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STRUCTURE OF CAVOXIN, A NEW PHYTOTOXIN FROM *PHOMA CAVA* AND CAVOXONE, ITS RELATED CHROMAN-4-ONE

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ABSTRACT.—Cavoxin (**1**), a new tetrasubstituted benzoic acid derivative showing phytotoxic activity, has been isolated from *Phoma cava* together with cavoxone (**2**), an inactive chroman-4-one structurally related to cavoxin. Structures of both compounds were elucidated by extensive nmr studies and preparation of some key derivatives.

A phytotoxic compound, cavoxin (**1**)[1], was isolated from culture filtrates of *Phoma cava* Schulzer from *Castanea* spp., a fungus belonging to a toxigenic genus (1-3). Supplied to tomato cuttings (4,5), cavoxin caused a vascular browning and a rapid wilting of leaflets. Together with the phytotoxic compound (**1**), a smaller amount of cavoxone (**2**)[1] was present in the culture filtrate of the same fungus. Cavoxone was inactive to tomato cuttings (4,5). The toxin (**1**) is a new tetrasubstituted benzoic acid derivative, and cavoxone (**2**) is a related chroman-4-one (6,7). Cavoxone is particularly interesting, as very few naturally occurring chroman-4-ones have been isolated so far (8).

RESULTS AND DISCUSSION

The fungus was cultured at 25° for 5 days in flasks containing a semisynthetic medium. The culture filtrate was extracted with $CHCl_3$ and taken to dryness. The residue left from the organic extract was chromatographed on Sephadex LH-20. Each fraction was checked by bioassay using the usual techniques (4,5).

Cavoxone (**2**) was obtained as a pure compound that crystallized as white needles (12.1 mg/liter) from EtOAc. The toxic eluate afforded cavoxin (**1**) as an oily residue that crystallized as pale yellow needles (108.7 mg/liter) from EtOAc-petroleum ether.

Cavoxin exhibited no optical rotation and had a molecular formula $C_{17}H_{20}O_6$ from hrms *m/z* 320.1263 (M^+, calcd. 320.1260). The fragmentation peaks at *m/z*: 277.0718 ($C_{14}H_{13}O_6$), 233.0829 ($C_{13}H_{13}O_4$), 224.0317 ($C_{10}H_8O_6$), 206.0246 ($C_{10}H_6O_5$, base peak), and 196.0375 ($C_9H_8O_5$) indicated the presence of an *ortho*-hydroxy aromatic ketone, an hydroxyl, and a carbonyl group in addition to an heptadienyl residue. Cavoxin gave a red color reaction with 1% aqueous $FeCl_3$. These results were in agreement with the characteristic absorption frequencies observed in the ir spectrum of **1**.

The ^{1}H-nmr (Table 1) and the proton noise decoupled (pnd), the single frequency off-resonance decoupled (sford), and the single frequency selective decoupled (sfsd) ^{13}C-nmr (Table 2) spectra provided initial insights into the structure of **1**. The compound contained a methyl, a methoxyl, and an hydroxymethyl group; the signal of a pentasubstituted benzene ring was also observed. Extensive ^{1}H-nmr analyses carried out with **1** suggested the presence of an octadienonyl residue attached to the aromatic ring. In addition, both olefinic bonds possessed a *trans*-isomerization (9). The intense uv ab-

[1]Nomenclature, cavoxin: 2-hydroxy-3-(1-oxo-2*E*,4*E*-octadienyl)-4-methoxy-6-hydroxymethyl benzoic acid; cavoxone: 2-(1*E*-pentenyl)-5-methoxy-7-hydroxymethyl-8-carboxylic acid chroman-4-one.

TABLE 1. ^{1}H-nmr Data of Cavoxin (**1**), Cavoxone (**2**), and the Corresponding Methyl Esters (**4** and **8**). Chemical Shifts are in δ-Values (ppm) from TMS.

