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THE STRUCTURE OF FUNICONE

A NEW METABOLITE FROM *PENICILLIUM FUNICULOSUM* THOM

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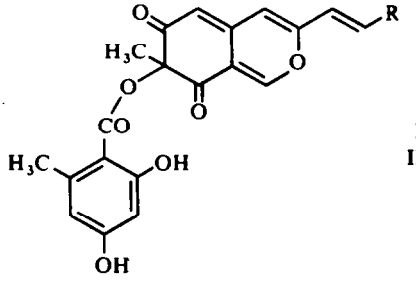
Abstract—Funicone, a new $C_{19}H_{18}O_8$ metabolite from a strain of *Penicillium funiculosum* Thom, is shown to be 2-*trans*-propenyl-3-hydroxy-5-(2′-carbomethoxy-4′,6′-dimethoxybenzoyl-)-4-pyrone.

IN A PRECEDING paper[1] we reported the isolation from a strain (IPV 2) of *Penicillium funiculosum* Thom of orsellinic acid, mitorubrin (I),[2] mitorubrinol (II),[4] mitorubrinic acid (III), and of a new metabolite $C_{19}H_{18}O_8$.



I R = CH_3
II R = CH_2OH
III R = COOH

We wish to report here the structural elucidation of this compound (IV), for which we propose the name funicone.

Elemental analysis and high-resolution mass spectrum gave the composition $C_{19}H_{18}O_8$. The UV spectrum, with maxima at 245 and 310 nm, which are shifted bathochromically in basic medium, indicated a conjugated aromatic chromophore. From the IR spectrum (KBr) the presence of OH (3·1 μ), and of CO groups at 5·83, 5·95 and 6·05 μ was inferred. The analysis of the NMR spectrum at 60 MHz ($CDCl_3$) allowed the assignment of all the H atoms of the molecule, which could be arranged as follows: a chain CH_3—CH=CH—C—, *trans*, 3 methoxy groups, one OH at 6·0 δ, 2 aromatic *meta* protons at 6·60 and 7·10 δ, and a —OCH= group, which appears as a singlet at low field (8·50 δ).

Acetylation of funicone with acetic anhydride in pyridine gave a monoacetate V, whereas treatment with CH_2N_2 in CH_2Cl_2 gave a mixture, from which a monomethylether VI could be isolated. Both these experiments confirmed the presence of an enolic OH, in agreement with the positive $FeCl_3$ test on IV itself.

Hydrogenation with $Pd/BaSO_4$ in ether afforded a dihydro derivative VII, where the unsaturated chain had been reduced to CH_3(1)—CH_2(2)—CH_2(3)—, as it appeared from the NMR spectrum. The absence of further coupling of CH_2(3) in VII and of =CH(3) in IV indicated that there are no hydrogen atoms on the carbon bearing

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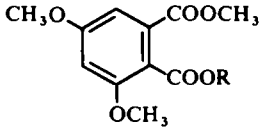
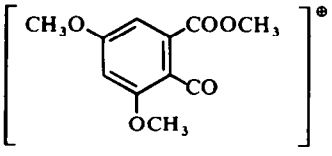
the chain. The values of the chemical shifts of the olefinic protons in IV and V rule out the conjugation with a carbonyl group, while that of —CH_2(3) in VII (2·70 δ) suggests that the chain is most probably attached to an unsaturated carbon.

The mass spectrum of funicone (IV) shows an important peak at m/e 223 (*a*, see Scheme 1) with composition $C_{11}H_{11}O_5$. The labelling of the OH group by D_2O exchange, as well as the hydrogenation of the chain double bond do not affect this fragment, that therefore cannot contain the OH or the chain groups. Accordingly, the mass spectra of the monoacetate V and of the methylether VI show the peak at m/e 223 too. Selenium dehydrogenation and ozonolysis of IV gave evidence on this C_{11} fragment. The former reaction afforded an aromatic compound, (VIII), that from all the spectral data appeared to be methyl 3,5-dimethoxybenzoate, as was confirmed by comparison with a synthetic sample. Ozonolysis gave instead a mixture, from which the acid (IX) was isolated. Its mass spectrum does not have a visible molecular ion peak, but shows significant peaks at m/e 223 (M − OH), 208 (M − CH_3OH), 164 (208 − CO_2), 134 (164 − CH_2O) and 106 (134 − CO). By treating IX with CH_2N_2, another compound (X) was obtained, whose mass spectrum shows an intense parent peak at m/e 254 and a stronger one (base peak) at m/e 223 (M − OCH_3). Thus the compound IX must have the same formula as VIII, plus a —COOH group, which is



