THE JOURNAL OF Organic Chemistry

VOLUME 49, NUMBER 26 DECEMBER 28, 1984

Demonstration of an Alternative Chain Folding in the Pyranonaphthalene Fonsecin. Use of Long-Range Heteronuclear Couplings in the Naphthalene Ring for Structural Assignment[1,2]

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Received May 1, 1984

A complete assignment of resonances in the ^{13}C magnetic resonance spectrum, based on empirical δ_c calculations, off-resonance decoupling, and extensive analysis of ^{13}C–^{1}H heteronuclear long-range couplings, has been carried out for fonsecin **3**. Optimization studies for growth, precursor uptake, metabolite production, and percentage incorporation of acetate were performed on *Aspergillus carbonarius* NRRL 8740, strain 0-16-1.[3,4] The ^{13}C NMR spectra of samples of fonsecin **3** derived from sodium [1-^{13}C]acetate and sodium [1,2-^{13}C]acetate provide the first experimental evidence for the class of polyketide depicted in **8**. Optimization of acetate incorporation was achieved by adjusting the concentration of mixtures of acetate and malonic acid in replacement media.

Introduction

Nuclear magnetic resonance, as a tool for biosynthetic study, has usurped the position formerly held by radiochemical degradative methods.[5-7] The utility of carbon-13 magnetic resonance (^{13}C NMR) in biosynthetic studies has been particularly striking when applied to polyketides, where good incorporations of simple precursors such as acetate are generally obtainable. Since, with few exceptions, the sequence of transformations of the polyketide chain leading to the metabolite actually isolated is obscure, ^{13}C NMR techniques are especially attractive in that they allow a rapid determination of the chain folding mode by the use of ^{13}C NMR double-label techniques (^{13}C NMRDL). The usual precursor for ^{13}C NMRDL studies is sodium [1,2-^{13}C]acetate. The ^{13}C NMRDL studies are not without their own peculiar difficulties, however, since a rigorous and unambiguous analysis of all carbon resonances is a necessary prerequisite for such studies. On occasion, interchange of one or two pairs of resonances may lead to completely different interpretation of a chain folding.[8-14] This situation is not, however, as intellectually hazardous as it first appears, owing to the wealth of information obtainable from the ^{13}C NMR spectrum—especially of simple aromatic systems.

The ^{13}C NMR spectra of aromatic systems presents unusual opportunities for rigorous structural assignment owing to several factors which affect both the peak position and the heteronuclear ^{13}C–^{1}H coupling. In simple benzene derivatives, especially, the δ_C may be calculated for each resonance with some confidence by the use of empirical constants—making the assumption that the effects of substituents are additive.[15] An impressive tabulation of

(1) This paper should be considered as Part 13 of our series on microbial metabolites. For Part 12 see ref 2, which is a preliminary communication of the chain folding results.

(2) Bloomer, J. L.; Caggiano, T. J.; Smith, C. A. *Tetrahedron Lett.* **1982**, *23*, 5103–5106. See note 36b below.

(3) Obtained from the Northern Regional Research Laboratories of the U. S. Department of Agriculture. Peoria, IL. The organism was previously named *Aspergillus fonsaeceus*, hence the name fonsecin. For the original isolation and structural studies, see ref 4.

(4) Galmarini, O. L.; Stodola, F. H., *J. Org. Chem.* **1965**, *30*, 112–115. The stereochemistry of fonsecin was not specified.

(5) For a review of the literature to 1978, see the following reference. Another recent short review of interest is ref 7.

(6) Simpson, T. J. *Spec. Period. Rep.: Biosynthesis* **1980**, *6*, 1–39.

(7) Torssell, K. B. G. "Natural Products Chemistry"; Wiley: New York, 1983; pp 123–132.

(8) An example where the reassignment of but two resonances led to a completely different biosynthetic conclusion is sterigmatocystin, as described in the following reference.

(9) Pachler, K. G. R.; Steyn, P. S.; Vleggaar, R.; Wessels, P. L.; Scott, DeBuys, *J. Chem. Soc. Perkin Trans. 1* **1976**, 1182–1189.