	1[a]	**4**[b]		**2**[a]	**8**[b]
H-5	6.47 s	6.41 s	H-2	4.94 ddd	4.91 ddd
H-9	6.61 d	6.55 d	2H-3	2.80 dd AB	2.79 dd AB
H-10	7.21 dd	7.23 ddd		2.69 dd	2.66 dd
H-11	6.32 dd	6.27 dd	H-6	6.46 s	6.39 s
H-12	6.20 dt	6.23 ddt	H-9	5.72 dd	5.67 dd
2H-13	2.17 td	2.17 td	H-10	5.88 dt	5.84 dt
2H-14	1.46 tq	1.46 tq	2H-11	2.07 td	2.06 td
3H-15	0.91 t	0.92 t	2H-12	1.44 tq	1.42 tq
2H-17	3.56 s	3.73 s	3H-13	0.92 t	0.89 t
OMe	3.89 s	3.91 s	2H-15	3.96 d AB	3.97 d AB
OMe	—	3.68 s		3.88 d	3.88 d
			OMe	3.94 s	3.93 s
			OMe	—	3.69 s

[a]Run in $CDCl_3$-CD_3OD, 2:1.

[b]Run in $CDCl_3$.

J (Hz), **1**: 9, 10=15.4; 10, 11=9.2; 11, 12=15.1; 12, 13=6.6; 13, 14=14, 15=7.0; **2**: 2, 3A=10.7; 2, 3B=4.0; 2, 9=10, 11=6.6; 3A, 3B=16.7; 9, 10=15.4; 11, 12=12, 13=7.0; 15A, 15B=16.9; **4**: 9, 10=11, 12=15.1; 10, 11=12, 13=6.6; 10, 12=3.7; 13, 14=14, 15=7.3; **8**: 2, 3A=11.0; 2, 3B=3.7; 2, 9=10, 11=6.6; 3A, 3B=15A, 15B=16.9; 9, 10=15.4; 11, 12=12, 13=7.0.

TABLE 2. ^{13}C-nmr Data of Cavoxin (**1**), Cavoxone (**2**), and the Corresponding Methyl Esters (**4** and **8**). Chemical Shifts are in δ-Values (ppm) from TMS.

	1[a,b]	**4**[b,c]		**2**[a,d]	**8**[b,c]
C-1	125.3 s[e]	125.5 s	C-2	78.8 d	78.8 d
C-2	145.8 s[e]	147.2 s	C-3	41.3 t	40.7 t
C-3	133.1 s[e]	132.4 s	C-4	193.9 s[e]	192.0 s
C-4	150.1 s[e]	149.3 s	C-4a	134.0 s[e]	133.1 s
C-5	106.6 d	106.7 d	C-5	152.4 s[e]	150.7 s
C-6	121.0 s[e]	118.6 s	C-6	109.7 d	109.1 d
C-7	174.3 s[e]	171.8 s	C-7	114.4 s[e]	113.9 s
C-8	197.3 s[e]	195.5 s	C-8	128.7 s[e]	128.2 s
C-9	129.6 d[e,f]	128.3 d	C-8a	151.3 s[e]	150.0 s
C-10	146.4 d[e,f]	145.3 d	C-9	127.7 d[e,f]	127.0 d
C-11	129.7 d[e,f]	129.2 d	C-10	136.3 d[e,f]	136.2 d
C-12	147.3 d[e,f]	147.0 d	C-11	34.6 t	34.2 t
C-13	35.6 t	35.2 t	C-12	22.3 t	21.8 t
C-14	22.2 t	21.8 t	C-13	13.7 q	13.5 q
C-15	13.8 q	13.6 q	C-14	56.4 q	56.2 q
C-16	56.2 q	56.1 q	C-15	44.3 t	44.1 t
C-17	40.1 t	39.8 t	C-16	174.7 s	172.0 s
OMe	—	52.1 q	OMe	—	51.7 q

[a]Run in $CDCl_3$-CD_3OD, 2:1.

[b]Multiplicities were determined by sford spectrum.

[c]Run in $CDCl_3$.

[d]Multiplicities were determined by sford and dept (distortionless enhancement by polarization transfer) spectra.

[e]Attributions made by long-range ^{13}C-^{1}H couplings observed in the gated-decoupling spectrum.

[f]Assignments made by sfsd spectra.

sorption at 280 and 325 nm corroborated the presence in **1** of a conjugated chromophore. These findings indicated that **1** is a tetrasubstituted benzoic acid derivative.