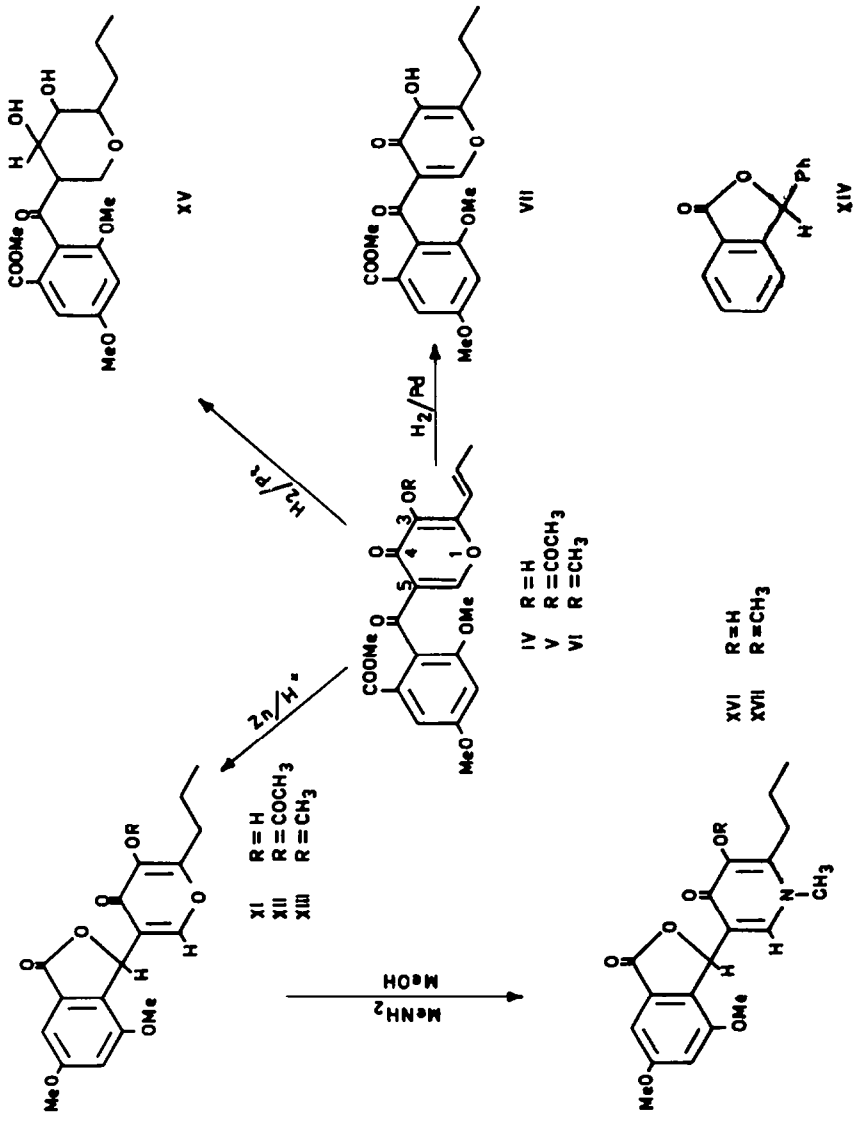
IX R = H
X R = CH_3

methylated by CH_2N_2 to give the ester X. The position of the COOH in IX is established from the NMR spectrum of funicone, where the two aromatic protons are in *meta* position. It follows that the fragment $C_{11}H_{11}O_5$ (m/e 223) in the mass spectrum of funicone and its derivatives must have the structure



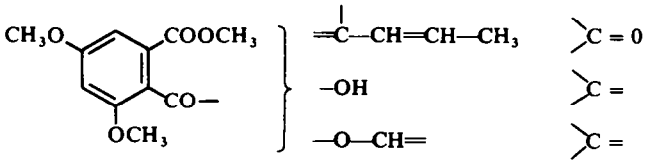
which corresponds also to a part of the structure of IV.

Another piece of evidence in favour of this partial structure came from Zn and acid reduction of IV in methanol, which gave a compound $C_{18}H_{18}O_7$ (XI). In the IR spectrum of XI a new band appears at 5·65 μ (lactone CO), while the ester band at 5·85 μ is absent. The NMR spectrum of XI shows: the saturated chain CH_3—CH_2—CH_2— as well as in VII, only two methoxy groups, the two aromatic *meta* protons, a new singlet (1H) at 6·40 δ, whereas the singlet at low field is now shifted to 7·49 δ. These results can be interpreted by reduction of the *ortho* carbonyl to a secondary alcohol, and ring closure to a phthalide lactone (XI). Under the reaction conditions the double bond of the chain was reduced. Consistent with this interpretation is the chemical shift of the corresponding proton —CHOCO— in phenylphthalide (XIV) which appears at 6·39 δ too. Similarly to funicone, XI gave the corresponding monoacetate (XII) and monomethylether (XIII). These rule out the intervention of the enolic


XV
VII
XIV
IV R=H
V R=COCH3
VI R=CH3
XI R=H
XII R=COCH3
XIII R=CH3
XVI R=H
XVII R=CH3
H2/Pd
Zn/H+
MeNH2
MeOH

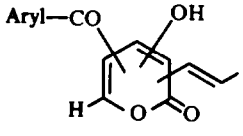
OH in the lactone formation. The mass spectra of XI, XII and XIII are consistent with the phthalide structure, since they do not show any peak at m/e 223, but a significant one at m/e 193, corresponding to fragment **p** (Scheme 2).