(10) Some of the few examples of extensive long-range coupling analysis are the preceding reference, which deals with aflatoxin B, as well as sterigmatocystin, and the following reference, which deals with averufin.

(11) Gorst-Allman, C. P.; Pachler, K. G. R.; Steyn, P. S.; Wessels, P. L.; Scott, D. *J. Chem. Soc. Perkin Trans. 1* **1977**, 2181–2188.

(12) The following reference describes how interpretation of a certain long-range coupling in xanthomegnin led to a significant structural revision, necessitating a complete reinterpretation of the previous chain folding studies. Reference 14.

(13) Hofle, G.; Roser, K. *J. Chem. Soc., Chem. Commun.* **1978**, 611–612.

(14) Simpson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1977**, 592–595.

(15) Levy, G. C.; Lichter, R. L.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", 2nd ed.; Wiley: New York, 1980; pp 102–135.

0022-3263/84/1949-5027$01.50/0

Table I. Initial Peak Assignments for Fonsecin 3 Based on δ Values and Multiplicities in Off-Resonance Decoupled Spectra

peak	δ_C	ORD[a]	assign[b]
A	195.6	S	4
M	53.5	Q	15
N	45.7	T[c]	3
O	25.5	Q	1
K	98.1	S	2

[a] ORD = off-resonance decoupled spectrum. Multiplicites are given as follows: S = singlet, D = doublet, T = triplet, Q = quartet, M = multiplet. [b] Carbon number in formula 3. [c] Actually D × D (see Table VIII below).

these constants is given in the standard reference work on ^{13}C NMR by Levy and Nelson.[15] In addition to the possibility of calculation of δ_C, simple benzene and naphthalene systems exhibit heteronuclear ^{13}C–^{1}H couplings across as many as four bonds.[16] The magnitude of the coupling constants is affected not only by the spatial relationship of the coupled nuclei but also the electronic effects of the substituent groups. For simple benzene derivatives, these substituent effects have been shown to be additive, and a set of empirical constants may be used to calculate the magnitude of the heteronuclear couplings for polysubstituted benzene derivatives.[17]

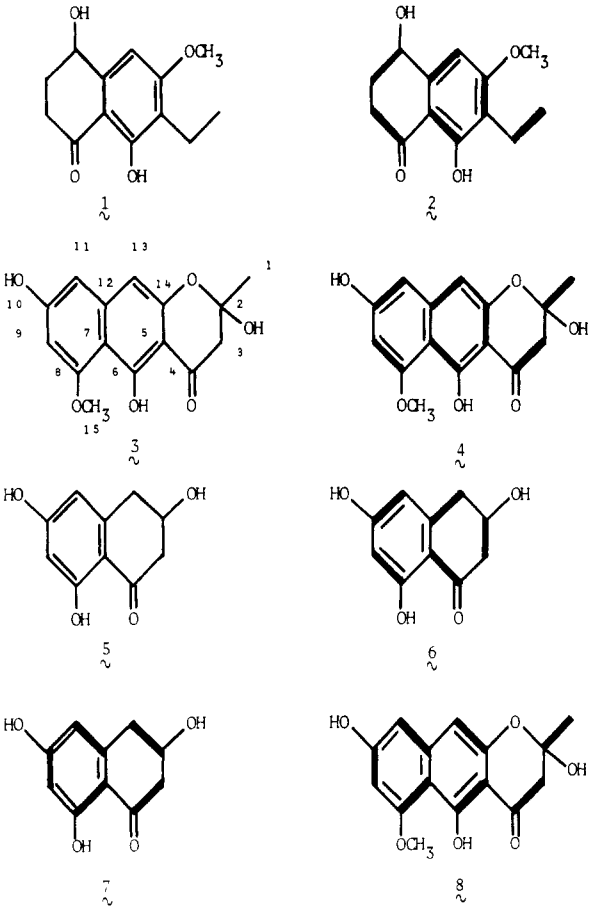
As noted above, one of the few accessible biosynthetic parameters for polyketides is their manner of chain folding, and so identification of hitherto undemonstrated chain folding modes is of some considerable interest for this class of compound. A survey of possible chain foldings vs. those rigorously demonstrated revealed a number of significant gaps.[18-21] In particular, we noted that in the few instances in which naphthalene-based polyketides had been subjected to ^{13}C NMRDL experiments, the chain foldings indicated were of unusual type.[22-25] We therefore targeted the simple pyranonaphthalene fonsecin **3** for ^{13}C NMRDL study in the hopes of identifying its chain folding pattern, and this paper deals with the results of those studies and in particular with the assignment techniques and microbiological aspects of the work.[26]