A gated-decoupling ^{13}C-nmr experiment (10) with **1** provided precise measurements of the long-range ^{13}C-^{1}H coupling constants and, therefore, was used for the structural determination of cavoxin. The multiplicities and long-range ^{13}C-^{1}H couplings observed for the quaternary carbons and for the carbons of the groups attached to the aromatic ring are summarized in Table 3. From these results, we deduced the location of the substituent groups on the benzene ring and suggest the structure **1** for cavoxin.

TABLE 3. Multiplicities and Long-Range ^{13}C-^{1}H Coupling Constants Measured in the Gated-Decoupling ^{13}C-nmr Spectrum of Cavoxin (**1**)[a]

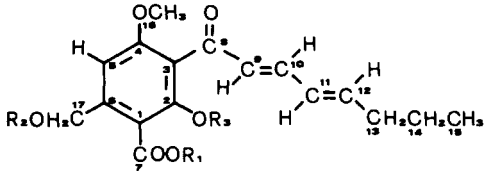
	δ	m	$J_{C\text{-}H}$[b]		δ	m	$J_{C\text{-}H}$[b]
C-1	125.3	t	^{3}J=5.7	C-6	121.0	dt	^{2}J=5.7, ^{2}J=3.4
C-2	145.8	s	—	C-7	174.3	t	^{4}J=7.9
C-3	133.1	d	^{3}J=6.8	C-8	197.3	dd	^{4}J=4.5, ^{3}J=5.7
C-4	150.1	dq	^{3}J=3.4, ^{2}J=2.7	C-17	40.1	td[c]	^{3}J=4.5
C-5	106.6	dt[c]	^{3}J=6.8				

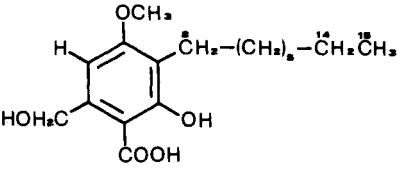
[a]Run in $CDCl_3$-CD_3OD, 2:1, at 67.88 MHz (temp. 27°, pulse 45°, repetention time 1.5 sec, data points 16 K).

[b]Long-range ^{13}C-^{1}H coupling constants in Hz.

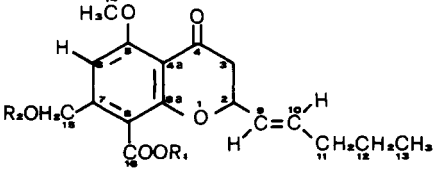
[c]$^{1}J_{C\text{-}H}$ was 182.5 Hz for C-5 and 147.3 Hz for C-17.

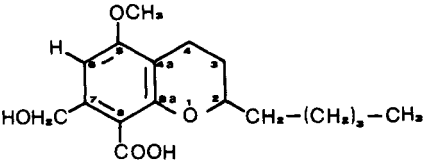
Further support for the structure of **1** was obtained using long-range selective proton decoupling (lspd) (10, 11). The application of this technique allowed us, by keeping with the decoupler at a weak power level, to eliminate the fine splitting due to the long-range ^{13}C-^{1}H coupling, leaving a simplified or a better defined signal. In fact, irradiation at δ 6.47 (H-5) has converted the doublet at δ 133.1 (C-3) into a singlet, the double quartet at δ 150.1 (C-4) into a quartet ($^{3}J_{C\text{-}H}$=3.4), the double triplet at δ 121.0 (C-6) into a triplet ($^{2}J_{C\text{-}H}$=3.4), and the triple doublet at δ 40.1 (C-17) into a triplet ($^{1}J_{C\text{-}H}$=147.3). The irradiation of the methoxyl group at δ 3.89 has collapsed the double quartet at δ 150.1 (C-4) to a doublet ($^{2}J_{C\text{-}H}$=2.7), while irradiation at δ