Thus a partial formula A can be written for funicone:

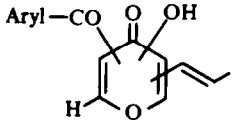


A

Consideration of the remaining carbon atoms and of the substituents clearly requires the presence of at least one ring and of four unsaturated functions (or the equivalent). In the IR spectrum of IV there is a —CO— band at 6·05 μ, which disappears by exhaustive hydrogenation with PtO_2 in MeOH. This reaction gave in very small yields an octahydro derivative $C_{19}H_{26}O_8$ (XV), the IR spectrum of which shows only the CO bands at 5·83 and 5·90. Moreover, the mass spectrum of XV has the base peak at m/e 223, thus showing that the aromatic fragment **a** (see Scheme 3) is unaltered, while the saturation of the remaining part makes the benzoylic bond cleavage easier. Assuming thus that the C_8 part contains 3 double bonds, one ring and one CO, and considering that the vinylic hydrogen at low field must undoubtedly be bonded to an oxygen atom, only the two following formulae become possible for this moiety:



B



C

The pyrone formulation is consistent with the difficulty and the numerous products of the hydrogenation, and with the reaction of XI with methylamine, which gave a compound $C_{19}H_{21}NO_6$, most probably the pyridone XVI, also easily methylated to the ether XVII. It was, however, obtained in small amount, and no additional information, besides molecular weight, could be obtained from it.

Failure of other degradation reactions, e.g. alkali cleavage, to give useful products, and lack of supply of IV compelled us to rely mostly on spectral data to derive a structure for funicone.

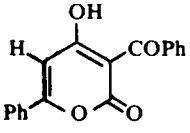
The formula B can be excluded on the basis of the following evidence:

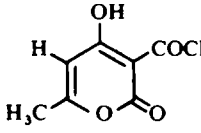
(i) the α-pyrone structure requires a lactone band in the IR at 5·75–5·85 μ,[3] whereas the band at 6·05 μ in IV seems much more consistent with the γ-pyrone structure;

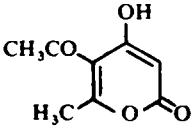
(ii) the interpretation of the UV spectrum of IV is not easy, because of the presence of the conjugated side chain and aromatic carbonyl. However, the maximum of XI, where these additional conjugations should be eliminated, appears only at 285 nm, which is lower than the usual absorption of α-pyrones (around 300 nm),[3] moreover,

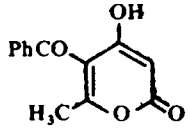
the strong bathochromic shift in alkali of the long wavelength maximum of IV and XI, and the small hypsochromic shift on acetylation of IV would indicate a 3-hydroxy-4-pyrone chromophore.

(iii) the absence of chelation between the aryl-CO and the OH groups in IV, VI and XI (OH at 6·0, 6·5 and 5·7 δ in $CDCl_3$), which excludes the formation of an H-bonded 6-membered ring, rules out any 2-hydroxy-4-pyrone or 4-hydroxy-2-pyrone formula. This is supported by comparison with the appropriate models XVIII, XIX


XVIII

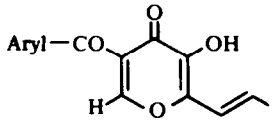

XIX

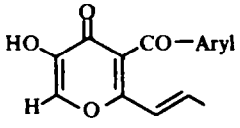

XX

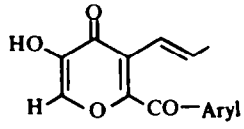

XXI

and XX,[4,5] that all show a strongly chelated OH (14·6, 15·0, 12·0 δ in $CDCl_3$). This chelation probably stabilizes the α-pyrone structure, which appears to be predominant in $CHCl_3$ solution (CO band at 5·72, 5·72, 5·75 μ in the IR spectrum). This holds for XVIII, XIX and XXI[4,6] also in the solid state (CO at 5·74, 5·81, 5·79 μ, whereas XX has probably a γ-pyrone structure (CO at 5·90[4] or 6·02[5]).

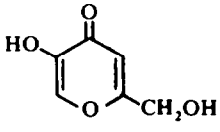
The absence of chelation also requires that in the other γ-pyrone structure C the two groups aryl-CO and OH cannot be adjacent. This, together with the discussion at point (iii) above, reduces the possible formulae to the three D, E and F. Consistent

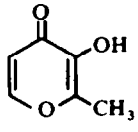

D


E


F

with these 3-hydroxy-4-pyrone formulae is again the comparison of the chemical shift of the OH (6·0 in $CDCl_3$ and 9·35 δ in DMSO for IV) with that in kojic acid


