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CAVOXININE, A MINOR METABOLITE FROM *PHOMA CAVA*, AND CAVOXINONE, ITS CORRESPONDING CHROMAN-4-ONE

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ABSTRACT.—A new metabolite named cavoxinine [**3**] was isolated from the culture filtrate of *Phoma cava* together with cavoxinone [**4**], its related chroman-4-one. Both structures were established by nmr and hrms studies by correlating their spectroscopic and chemical properties with those of cavoxin [**1**] and cavoxone [**2**] and on the basis of biogenetic considerations.

In a previous paper we (1) reported the structure of cavoxin [**1**], a new phytotoxin isolated from *Phoma cava* Schulzer (Sphaeropsidaceae), together with cavoxone [**2**], the corresponding chroman-4-one.

This note describes the structural elucidation of a minor metabolite named cavoxinine [**3**] and of cavoxinone [**4**], its related chroman-4-one, both present at very low levels (0.6 and 0.4 mg/liter, respectively) in the culture filtrate of the same fungus.

RESULTS AND DISCUSSION

The crude $CHCl_3$ extract obtained from the culture filtrates of *P. cava* was fractionated on a Sephadex LH-20 column as previously reported (1). The eluate showing phytotoxic activity yielded cavoxin [**1**]. Cavoxone [**2**] was obtained by recrystallization from the inactive fractions. The resulting mother liquors contained two other uv absorbing compounds both possessing higher Rf values than **2** by tlc analysis (SiO_2, $CHCl_3$-iPrOH, 9:1).

An initial fractionation by preparative tlc (SiO_2, $CHCl_3$-iPrOH, 9:1) yielded the two minor components as crude oils. Cavoxinine [**3**] and cavoxinone [**4**] were further purified by an additional tlc step (SiO_2, C_6H_6-Me_2CO, 6:4).

Cavoxinine [**3**] had a molecular formula $C_{17}H_{20}O_5$ from the hrms data *m/z* 304.1185 (M^+, calcd. 304.1311). The significant peaks at *m/z* 261.0795 ($C_{14}H_{13}O_5$), 190.0227 ($C_{10}H_6O_4$), 180.0437 ($C_9H_8O_4$), and 164.0507 ($C_9H_8O_3$) were consistent with the fragmentation pattern already proposed for cavoxin (1). These data indicated the presence of an *ortho*-hydroxy aromatic ketone and a heptadienyl residue in cavoxinine.

The ir spectrum showed the characteristic absorptions of hydroxyl, carboxylic, conjugate carbonyl, and benzene groups. Furthermore, cavoxinine reacted with $FeCl_3$ and had no optical activity.

An accurate inspection of its ^{1}H-nmr spectrum and the comparison with that of **1** (Table 1) suggested a cavoxin-like structure for this new metabolite. The singlet at δ 3.80 attributed to the methoxyl group was present in addition to the signal pattern of the octadienonyl residue. A substantial difference between **3** and **1** was observed in the region of the aromatic proton shifts; **3** showed two *meta*-coupled doublets at δ 6.42 and 6.40, whereas cavoxin exhibited only a singlet at δ 6.50. Moreover, a downfield shift was recorded for the signal due to the benzylic methylene group which now appeared as two doublets (an AB system) at δ 3.84 and 3.76.

The ^{13}C-nmr data of **3** are reported in Table 2. The carbon shifts, assigned using the pnd (proton noise decoupled) and the sford (single frequency off-resonance decoupled) techniques, resembled those reported for **1** (1). The carbon shifts of the carboxylic and methoxyl groups and those of the octadienonyl residue were very close to the resonance frequencies reported for **1**, whereas some differences were noticed in the

TABLE 1. ^{1}H-nmr Data of Cavoxin [**1**], Cavoxinine [**3**], Cavoxinone [**4**], and the Corresponding Methyl Ester [**5**][a]

Proton No.	Compounds		Proton No.	Compounds	
	1	**3**		**4**	**5**
H-1[b]	—	6.42, d	H-2	4.87, ddd	4.87, ddd
H-5[b]	6.50, s	6.40, d	2H-3	2.81, dd } AB	2.75, dd } AB
H-9	6.71, d	6.59, d		2.69, dd	2.60, dd
H-10	7.37, ddd	7.31, ddd	H-6[b]	6.40, d	6.36, d
H-11	6.32, dd	6.27, dd	H-8[b]	6.47, d	6.43, d
H-12	6.27, ddt	6.22, ddt	H-9	5.64, dd	5.68, dd
2H-13	2.18, td	2.17, td	H-10	5.87, dt	5.86, dt
2H-14	1.47, tq	1.46, tq	2H-11	2.08, td	2.07, td
3H-15	0.92, t	0.92, t	2H-12	1.44, tq	1.44, tq
2H-17	3.57, s	3.84, d } AB	3H-13	0.92, t	0.92, t
	—	3.76, d	2H-15	3.93, s	3.99, d } AB
OMe	3.86, s	3.80, s		—	3.89, d
			OMe	3.82, s	3.81, s
			OMe	—	3.71, s