1 R_1=R_2=R_3=H **4** R_1=CH_3, R_2=R_3=H **3**
5 R_1=R_3=CH_3, R_2=H **6** R_1=R_2=CH_3, R_3=H
7 R_1=R_2=R_3=CH_3





2 R_1=R_2=H **8** R_1=CH_3, R_2=H **11**
9 R_1=R_2=CH_3 **10** R_1=H, R_2=$COCH_3$

7.21 (H-10) simplified the double doublet at δ 197.3 (C-8) into a doublet ($^4J_{C-H}$=4.5). Finally, irradiation of the hydroxymethyl group (2H-17) at δ 3.56 has converted both the triplet at δ 125.3 (C-1) and the one at δ 174.3 (C-7) into two singlets, the double triplet at δ 106.6 (C-5) into a doublet ($^1J_{C-H}$=182.5), and the double triplet at δ 121.0 (C-6) into a doublet ($^2J_{C-H}$=5.7). Moreover, in the gated-decoupling ^{13}C-nmr spectrum of the 8-deoxyhexahydroderivative of **1** (**3**) the signal of the C-2 appeared as a triplet at δ 143.9 ($^3J_{C-H}$=4.5), while the splitting of the other carbons remained unchanged, as compared with those of **1**, except for the signal due to C-4; this carbon now appeared as a more complex system due to a further coupling with the benzylic methylene H_2C-8. Consequently, irradiation at δ 2.58 (2H-8) in the lspd ^{13}C-nmr spectrum of **3** caused the collapse of the triplet at δ 143.9 into a singlet.

The location of the methoxyl and the hydroxymethyl groups in the *ortho*-position with respect to the aromatic proton was confirmed by the evidence obtained from the ^{1}H-nOe difference spectra (Table 4) carried out with the methyl ester of **1** (**4**). The results *a*, *b*, *c*, and *d*, have established the spatial proximity between H-5 and both $HOCH_2$-17 and OMe groups.

TABLE 4. Nuclear Overhauser Effects Measured on Compound **4** ($CDCl_3$)

Irradiated		Observed	
6.41	(H-5)	*a* 3.73	(2H-17)
		b 3.91	(OMe)
3.73	(2H-17)	*c* 6.41	(H-5)
3.91	(OMe)	*d* 6.41	(H-5)

Confirmation of the structure assigned to **1** was also obtained by the synthesis of some derivatives. Treatment of cavoxin with CH_2N_2 for 6 h gave the corresponding methyl ester **4**. When the reaction was performed for 2 days with successive additions of CH_2N_2, **1** was converted into the three derivatives **5**, **6**, and **7**. Only the compounds **4** and **6** gave a red color reaction with $FeCl_3$. The ^{1}H-nmr spectra of these four derivatives were very similar to that of **1**, except for the presence of the singlets due to the other methoxyl groups, which were observed in **4** at δ 3.68; in **5** at δ 3.78 and 3.61; in **6** at δ 3.90 and 3.60, and in **7** at δ 3.87, 3.80, and 3.62. Hydrogenation of cavoxin afforded the 8-deoxyhexahydroderivative **3**. The ^{1}H-nmr spectrum of **3**, as compared to that of **1**, lacked the signals caused by the octadienonyl residue and showed the presence of a very broad signal at δ 1.26 attributed to six aliphatic methylenes, and a triplet at δ 2.58 due to the benzylic methylene (2H-8). In the ^{13}C-nmr spectrum of **3**, with respect to that of **1**, the signals of the carbonyl and the olefinic carbons were absent, while at δ 30.3, 30.2, 29.9, and 29.7 the signals due to the four aliphatic methylene carbons and the signal at δ 26.7 attributed to the H_2C-8 appeared.

Cavoxone (**2**) had a molecular formula $C_{17}H_{20}O_6$ from hrms *m/z* 320.1237 (M^+, calcd. 320.1260). Its ir spectrum exhibited the absorption frequencies typical of the carboxylic, carbonyl, and hydroxyl groups; because no color was observed by reaction with $FeCl_3$, the presence in **2** of the phenolic hydroxyl groups was ruled out. Cavoxone showed uv absorptions at 288 and 242 nm in agreement with the values indicated in the literature for chroman-4-one derivatives (12). The ^{1}H- and ^{13}C-nmr data of **2** are shown in Tables 1 and 2, respectively.