XXII


XXIII

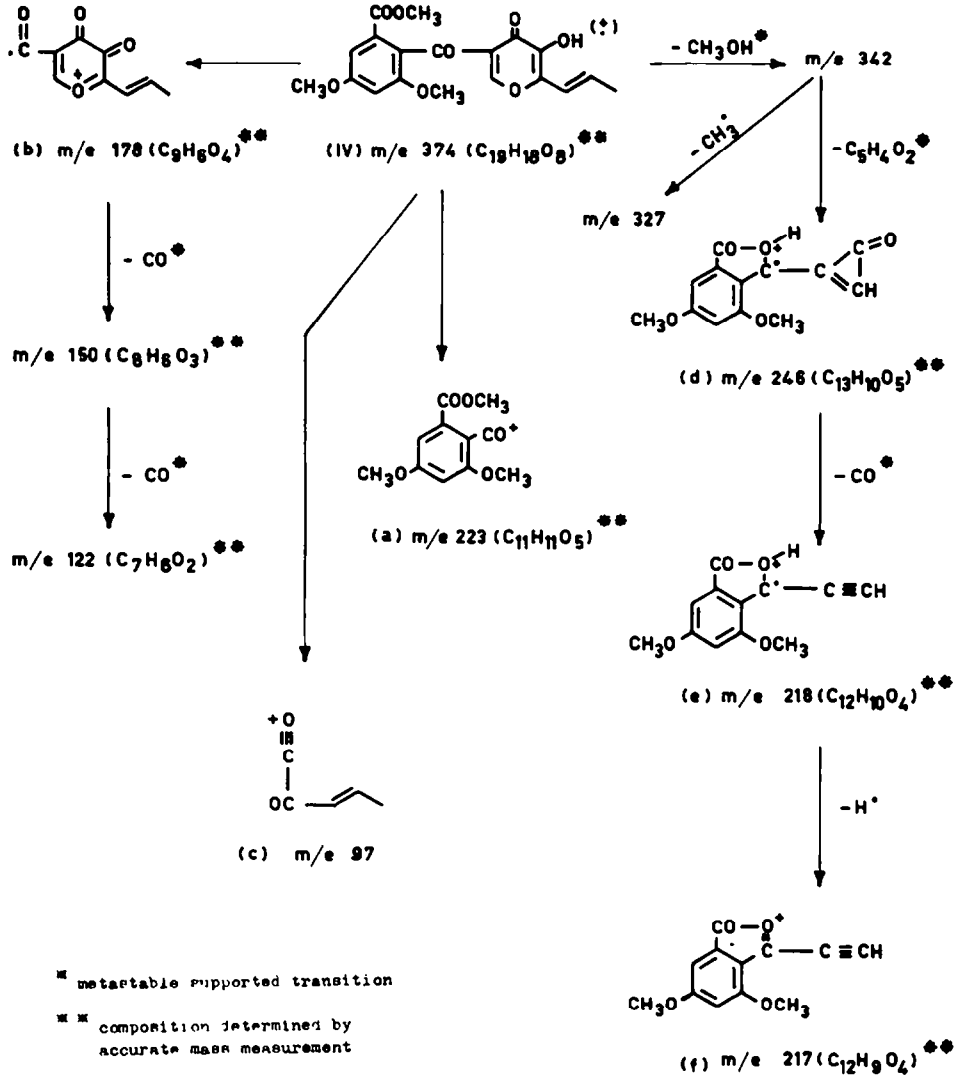
(XXII) and maltol (XXIII). The OH signals appear at 5·6 and 9·0 δ (XXII) and 8·85 δ (XXIII) in DMSO, and at 7·0 (XXIII) in $CDCl_3$.

The study of the mass spectra of funicone itself and of its derivatives gives strong evidence in favour of a structure with the C_3 chain in position 2, and particularly of D. The following considerations support the choice of a structure with the chain in position 2:

(i) the fragmentation of the octahydro derivative XV (Scheme 3), with the formation of the ion **h**;

(ii) the spectrum of the phthalide derivative XIII shows very intense peaks at m/e 290 and 275, due respectively to the ions **n** and **o**, the origin of which is well established