J (Hz), **1**, **3**: 9, 10=11, 12=15.1; 10, 11=12, 13=6.3; 10, 12=2.9; 13, 14=14, 15=7.3; **3**; 1, 5=2.6; 17A, 17B=16.9; **4**, **5**: 2, 3A=11.7; 2, 3B=3.68; 2, 9=10, 11=6.6; 3A, 3B=16.8; 6, 8=2.2; 9, 10=15.4; 11, 12=12, 13=7.3; **5**: 15A, 15B=16.8.

[a]Chemical shifts are in δ-values (ppm) from TMS.

[b]These assignments may be reversed.

region of the aromatic carbon shifts. In particular, the signals at δ 111.2 and 100.6 attributed to C-5 and C-1, respectively (both doublet in the sford spectrum), were consistent with a tetrasubstituted benzene ring. Cavoxin having a pentasubstituted ring showed only one signal at δ 106.6 due to an aromatic secondary carbon.

TABLE 2. ^{13}C-nmr of Cavoxinine [**3**][a]

Atom		Atom	
C-1	100.6, d[b]	C-10[c]	145.2, d[b]
C-2	163.1, s	C-11[c]	129.1, d
C-3[c]	135.7, s	C-12[c]	147.2, d
C-4	163.7, s	C-13	35.2, t
C-5	111.2, d	C-14	21.8, t
C-6[c]	117.1, s	C-15	13.5, q
C-7	174.5, s	C-16	55.4, q
C-8	195.3, s	C-17	40.6, t
C-9[c]	127.9, d		

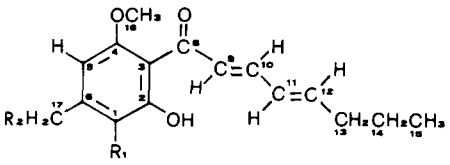
[a]Chemical shifts are in δ-values (ppm) from TMS.

[b]Multiplicities were determined by sford spectrum.

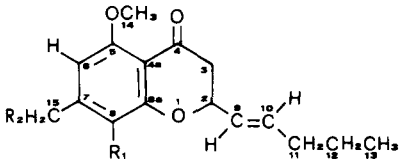
[c]Assignments made also by comparison with data reported for **1** (1).

The downfield chemical shift of the C-2 and C-4 in the ^{13}C-nmr spectrum of **3** ($\Delta\delta$=17.3 ppm and 13.6 ppm, respectively), as compared with the resonance frequencies of the corresponding carbons in **1**, could be ascribed to the disappearance of the mesomeric effect (2) governed in **1** by the electron attracting COOH group on C-1. In fact, the absence of this group on C-1 of **3** determines a less effective delocalization of the unshared electron pair of the hydroxyl and the methoxyl groups; this, in turn, resulting in a decrease of charge density at C-2 and C-4, respectively. These findings suggested structure **3** for cavoxinine.

The location of the phenolic hydroxyl group on C-2 and of the octadienonyl side chain on C-3 was consistent with the conversion of **3** to the corresponding chroman-4-one [**4**]. In fact, cavoxinone [**4**] was obtained with quantitative yielded by acid treatment (5N HCl at reflux) of cavoxinine [**3**]. A partial conversion was also achieved when cavoxinine was left for a long period in moist air or in solution. Identical reactions have been already observed for cavoxin (1).



1 R_1=^{7}COOH, R_2=OH
3 R_1=H, R_2=^{7}COOH



2 R_1=^{16}COOH, R_2=OH
4 R_1=H, R_2=^{16}COOH
5 R_1=H, R_2=^{16}COOCH$_3$

The assignment of the phenolic hydroxyl group on C-2 and of the octadienonyl side chain group on C-3 was supported by the following evidence: (a) The very close chemical shifts of H-1 and H-5 (δ 6.42 and 6.40, respectively) in the ^{1}H-nmr spectrum of **3** indicates that very similar functional groups were attached on the *ortho*-positions of C-1 and C-5. Moreover, the δ values were in agreement with the location of the phenolic hydroxyl, the methoxyl, and the carboxymethyl group on C-2, C-4, and C-6, respectively (3). The same considerations justified the very close chemical shifts of H-6 and H-8 (δ 6.40 and 6.47, respectively) observed in the ^{1}H-nmr spectrum of **4**. (b) The carbon shifts of C-3 and C-6 in the ^{13}C-nmr spectrum of **3** (δ 135.7 and 117.1, respectively) were very similar to the values observed for the same carbons in **1** (δ 133.1 and 121.0 for C-3 and C-6, respectively) (1). These results were consistent with the presence on C-2, C-3, and C-4 of **3** of the same functional groups found in **1** (1,2).

