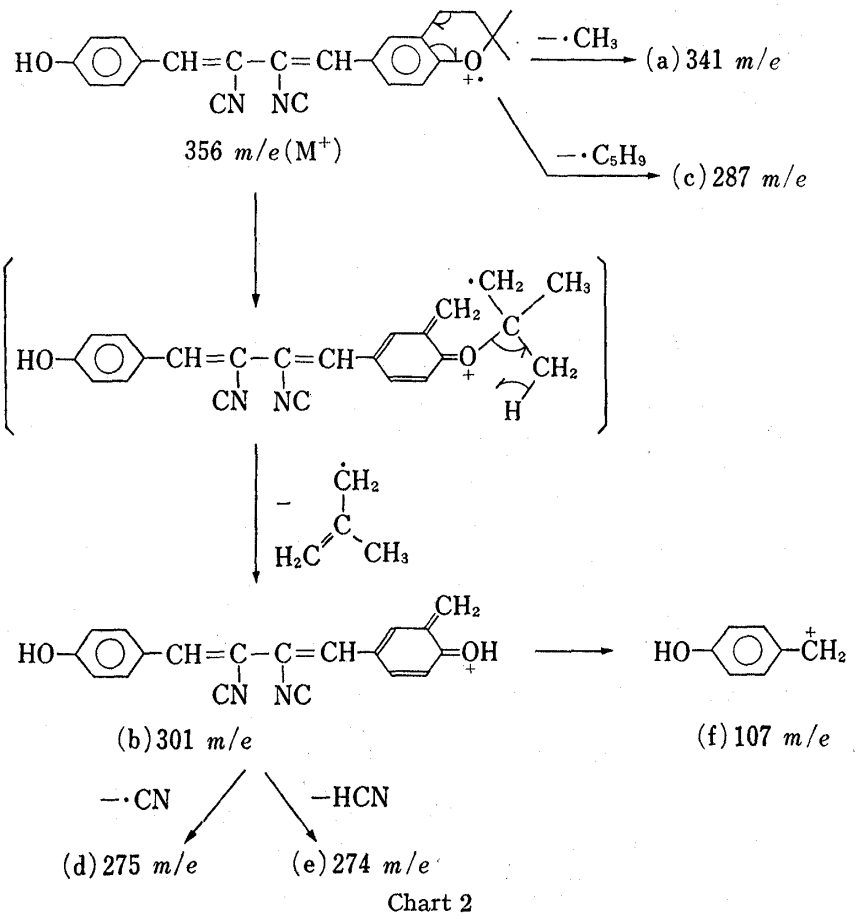

Chart 2

Thus the structure of the toxin was proved to be 1-(4-hydroxyphenyl)-4-(2,2-dimethyl-6-chromanyl)-2,3-diisocyano-1,3-butadiene (**3**).

Five metabolites of *Aspergillus candidus* so far characterized, two flavones,[15,16] two terphenyls (**1**, **2**),[1,16] and xanthoascin (**3**), are all assumed to be derived from shikimic acid[17] and the fact may suggest a characteristic metabolic feature of the mold as a fungi imperfecti.

Xanthoascin and other xanthocillin analogs exhibit cytotoxicity to cultured HeLa cells (Table II). Xanthoascin has been found to exhibit not only hepato- and cardio-toxicity but

14) Q.N. Porter and J. Baldes, "Mass Spectrometry of Heterocyclic Compounds," Wiley-Interscience, New York, 1971, p. 80.
15) A.E. Bird and A.C. Marshall, *J. Chem. Soc.*, (*C*), **1969**, 2418.
16) R. Marchelli and L.C. Vining, *J. Chem. Soc., Chem. Commun.*, **1973**, 555; *Idem*, *Canad. J. Biochem.*, **51**, 1624 (1973); *Idem*, *J. Antibiotics*, **28**, 328 (1975).
17) The strain of the fungus once reported to produce citrinin (M.I. Timonin and J.W. Ronatt, *Canad. J. Public Health*, **35**, 80 (1944)) has been identified as *Aspergillus niveus* (K.B. Raper and D.I. Fennell, "The Genus Aspergillus," Williams and Wilkins Co., Baltimore, 1965, p. 356).

also teratogenecity to experimental animals and these pathological observations will be reported precisely in a separate paper by our coworkers.[18] Recently bioproduction of terphenyllin and xanthoascin by rather wide range of the strains of *Aspergillus candidus* has been proved[19] and the mold should hereafter be paid attention as one of the mycotoxin-producing fungi.