SCHEME 1



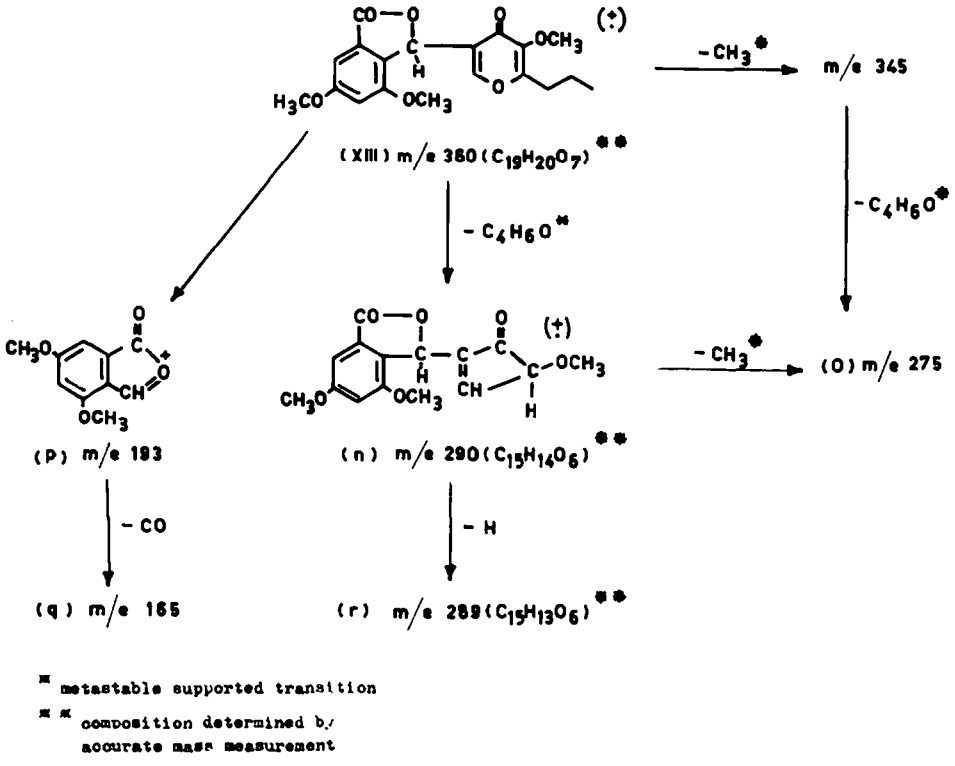
* metastable supported transition

** composition determined by accurate mass measurement

by the corresponding metastables (see Scheme 2). This fragmentation, involving the loss of a C_4H_6O neutral fragment, is possible only if the chain is in position 2.

The funicone molecular ion, after loss of CH_3OH, involving the H atom of the OH group, as proved by D_2O exchange, loses a $C_5H_4O_2$ neutral fragment, through a metastable supported transition, giving the fragment **d**, from which **e** and **f** are derived (see Scheme 1). The **c** ion moreover, very likely corresponds to the protonated $C_5H_4O_2$ neutral fragment. The same fragmentation is observed for the acetate V. The proof that the C_3-chain is lost together with two carbon and two oxygen atoms

SCHEME 2



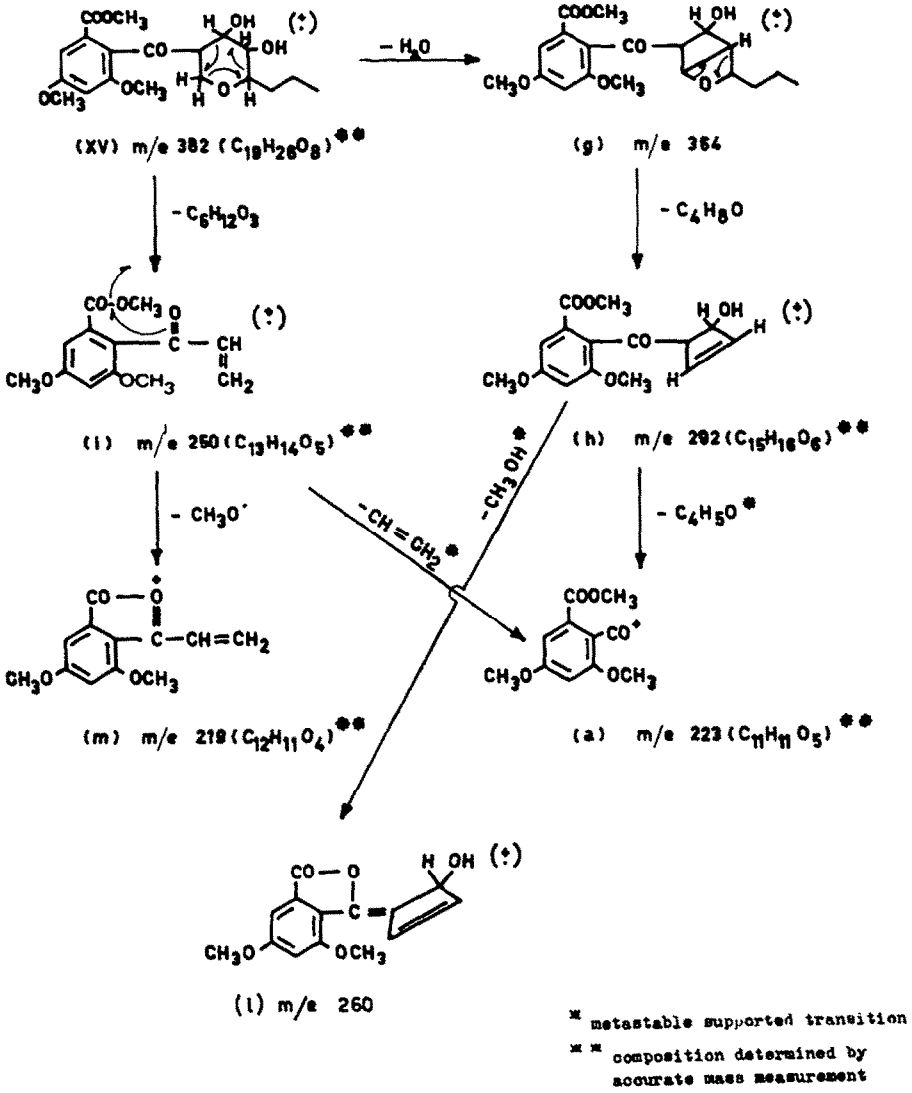
* metastable supported transition

** composition determined by accurate mass measurement

comes from the spectrum of dihydrofunicone VII, which shows the same ions **d**, **e** and **f**. This behaviour is consistent with the suggested structure D and cannot be explained on the basis of E or F.