The structural features of cavoxinine and its corresponding chroman-4-one indicated an acetogenin nature for these two compounds in agreement with literature data (4,5).

In the acetogenins biosynthesis incorporating at least one benzene ring, two pathways have been proposed for the polyacetyl chain cyclization. The route leading to acylphloroglucinols (4) may be invoked for the cavoxinine biosynthesis. In fact, the cyclization of an appropriate acetate-derived precursor via acylphloroglucinol is in agreement with the presence of the phenolic hydroxyl group on C-2 and of the octadienonyl residue on C-3 of cavoxinine. Moreover, this pathway suggests the presence of the methoxyl group on C-4 previously found on C-4 in **1** and on C-5 in **2** (1). Since the two aromatic protons (H-1 and H-5) were *meta*-coupled, the carboxymethyl group is thus on C-6 in **3**.

Cavoxinone obtained from **3** described as above was identical in all respects to the compound **4** isolated together with **3** from the culture filtrate of the fungus. The optical inactivity of natural cavoxinone suggests that it is a racemic mixture probably formed from the open precursor **3**. Therefore, **4** may be considered an artifact as previously demonstrated for **2** produced from **1** (1). However, **4** represents a further example of a chroman-4-one (6,7) which are rare as naturally occurring compounds (8).

Further support of the structure assigned to cavoxinone and, consequently, to that of **3** was given by the chemical and the spectroscopic properties of this chroman-4-one. The uv spectrum of **4** showed a maximum absorption at 275 nm in agreement with the value previously observed for cavoxone (1) and with those reported for other chroman-4-ones (9). The ^{1}H-nmr spectrum of **4** was very similar to that of cavoxone [**2**] except for

the presence of the signals at δ 6.47 and 6.40 assigned to the two *meta*-coupled aromatic protons. The hrms of **4** gave a molecular formula $C_{17}H_{20}O_5$ at *m/z* 304.1294 (M^+, calcd. 304.1311). The prominent peaks at *m/z* 190.0292 ($C_{10}H_6O_4$), 180.0414 ($C_9H_8O_4$), and 164.0465 ($C_9H_8O_3$) were consistent with the fragmentation pattern reported in the literature for chroman-4-one (10) and previously observed for **2** (1).

Compound **4** was not acetylated by treatment with Ac_2O, whereas **2** under the same experimental conditions gave the corresponding monoacetyl derivative (1). As expected, **4** afforded the methyl ester [**5**] by reaction with ethereal CH_2N_2 while **5** was reconverted into **4** by hydrolysis performed with 3N ethanolic HCl.

The ^{1}H-nmr spectrum of **5** (Table 1) with respect to that of **4** showed a singlet at δ 3.71 attributed to the other methoxyl group as the only difference. Its hrms gave a molecular formula $C_{18}H_{22}O_5$ at *m/z* 318.1477 (M^+, calcd. 318.1468).

Significant peaks were recorded at *m/z* 275.0950 ($C_{15}H_{15}O_5$), 222.0517 ($C_{11}H_{10}O_5$), 194.0600 ($C_{10}H_{10}O_4$), 179.0326 ($C_9H_7O_4$), and 135.0443 ($C_8H_7O_2$). The peaks at *m/z* 222.0517 and 194.0600 were in agreement with the fragmentation mechanism described for chroman-4-ones (10). The ion at *m/z* 194.0600 produced, by loss of methyl, the fragment at *m/z* 179.0326 which, in turn, by successive loss of CO_2 yielded the ion at *m/z* 135.0443. With this evidence, the cavonxinine and cavoninone are assigned structures **3** and **4**, respectively.

The very low level of cavoxinine present in the culture filtrate of *P. cava* and the low amount of the pure compound available have not allowed a test of its phytotoxic activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 684 instrument for solutions in $CHCl_3$; uv spectra were measured on a Perkin-Elmer 550 S spectrometer in EtOH solutions; ^{1}H- and ^{13}C-nmr spectra were recorded at 270 and 67.88 MHz, respectively, on a Bruker spectrometer; mass spectra and high resolution mass spectra were recorded at 70 eV on an AEI-30 and on a Kratos-50 mass spectrometer, respectively. Analytical and preparative tlc were performed on SiO_2 plates (Merck, Kieselgel 60 F_{254}, 0.25 and 2 mm, respectively; the spots were visualized by spraying the plates first with 10% H_2SO_4 in MeOH and then with 3% phosphomolybdic acid in MeOH followed by heating 5 min at 105° or by exposure to uv radiation. Column chromatography was carried out on Sephadex LH-20 (Pharmacia 25-100 μm).