After treatment with CH_2N_2, cavoxone gave the corresponding methyl ester **8**. When the reaction was performed with a large excess of reagent for a longer time, **2** afforded essentially the compound **9**. The two derivatives showed a ^{1}H-nmr spectrum very similar to that of **2**, except for the presence of the singlets due to the other

methoxyl groups appearing in **8** at δ 3.69 and in **9** at δ 3.85 and 3.72. Reaction of pyridine-dissolved cavoxone with equimolar amounts of Ac_2O under the described conditions gave the 15-*O*-acetyl derivative of **2** (**10**); a longer reaction time or a larger amount of reagent gave degradative products. The ^{1}H-nmr spectrum of **10** showed a singlet at δ 2.28 assigned to the acetyl group, as the only difference from the spectrum of **2**. Catalytic hydrogenation of **2** afforded the 4-deoxytetrahydroderivative of **2** (**11**) which had a 2-alkyl-chroman-type structure (6,7). The analysis of its ^{1}H-nmr spectrum evidenced, as compared to that of **2**, some complex system in the region of the aliphatic protons and two doublets of double doublets at δ 3.00 and 2.91 attributed to H-4A and H-4B, respectively. The ^{13}C-nmr spectrum of **11** lacked, with respect to that of **2**, the signals of the carbonyl and the olefinic carbons but showed signals at δ 35.1, 27.4, and 25.3 assigned to C-4, C-9, and C-10, respectively.

The chemical and the spectral evidence presented herein, in addition to the ^{1}H-nmr experiments carried out with **2**, suggested a chroman-4-one-type structure for cavoxone.

The hrms of **2** showed a fragmentation pathway very similar to that described for the chroman-4-one nucleus (13). In fact, the most important fragment ions were observed at *m/z* 224 ($C_{10}H_8O_6$), produced from the molecular ion (*m/z* 320, $C_{17}H_{20}O_6$) by a retro Diels-Alder reaction, and at *m/z* 206 ($C_{10}H_6O_5$, base peak) and 196 ($C_9H_8O_5$) both deriving from further fragmentation of the ion at *m/z* 224 by loss of H_2O and CO, respectively. Similarly, the hrms of **11** showed a fragmentation pathway very close to that reported for the 2-alkylchromans (13). In fact, the base peak present at *m/z* 211 ($C_{10}H_{11}O_5$) was produced from the molecular ion (*m/z* 308.1633, $C_{17}H_{24}O_5$, calcd. 308.1624) by a retro Diels-Alder reaction with hydrogen transfer. Further fragments observed were those at *m/z* 210 ($C_{10}H_{10}O_5$) and 183; this latter was formed from the ion at *m/z* 211 by further loss of CO.

Cavoxin (**1**), by treatment with 6 N HCl at reflux, gave cavoxone with quantitative yield; this result allowed us to assign definitely the structure **2** to cavoxone. The structure **2** is in full agreement with the evidence provided from the gated-decoupling ^{13}C-nmr spectrum (10) obtained with natural **2**. As expected, the attach position of the substituent groups on the benzene ring, deduced from the multiplicity of the quaternary carbons and from the value of their long-range ^{13}C-^{1}H coupling constants, were coincident to those assigned in **2**.

The appearance of **2**, when the cavoxin was left for a long period in moist air or in solution, suggested that **1** spontaneously converts to **2**. That **2** is an artifact formed from **1** was confirmed by measurements of its optical activity. At 589 (Na) and 578, 546, and 436 (Hg) nm, **2** exhibited no optical rotation. Finally, when exogenous **1** was added to the culture medium, in the absence of fungus, it was transformed progressively into **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Melting points are uncorrected; optical rotations were measured on a Perkin-Elmer 141 polarimeter on $CHCl_3$-MeOH (2:1) solutions; ir spectra were recorded on a Perkin-Elmer 399 instrument for solutions in $CHCl_3$; uv spectra were measured on a Perkin-Elmer 550 S spectrophotometer 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 Ltd.-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 either on SiO_2 (Merck, Kieselgel 60, 0.063-0.2 mm) or on Sephadex LH-20 (Pharmacia 25-100 μm).