Another argument in favour of the structure D is the observation that the reduction of the bridge CO group in the formation of XI shifts the —OCH= proton 1 ppm upfield in the NMR spectrum, probably as a consequence of the elimination of the

SCHEME 3

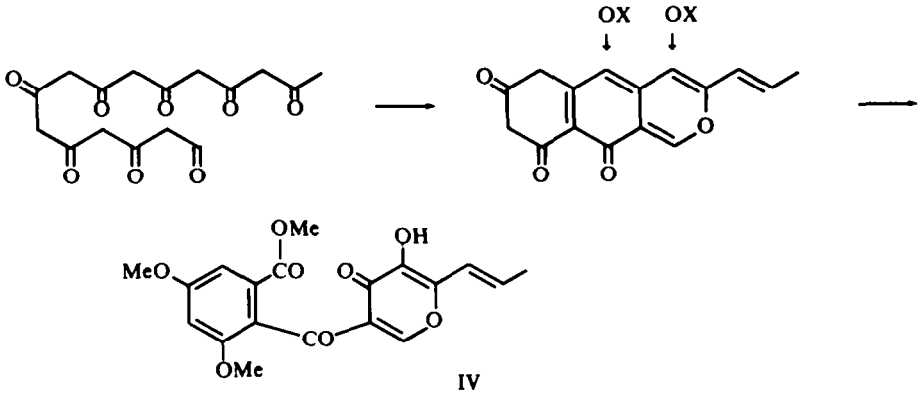


* metastable supported transition

** composition determined by accurate mass measurement

deshielding effect of the electron-withdrawing carbonyl group. There is no reason for such an effect in the corresponding structures E and F.

Formula IV is thus assigned to funicone. Its structure most probably represents a new example of modification of a polyketide chain skeleton. An attractive, even if rough and tentative, scheme of biosynthesis could be the following:



IV

EXPERIMENTAL

UV spectra (λ_{max} in nm) were measured in 95% EtOH soln., with a Beckman DK-2, NMR spectra (values in δ, J in Hz) with a Varian A-60 (TMS as internal standard), and mass spectra with a Hitcahi RMU6D instrument at 70 eV (80 μA), samples being directly introduced in the ion source at 250°. Accurate mass measurements were carried out with an AEI MS9 instrument and with an Hitachi RMU6D double focusing instrument, using the peak matching technique. M.ps are uncorrected. Column chromatography was performed with Merck 0·05–0·20 mm silica gel, and TLC with Merck HF_{254}.

The isolation, characteristics and cultivation of the IPV 2 strain of *Penicillium funiculosum* Thom were described in the preceding paper.[1] The crude extract from AcOEt extn. of 100 Roux flasks was adsorbed on silica gel and chromatographed through the same silica gel with ether/AcOEt mixtures. The four metabolites already described[1] were eluted. Subsequent elution with AcOEt/MeOH 95/5 gave crude funicone, which was purified by rechromatography through HCl-washed Bender & Hobein silica gel with hexane/$CHCl_3$ 3/1 as eluent, to give 250 mg of *funicone* (IV), white crysts., m.p. 176–178° (ether) (Found: C, 60·33; H, 4·79. $C_{19}H_{18}O_8$ requires: C, 60·96; H, 4·85%), UV: 245, 310 (ε21000, 18000) 245sh, 310sh, 368 (ε 16400, 8200, 12800) in alk. EtOH; IR (KBr): 3·1 (OH), 5·83 (—COOR), 5·95 (Aryl-CO—), 6·05 μ (γ-pyrone CO); IR ($CHCl_3$): 5·82, 5·95, 6·02 μ. NMR ($CDCl_3$): $C\underline{H}_3$—CH=CH— (1·95, d with further small coupling. J = 6). 3 OCH_3 (3·72; 3·76; 3·86), CH_3—C<u>H</u>=C<u>H</u>— (m, 6·2–7·2), 2 *meta* arom H (6·60, 7·10; J = 2), =CHO— (s, 8·50), 1 OH broad at approx. 6·0. Mass: m/e (% I): 375(12), 374(56), 359(10), 344(6), 343(13), 342(23), 328(8), 327(41), 315(6), 299(20), 247(5), 246(14), 223(31), 219(5), 218(22), 217(14), 180(6), 179(9), 178(72), 177(6), 165(16), 154(11), 163(41), 152(10), 151(11), <u>150</u>(100), 149(9), 137(8), 135(5), 122(20), 97(14), 69(63), 57(11), 53(16), 43(13), 41(21). Accurate mass measurements:

Found	Calc	Composition
374·1015 ± 0·0019	374·1004	$C_{19}H_{18}O_8$
246·0521 ± 0·0012	246·0528	$C_{13}H_{10}O_5$
223·0609 ± 0·0011	223·0606	$C_{11}H_{11}O_5$
218·0578 ± 0·0011	218·0579	$C_{12}H_{10}O_4$
217·0495 ± 0·0011	217·0500	$C_{12}H_9O_4$
178·0262 ± 0·0009	178·0266	$C_9H_6O_4$
163·0027 ± 0·0016	163·0031	$C_8H_3O_4$
150·0324 ± 0·0007	150·0317	$C_8H_6O_3$
122·0364 ± 0·0006	122·0368	$C_7H_6O_2$

Funicone acetate (V): 50 mg of IV were left for 48 h in 2 ml Ac_2O and 0·5 ml pyridine. Pouring in ice, washing the ppt with water, and crystallizing from ether/hexane gave 30 mg V, m.p. 187–190°, negative $FeCl_3$ test, UV: 250, 280sh, 290sh, 302sh (ε 25700, 18600, 15700, 10600), IR (KBr): 5·60, 5·75, 6·0–6·05; NMR ($CDCl_3$): $C\underline{H}_3$—CH_A=CH_B— (1·99, dd; J = 6 and 1), CH_3COO— (2·28), 3 OCH_3 (3·70, 3·76, 3·86), H_B (6·25; J = 16 and 1), H_A (6·73; J = 16 and 6), 2 *meta* arom H (6·68, 7·05; J = 2·2), =CHO— (s, 8·50). Mass: m/e (% I): 416(18), 385(5), 375(21), 374(95), 359(17), 343(17), 342(36), 328(10), 327(51), 315(8), 299(10), 246(17), 224(7), 223(52), 218(20), 217(15), 180(8), 179(13), 178(98), 177(9), 165(19), 164(8), 163(42), 152(7),

151(12), 150(100), 149(18), 137(9), 135(5), 121(20), 97(14), 69(81), 60(13), 53(21), 45(17), 43(74), 42(18), 41(30), 39(12).

Funicone methylether (VI): 10 mg IV in MeOH were treated with excess CH_2N_2 in CH_2Cl_2. By prep TLC with hexane/AcOEt 1/1 as eluent, a small amount of VI was isolated, mass: m/e (% I): 388(16), 358(5), 357(14), 356(9), 341(8), 329(17), 313(5), 299(6), 233(7), 224(7), 223(56), 218(5), 217(10), 195(5), 193(17), 192(95), 191(8), 180(8), 178(6), 177(30), 165(20), 164(22), 163(9), 152(8), 151(6), 150(18), 149(18), 138(6), 137(15), 136(20), 135(9), 134(6), 123(6), 122(10), 121(9), 109(11), 107(10), 106(11), 97(12), 95(23), 93(16), 91(10), 85(11), 83(22), 82(10), 81(17), 79(12), 77(17), 71(21), 70(11), 69(100), 67(22), 66(10), 65(12), 59(16), 57(36), 56(10), 55(38), 53(40), 44(11), 43(54), 41(61), 39(21).

Dihydrofunicone (VII): 50 mg IV in 100 ml ether were hydrogenated with 50 mg 10% $Pd/BaSO_4$ as a catalyst. Filtn. and evapn. gave VII, UV: 272, 312 (ε 8850, 6550); 316 (ε 10600) in alk EtOH; IR (KBr): 5·81, 5·95, 6·10 μ; NMR ($CDCl_3$): $C\underline{H}$—CH_2— (t, 1·00; J = 7), CH_3—$C\underline{H_2}$—CH_2— (m around 1·7), CH_3—CH_2—$C\underline{H_2}$— (t, 2·70; J = 7), 3 OCH_3 (3·72, 3·78, 3·86), 2 arom *meta* H (6·60, 7·10; J = 2·5), =CHO (8·55). Mass: m/e (%I): 377(10), 376(52), 349(5), 348(37), 346(6), 345(30), 344(9), 329(11), 318(8), 317(46), 316(48), 315(13), 301(8), 298(7), 288(7), 287(20), 276(13), 275(5), 248(10), 247(12), 246(12), 232(6), 224(6), 223(68), 219(7), 218(22), 217(27), 209(5), 189(5), 181(8), 180(47), 179(5), 165(31), 164(5), 153(9), 152(100), 151(28), 150(19), 149(6), 137(15), 135(7), 124(53), 123(28), 122(11), 106(10), 83(11), 77(10), 71(18), 69(14), 55(12), 53(22), 43(29), 41(10), 39(10).

Octahydrofunicone (XV): 40 mg IV in 8 ml MeOH were hydrogenated for 48 h with 20 mg PtO_2 as a catalyst Filtn., evapn, and chromatography through silica gel with hexane/ether 1/3 gave first a mixture of tetrahydro- and hexahydrofunicone (from mass spectral data), then a product, from which, by crystallization from hexane/ether, a few mg of IX were obtained, m.p. 122–123°, UV: 240, 265sh, 314 (ε 8400, 5000, 5970); 304 (ε 4000) in alk EtOH; IR ($CHCl_3$): 5·80, 5·90, 6·20 μ. Mass (m/e (% I)): 382 (0·2),364(0·2), 350(0·5), 346(0·5), 323(1), 305(1), 292(2), 279(1), 261(1), 260(4), 250(3), 249(8), 233(2), 224(12), 223(100), 221(2), 220(2), 219(13), 209(6), 196(6), 165(4).