FUNGUS CULTURES.—Most experimental details on the growth of the *P. cava* (CBS 535.66, Centraalbureau voor Shimmelcutures Baarn, the Netherlands) to obtain phytotoxic culture filtrates were reported previously (1).

EXTRACTION AND ISOLATION.—Lyophilized solid residue corresponding to 21.7 liters of culture filtrate of *P. cava* was dissolved in distilled H_2O (2.4 liters) and extracted with $CHCl_3$ (4×1 liter). The organic extracts were combined, dried (Na_2SO_4), filtered, and then evaporated under reduced pressure. The residue (3.5 g) was chromatographed on Sephadex LH-20 column eluted with $CHCl_3$-iPrOH (9:1). The first collected fractions yielded crude cavoxone [**2**]; the successive eluate contained pure cavoxin [**1**]. Both compounds were obtained as an oil after removal of the solvent under pressure. Cavoxin crystallized as pale yellow needles (1.8 g) from EtOAc/petroleum ether (40-70°); cavoxone was obtained as white needles (199.5 mg) by crystallization from EtOAc. The mother liquors of **2** were concentrated to a residue (73.8 mg) and further fractionated by preparative tlc on SiO_2, using $CHCl_3$-iPrOH (9:1) as eluent. Three uv asborbing bands were observed, scraped from the plates, and eluted with the same solvent system. Evaporation of the solvent gave the following residues: A (from the lower zone, 18.4 mg) monitored by tlc showed to contain cavoxone [**2**]; B (from the upper zone, 4.4 mg) contained compound **4**; C (from the intermediate zone, 17.2 mg) contained **4** and another compound. The residue C was further chromatographed on SiO_2 plates (C_6H_6-Me_2CO, 6:4) affording cavoxinine [**3**] (12.0 mg) and cavoxinone [**4**] (4.0 mg) both as homogeneous oils.

Cavoxinine [**3**].—Cavoxinine, a pale yellow oil resistant to crystallization, showed: ir ν max 3520, 3000, 1740, 1710, 1670, 1610, 1575, cm^{-1}; uv λ max nm (log ε) 315 (3.92), 280 (4.06); ^{1}H- and ^{13}C-nmr spectra are reported in Tables 1 and 2, respectively; ms *m/z* (rel. int.) 304 (M^+) (18), 286 (9), 262 (9), 261 (100), 243 (27), 217 (64), 208 (5), 190 (36), 180 (40), 165 (40), 164 (50).

Cavoxinone [**4**].—Cavoxinone, a colorless oil resistant to crystallization, had: ir ν max 3000, 1740, 1710, 1670, 1605, 1575, cm^{-1}; uv λ max nm (log ϵ) 309 (3.00), 275 (3.42), 230 (shoulder); ^{1}H-nmr spectrum is reported in Table 1; ms *m/z* (rel. int.) 304 (M^+) (12), 286 (20), 262 (15), 261 (100), 243 (45), 217 (70), 190 (28), 180 (40), 165 (35), 164 (55).

Methyl ester of **4** [**5**].—To a solution of **4** (5 mg) in Et_2O (1 ml) was added at 0° ethereal CH_2N_2 (1 ml). The mixture was allowed to stand at room temperature for 4 h and then evaporated under a N_2 stream. Purification of the residue by preparative tlc (C_6H_6-Me_2CO, 8:2) afforded **5** as an oily residue (3.6 mg, 72%): ir ν max 1780, 1670, 1605, 1575 cm^{-1}; uv λ max nm (log ϵ) 309 (2.76), 274 (3.14), 230 (3.22); ^{1}H-nmr spectrum is reported in Table 1; ms *m/z* (rel. int.) 318 (M^+) (32), 287 (14), 286 (13), 276 (19), 275 (100), 243 (7), 229 (6), 222 (6), 194 (43), 179 (42), 135 (14).

Conversion of cavoxinine [**3**] *to* **4**.—Cavoxinine (3 mg) in EtOH (0.2 ml) was treated with 5N HCl at reflux for 4 h. After cooling, the solution was diluted with H_2O (15 ml) and extracted with $CHCl_3$ (3 × 10 ml). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The pure product showed the same Rf as **4** by co-chromatography according to tlc analysis on SiO_2 ($CHCl_3$-iPrOH, 9:1 and C_6H_6-Me_2CO, 6:4) and on reverse phase (Stratocrom C-18, Whatman 0.2 mm, eluent H_2O-MeCN, 6:4).

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