TOXIN PRODUCTION.—A single spore culture of *P. cava* Schulzer (CBS 535.66)[2] was used. The fungus was cultured in flasks containing 300 ml of a semisynthetic liquid medium (14) incubated at 25° and 200 rpm for 5 days. The cultures were filtered, and the filtrate was lyophilized.

PHYTOTOXIC TEST.—The phytotoxicity of the culture filtrates and of the extracts purified was assayed on tomato cuttings by the usual technique (4,5).

TOXIN PURIFICATION.—Lyophilized solid residue corresponding to 9 liters of culture filtrate was dissolved in distilled H_2O (1 liter) and extracted with $CHCl_3$ (4×500 ml). After the extraction, the aqueous phase had no phytotoxic activity. The organic extracts were combined, dried (Na_2SO_4), filtered, and then evaporated under reduced pressure. The residue (1.762 g), which had a good phytotoxic activity, was chromatographed on Sephadex LH-20 column. The former compound eluted with $CHCl_3$-*i*PrOH (9:1) was cavoxone (**2**), while the successive toxic eluate contained cavoxin (**1**). After removal of the solvent under reduced pressure, both compounds **1** and **2** were obtained as an homogeneous oil. Cavoxin (**1**) crystallized as pale yellow needles (979 mg, 108 mg/liter) from EtOAc-petroleum ether (40-70°); cavoxone was obtained as white needles (101.9 mg, 12.1 mg/liter) by crystallization from EtOAc.

Cavoxin (**1**).—Pale yellow needles had mp 125-128° (from EtOAc-petroleum ether, bp 40-70°); ir ν max 3540, 3000, 1745, 1640, 1620, 1590 cm^{-1}; uv λ max nm (log ε) 325 (4.19), 286 (4.38); ^1H- and ^{13}C-nmr spectra are reported in Tables 1 and 2, respectively; ms *m/z* (rel. int.) 320 (M^+) (23), 302 (4), 278 (9), 277 (87), 224 (21), 206 (100), 150 (23), 121 (11).

Cavoxone (**2**).—White needles had mp 210-213° (from EtOAc); ir ν max 3540, 2950, 1750, 1710, 1675, 1610 cm^{-1}; uv λ max nm (log ε) 288 (3.87), 242 (3.95); ^1H and ^{13}C nmr are reported in Tables 1 and 2, respectively; ms *m/z* (rel. int.) 320 (M^+) (31), 278 (22), 277 (85), 224 (28), 206 (100), 196 (20), 150 (31), 121 (27).

Hydrogenation of cavoxin.—Cavoxin (30 mg) in THF (6 ml) was hydrogenated overnight with 5% Pt on charcoal at room temperature and atmospheric pressure, under stirring. The reaction was stopped by filtration, the clear solution was evaporated under reduced pressure, and the residue (28 mg) was chromatographed on preparative tlc ($CHCl_3$ *i*PrOH, 9:1). The compound **3**, obtained as a crude oil, crystallized from Et_2O-petroleum ether (40-70°) (19 mg, 63%): mp 105-106°; ir ν max 3515, 2900, 1700, 1610 cm^{-1}; uv λ max nm (log ε) 319 (2.86), 271 (3.73); ^1H nmr ($CDCl_3$) δ 6.35 (s, H-5), 3.83 (s, 3H, OMe), 3.59 (s, 2H, H-17), 2.58 (t, J=7.3 Hz, 2H, H-8), 1.26 (br s, 12H, six CH_2), 0.87 (t, J=7.0 Hz, 3H, Me-15). ^{13}C nmr ($CDCl_3$) δ 175.6 (C-7), 146.1 (C-4), 143.9 (C-2), 132.7 (C-3), 124.6 (C-6), 122.5 (C-1), 105.7 (C-5), 56.2 (OMe), 38.7 (C-17), 32.3 (C-13), 30.3, 30.2, 29.9, 29.7 (four CH_2), 26.7 (C-8), 23.0 (C-14), 14.2 (C-15); ms *m/z* (rel. int.) 310 (M^+) (45), 292 (0.4), 266 (1.1), 251 (2), 211 (100), 183 (18).