Table II. Cytotoxicity to HeLa Cells of Xanthocillin Derivatives[a]

Compound	100	32	10	3.2	1.0 μg/ml
Xanthoascin (the compound B) (**3**)	4	4	4	1	
Xanthoascin acetate (**4**)	4	1	0	0	
Xanthoascin methyl ether (**5**)	3	2.5	2	0	
Xanthocillin-X[5] (**6**)	4	4	4	4	2
Xanthocillin-X monomethyl ether[8] (**10**)	4	4	4	1	
Xanthocillin-X dimethyl ether[8] (**11**)	3	2.5	2.5	2	
Methoxyxanthocillin-X dimethyl ether[8] (**12**)	4	3	2	0	

a) The degree of the cytotoxicity was estimated on scale 0 (no cellular damage) through 4 (complete cytolysis) by the same method shown in the previous paper.[1]

Experimental[20]

Compound B (Xanthoascin) (**3**)——Pale yellow needles from chloroform, mp 165—170° (decomp.).[1] It changes into dark color under exposure to sun-light and acids. UV λ_{max}^{MeOH} nm (log ε): 244, 302, 365, 385 (4.18, 4.12, 4.73, 4.65), $\lambda_{max}^{CHCl_3}$ (log ε): 302, 372, 390 (4.12, 4.75, 4.67). IR ν_{max}^{KBr} cm^{-1}: 3300, 2150, 1615 (sh), 1600 (s), 1575 (sh), 1520, 1495, 1440, 1375, 1350, 1280, 1265, 1220, 1175, 1160 1125, 1000, 945, 890, 820. PMR (Table I). Mass Spectrum *m/e*: 356.1502 (Calcd. for $C_{23}H_{20}O_2N_2$, 356.1523, M$^+$) (Chart 2 and Fig. 1). *Anal.* Calcd. for $C_{23}H_{20}O_2N_2$: C, 77.50; H, 5.66; N, 7.86. Found: C, 77.12; H, 5.61; N, 7.89.

Xanthoascin Monoacetate (**4**)——Xanthoascin (51 mg) was acetylated by acetic anhydride (1 ml) and pyridine (1 ml). Recrystallization from benzene gave pale yellow needles of mp 160—161°. IR ν_{max}^{KBr} cm^{-1}: 2110, 1762, 1603, 1569, 1498, 1272, 1226, 1194, 1170, 1157, 1120, 1020, 947, 914, 878, 812. PMR (Table I). ^{13}C-NMR (in $CDCl_3$) δ: 21.1, 22.5, 27.0 (×2), 32.7, 77.0, 115.2, 118.2, 118.9, 121.7, 122.2 (×2), 123.7, 126.3, 129.1, 129.9, 130.0, 131.2 (×2), 132.1, 151.9, 156.7, 168.9, 174.0, 174.3. Mass Spectrum *m/e*: 398.1563 (Calcd. for $C_{25}H_{22}O_3N_2$, 398.1629, M$^+$).

Xanthoascin Monomethyl Ether (**5**)——Xanthoascin (67 mg) was treated with excess amount of ethereal diazomethane for 1 hr and, after the addition of MeOH (1 ml), the reaction mixture was kept standing for 4 hr. After evaporation the residue was applied for preparative TLC using benzene as the developer. The main spot below the solvent front was extracted and recrystallized from benzene to yellow needles (20 ml) of mp 110°. IR ν_{max}^{KBr} cm^{-1}: 2150, 1600, 1575, 1495, 1460, 1445, 1350, 1320, 1300, 1260 1220, 1180, 1155, 1125, 1025, 945, 885, 825. Mass Spectrum *m/e*: 370.1701 (Calcd. for $C_{24}H_{22}O_2N_2$, 370.1681, M$^+$).