Accurate mass measurements:

Found	Calc	Composition
292·0933 ± 0·0015	292·0947	$C_{15}H_{16}O_6$
250·0836 ± 0·0013	250·0841	$C_{13}H_{14}O_5$
223·0616 ± 0·0011	223·0606	$C_{11}H_{11}O_5$
219·0666 ± 0·0011	219·0657	$C_{12}H_{11}O_4$

Dehydrogenation of IV. A mixture of 30 mg IV and 20 mg Se were heated for 10 min at 300°. Extn. with ether, and prep TLC with hexane/AcOEt 4/1 gave methyl 3,5-dimethoxybenzoate, identified by comparison of spectra and TLC behaviour with an authentic sample.

Ozonolysis of VII. Ozonized O_2 was bubbled for 10 min into a soln of 20 mg VII in 10 ml AcOEt at 0°. Diln. with water and AcOH, evapn. of the org layer and TLC gave a few mg of IX, mass: m/e (% I): 223(37), 208(59), 164(74); 134(36), 106(100). Methylation with CH_2N_2 in ether and TLC gave X, mass: m/e (% I): 254(29), 223(100).

Reaction of IV *with Zn* and acid. To 100 mg IV in 13·5 ml MeOH and 1·5 ml H_2SO_4 excess of powdered Zn was added, and the mixture was refluxed for 2 h Evapn., taking up with water and ether extn. yielded XI, m.p. 99–102° (Found: C, 61·64; H, 5·34. $C_{18}H_{18}O_7$ requires: C, 62·42; H, 5·24%), UV: 254, 285 (ε 5150, 6300); 318 (ε 7100) in alk EtOH; IR ($CHCl_3$): 5·65 (lactone CO), 6·0 (γ-pyrone), 6·12 μ; NMR ($CDCl_3$): $C\underline{H_3}$—CH_2—CH_2— (t, 0·96; J = 6·5), $CH_3C\underline{H_2}CH_2$— (m centered at 1·67), $CH_3CH_2C\underline{H_2}$— (t, 2·70; J = 7), 2 OCH_3 (3·78, 3·88), aryl-CH(O)—CO (s, 6·40), 2 *meta* arom H (6·70, 6·97), =CHO— (s, 7·49), 1 OH (5·7, broad). The same product was obtained by treatment of dihydrofunicone (VII) in the same conditions.

Acetylation with Ac_2O in pyridine of 20 mg XI gave XII, NMR ($CDCl_3$): $\underline{CH_3}CH_2CH_2$— (t, 0·96; J = 6·5), $CH_3\underline{CH_2}CH_2$— (m centered at 1·67), $CH_3CH_2\underline{CH_2}$— (t, 2·53; J = 7), CH_3COO— (2·30), 2 OCH_3 (3·78, 3·88), aryl-CH(O)—CO— (s, 6·44), 2 *meta* arom H (6·70, 6·96), =CHO— (s, 7·48). Mass: 388(36), 276(100), 275(20), 193(25).

Treatment of XI with CH_2N_2 in ether gave the ether XIII, IR ($CHCl_3$): 5·65, 6·06 μ; NMR ($CDCl_3$): $CH_3CH_2CH_2$— (t, 0·96; J = 6·5; m centered at 1·63 and t, 2·64; J = 7), 3 OCH_3 (3·78, 3·88, 3·88), aryl-CH(O)—CO— (s, 6·45), 2 *meta* arom H (6·69, 6·97), =CHO— (s, 7·43). Mass (m/e; % I): 361(8), 360(35), 345(17), 301(5), 290(19), 289(11), 276(16), 275(100), 274(12), 247(9), 219(5), 218(7), 275(5), 193(5), 165(9).

Accurate mass measurements:

Found	Calc	Composition
289·0727 ± 0·004	289·0712	$C_{15}H_{13}O_6$
290·0790 ± 0·004	290·0809	$C_{15}H_{14}O_6$

Treatment of 50 mg XI with MeOH and 35% CH_3NH_2 (1/1) for 30 min on a steam bath, evapn., taking up with $CHCl_3$, and pptn with hexane gave XVI, m.p. 147–150°, UV: 254sh, 290 (ε 4970, 7200); UV: 315 (ε 7300) in alk EtOH; IR($CHCl_3$): 5·70, 6·0 μ. NMR (CF_3COOH): $CH_3CH_2CH_2$— (t, 1·17', J = 6·5; m around 1·8, and t around 3·0), 2 OCH_3 plus 1 NCH_3 (3·94, 4·03, 4·19), aryl-CH(O)—CO (s, 6·86), 2 *meta* arom H (7·00, 7·21), =CHN (s, 8·03). Mass: 359. Methylation with CH_2N_2 of XVI gave a monomethylether, m.p. 140–142°, mass = 373.

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