Methyl ester of cavoxin (**4**).—To a solution of **1** (34 mg) in Et_2O (5 ml) was added, at 0°, ethereal CH_2N_2 (5 ml). The mixture was allowed to stand at room temperature for 6 h and then evaporated under a N_2 stream. Purification of the residue by preparative tlc (C_6H_6-Me_2CO, 8:2) afforded an oil (30.7 mg, 90%) which crystallized from Et_2O-petroleum ether (40-70°): mp 89-91°; ir ν max 3540, 1740, 1640, 1620, 1580 cm^{-1}; uv λ max nm (log ε) 325 (4.05), 284 (4.36); ^1H and ^{13}C nmr are reported in Tables 1 and 2, respectively; ms *m/z* (rel. int.) 334 (M^+) (25), 303 (5.5), 291 (100), 238 (19), 206 (69), 179 (25).

Compounds **5**, **6**, *and* **7**.—To a solution of **1** (55 mg) in Et_2O was added at 0°, ethereal CH_2N_2 (5 ml). The reaction was performed at room temperature with successive additions of the reagent. After 2 days, **1** was converted into the three derivatives **5**, **6**, and **7** and the reaction was stopped by evaporation under a stream of N_2. By repeated chromatography, first on SiO_2 column and then on tlc with the same eluent (C_6H_6-Me_2CO, 8:2), all the compounds, **5** (10.4 mg, 19%), **6** (14.3 mg, 26%), and **7** (6.2 mg, 11%), were obtained as pure oil. Compound **5** showed: ir ν max 3515, 1725, 1620, 1590 cm^{-1}; uv λ max nm (log ε) 283 (4.29); ms *m/z* (rel. int.) 348 (M^+) (53), 317 (13), 305 (100), 289 (73). Compound **6** had: ir ν max 3500, 1725, 1610, 1575 cm^{-1}; uv λ max nm (log ε) 286 (4.23); ms *m/z* (rel. int.) 348 (M^+) (20), 317 (6.6), 305 (100), 289 (1.4). Compound **7** exhibited: ir ν max 1720, 1620, 1585 cm^{-1}; uv λ max nm (log ε) 284 (4.26); ms *m/z* (rel. int.) 362 (M^+) (74), 331 (13), 319 (100), 303 (75). For **5**, **6**, and **7** the ^1H-nmr data ($CDCl_3$) were very close to that reported for **4**, except for the presence of the signals due to the other methoxyl groups: in **5** at δ 3.78, in **6** at δ3.90, and in **7** at δ 3.87 and 3.80. The ^{13}C-nmr spectra of **5**, **6**, and **7** were very similar to that of **4** except for the presence of the signal attributed to the other methoxyl carbons: in **5** at δ 61.7, in **6** at δ 60.9, and in **7** at δ 61.7 and 60.9.

Methyl ester of cavoxone (**8**).—Compound **8** was prepared from 60 mg of cavoxone (**2**) dissolved in MeOH (10 ml) according to the procedure used to obtain **4** from **1** but stopping the reaction after 4 h. The reaction afforded an oily residue that was purified by SiO_2 column (C_6H_6-Me_2CO, 8:2). Compound **8** crystallized from evaporation of the solvent (41 mg, 68%): mp 102-104°; ir ν max 3540, 1735, 1680, 1615

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cm^{-1}; uv λ max nm (log ε) 285 (4.18), 238 (4.28); ^1H and ^{13}C nmr are reported in Tables 1 and 2, respectively; ms *m/z* (rel. int.) 334 (M^+) (24), 303 (10), 291 (82), 238 (38), 210 (22), 206 (100).

Compound **9**.—Cavoxone (60 mg) dissolved in MeOH (10 ml) was treated with a large excess of ethereal CH_2N_2 for 8 h at room temperature. The reaction, stopped as described in the preparation of **4** from **1**, yielded a solid residue that was chromotographed on SiO_2 column (C_6H_6-Me_2CO, 8:2). The first compound to be eluted was **9**, which crystallized by evaporation of the solvent (57 mg, 95%) mp 101-103°; ir ν max 1725, 1670, 1590 cm^{-1}; uv λ max nm (log ε) 310 (3.54), 284 (4.11), 235 (4.07); ms *m/z* (rel. int.) 348 (M^+) (36), 317 (12), 305 (100), 289 (14), 252 (36), 220 (33); ^1H- and ^{13}C-nmr spectra were very similar to those of **8** except for the presence of the signals assigned to the other methoxyl group at δ 3.85 and 60.7, respectively.