Chromium Trioxide Oxidation of Xanthoascin——To a warm solution of xanthoascin (15.5 mg) in $CHCl_3$ (1 ml) and HOAc (2 ml) chromium trioxide (18.6 mg) in 80% HOAc (1.8 ml) was gradually added and the mixture was kept standing at a room temperature overnight. The excess amount of chromium trioxide was decomposed by MeOH (1 ml), the solvent was evaporated, and ice-H_2O was added. The reaction mixture was extracted with ether, the ethereal layer was washed with $NaHCO_3$ solution, and the aqueous layer was acidified and shaken with ether. The ethereal extract was evaporated and the residue was passed through a column of silica-gel using hexane–ether as the developer. The eluates were cheked by TLC and two main products were collected. The first fraction was recrystallized from EtOH–H_2O to colorless needles (3 mg) of mp 171—174° IR ν_{max}^{KBr} cm^{-1}: 2945, ca. 2580 (br), 1677, 1610, 1410, 1300, 1260, 1154, 1120, 942, 843, 775, and the second fraction was recrystallized from EtOH–H_2O to colorless needles (2 mg) of mp 215—218°, IR ν_{max}^{KBr} cm^{-1}: 3340, *ca.* 2600 (br), 1677, 1599, 1240, 1165, 926, 853. They were respectively identified with 2,2-dimethylchroman-6-carboxylic acid (lit. mp 170—175°,[11] 173—175°[12]) and 4-hydroxybenzoic acid by direct comparison with authentic samples by TLC, IR, and mixed fusions.

18) K. Ohtsubo and M. Saito, *Japan. J. Exptl. Med.*, "in press."
19) Unpublished data of our laboratory (*cf.* S. Natori, *Proc. Japan. Assoc. Mycotoxicology*, No. 2, 26 (1976).
20) The melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. For thin-layer chromatography (TLC) silica-gel HF_{254} or GF_{254} and column chromatography silicic acid (100 mesh, Mallinckrodt) were used. ^{1}H-NMR spectra were determined on a Varian HR 220 (220 MHz) or a JEOL Model C-60HL (60 MHz) and ^{13}C-NMR on a Bruker BSV3PX (69.7 MHz). Mass spectra were determined on a JEOL 01SG-2 High Resolution Mass Spectrometer with direct inlet system.

Hydrolysis of Xanthoascin——Xanthoascin (50 mg) in 80% HOAc (1 ml) was warmed on a water-bath for 40 min. The solution was diluted with ice-H_2O and extracted with EtOAc. The solvent was evaporated and the residue was applied for preparative TLC using $CHCl_3$–EtOAc–acetone (7 : 1 : 3) as the developer. The main band was collected and recrystallized from $CHCl_3$ to afford the diformylamino derivative (**14**), pale yellow leaflets (23 mg) of mp 146—147°, IR ν_{max}^{KBr} cm^{-1}: 3240, 2990, 1669, 1608, 1578, 1493, 1386, 1263, 1230, 1172, 1156, 1119, 947, 896, 820. PMR (in CD_3OD) δ: 1.23 (6H, s), 1.72 (2H, t, J=6 Hz), 2.69 (2H, t, J=6 Hz), 6.4—6.8 (5H), 7.0—7.4 (4H), 7.82, 7.85, and 8.12 (ca 1/2H, ca 1/2H, *ca.* 1H; *cis*- and *trans*-NH-CHO).[13] Mass Spectrum *m/e*: 392.1679 (Calcd. for $C_{23}H_{24}O_4N_2$, 392.1735, M^+). From the upper band of the plate pale yellow leaflets (15 mg) of mp 186° ($CHCl_3$) were obtained and assigned as the *O*-acetate of **14** from the spectral data (IR ν_{max}^{KBr} cm^{-1}: 1764. PMR (in CD_3OD) δ: 2.24 (3H, s)).

Acknowledgement This work was carried out as a part of the cooperative survays on mycotoxins and we thank Professor M. Saito and Dr. K. Ohtsubo, Institute of Medical Sciences, University of Tokyo, and Dr. M. Umeda, Yokohama City University School of Medicine, for the pathological part of the work and Drs. H. Kurata, S. Udagawa, and F. Sakabe, Laboratory of Mycology of this Institute, for the mycological part of the work. The authors are indebted to Professor Dr. H. Achenbach, Freiburg University, Professor G. Tamura, University of Tokyo, Professor S. Seto, Tohoku University, and Professor Y. Ogiwara, Nagoya City University, for their generous gifts of the samples and Dr. K. Kanohda and Mr. H. Abe, this Institute, for the mass spectral determinations.