15-O-acetylcavoxone (**10**).—Acetylation of **2** (60 mg) dissolved in dry pyridine (2 ml) was performed with Ac_2O (20 μl) at 0°. After 1 min, the reaction mixture was poured into ice-cold H_2O. The aqueous solution was acidified to pH 2-3 with 2N H_2SO_4 and extracted with Et_2O (3×100 ml). The combined extracts were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. Purification of the residue by preparative tlc ($CHCl_3$-*i*PrOH, 9:1) afforded an oil (25 mg, 41%) that crystallized from EtOAc-petroleum ether (40-70°): mp 139-142°; ir ν max 2920, 1750, 1710, 1670, 1595 cm^{-1}; uv λ max nm (log ε) 308 (3.63), 279 (4.17), 235 (4.17); ms *m/z* (rel. int.) 362 (M^+) (3), 345 (2), 320 (48), 277 (88), 233 (21), 224 (45), 206 (100), 196 (14); ^1H- and ^{13}C-nmr spectra were very similar to that of **2** except for the presence of the signals due to the acetyl group at δ 2.28 and at δ 20.4 and 169.5, respectively.

Hydrogenation of cavoxone.—Cavoxone (50 mg) in THF (10 ml) was hydrogenated with 5% Pd on $BaSO_4$ according to the procedure used to obtained **3** from **1**. The reaction afforded **11** as a chromatographically pure compound (48 mg, 96%). Compound **11** crystallized from EtOAc-petroleum ether (40-70°): mp 115-116°; ir υ max 3540, 2960, 1710, 1620 cm^{-1}; uv λ max nm (log ε) 272 (3.24); ^1H nmr (C_5D_5N) δ 6.91 (s, H-6), 3.94 (ddd, *J*=5.2, 4.0, 2.2 Hz, H-2), 3.82 (s, 3H, OMe), 3.00 (ddd, *J*=16.6, 10.7, 6.3 Hz, H-4A), 2.91 (ddd, *J*=16.6, 6.3, 4.0 Hz, H-4B), 1.94 (dddd, *J*=14.3, 6.3, 4.0, 2.2 Hz, H-3A), 1.69 (dddd, *J*=14.3, 10.7, 6.3, 5.2 Hz, H-3B), 1.65 (m, H-9), 1.49 (m, 2H, H-9 and H-10), 1.34 (m, H-10), 1.16 (m, 2H, H-11), 1.14 (tq, *J*=7.0, 7.0 Hz, H-12), 0.82 (t, *J*=7.0 Hz, 3H, Me-13); ^{13}C nmr ($CDCl_3$-CD_3OD, 2:1) 174.8 (C-16), 145.6 (C-5), 143.5 (C-8a), 133.7 (C-4a), 123.7 (C-8), 115.4 (C-7), 106.8 (C-6), 76.4 (C-2), 56.5 (OMe), 38.5 (C-15), 35.1 (C-4), 32.1 (C-3), 27.4 (C-9), 25.3 (C-10), 22.8 (C-11), 22.0 (C-12), 14.1 (C-13); ms *m/z* (rel. int.) 308 (M^+) (68), 237 (6), 211 (100), 210 (28), 183 (13), 165 (50).

Conversion of cavoxin **1** *to* **2**.—Cavoxin (10 mg) in EtOH (0.3 ml) was treated with 5N HCl at reflux for 4 h. After cooling, the solution was diluted with H_2O (30 ml) and extracted with $CHCl_3$ (3×25 ml). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The homogeneous product obtained showed, also by co-chromatography, the same Rf as **2** according to tlc analysis on SiO_2 ($CHCl_3$-*i*PrOH, 9:1) and on reverse phase (Stratocrom C-18, Whatman 0.2 mm, eluent CH_3CN-H_2O, 4:6) plates.

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