Table II. Comparison of Experimentally Obtained δ Values for Fonsecin 3 with Values Calculated Empirically

peak	δ_C obsd	δ_c calcd (C)[a]	obsd − calcd
A	195.6		
B	162.9	157.4 (10)	+5.5
C	160.0	157.2 (14)	+2.8
D	159.1	152.7 (8)	+6.4
E	151.8	149.5 (6)[b]	+2.3
F	141.7	134.4 (12)[b]	+7.3
G	104.1	107.7 (5)	−3.6
H	100.9	106.4 (7)	−5.5
I	100.0	100.8 (11)	−0.8
J	99.6	97.4 (13)	+2.2
K	98.1		
L	94.6	93.9 (9)[b]	+0.7
M	53.5		
N	45.7		
O	25.5		

[a] Note that the numbers in parentheses do not represent peak assignments, except where specifically noted. Empirical constants used were obtained from references. [b] Tentative assignments. These were confirmed in subsequent long-range coupling studies below.

In the previous literature, two other naphthalenic systems could be related to fonsecin **3**. These are *O*-methylasparvenone **1**[27] and scytalone **5**.[28]



The metabolite *O*-methylasparvenone **1**, in ^{13}C NMRDL studies,[29] gave a ^{13}C NMR spectrum in which the homo-

(16) The topic of carbon–proton couplings is the subject of a recent monograph. See the following reference. Marshall, J. L. "Carbon–Carbon and Carbon–Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis."; Verlag Chemie International: Deerfield Beach, FA, 1983; pp 11–64.

(17) See pp 42–51 of the preceding reference for the empirical constants. The magnitude and sign of the coupling constants vary considerably. A useful general rule is that the absolute magnitude of the three-bond coupling is significantly greater than either the two-bond or the four-bond coupling, which are similar. The sign of the four-bond coupling is usually negative.

(18) The following reference is a good source for surveying the different chain folding types. For experimentally verified chain foldings, see note 5, ref 6, and earlier review articles cited therein.

(19) Devon, T. K.; Scott, A. I. "Handbook of Naturally Occurring Compounds. Volume I. Acetogenins, Shikimates, and Carbohydrates."; Academic: New York, 1975; pp 309–332.

(20) It is noteworthy that most naphthalene-based polyketides are isolated as the naphthoquinones. The most useful reference source for this class of compound is pp 198–366 of the following reference. Related dimeric species based on naphthalenes are covered in pp 576–533.

(21) Thomson, R. H. "Naturally Occurring Quinones", 2nd ed.; Academic: New York, 1971.

(22) The naphthalenic systems which have been subjected to ^{13}C NMRDL study are *O*-methylasparvenone, ref 23, scytalone, ref 24, xanthomegnin and viomellein, ref 14, and mollisin, ref 25.

(23) Simpson, T. J.; Stenzel, D. J. *J. Chem. Soc., Chem. Commun.* **1981**, 239–240.

(24) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T.; Yamasaki, K. *Tetrahedron Lett.* **1977**, 483–486. The same result was reported in the accompanying communication: Seto, H.; Yonehara, H. *Tetrahedron Lett.*, **1977**, 487–488.

(25) Casey, M. L.; Paulick, R. C.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 2636–2640.

(26) See ref 4 for the original isolation and ref 2 for a preliminary communication of our chain folding results.

(27) Simpson, T. J.; Stenzel, D. J. *J. Chem. Soc., Chem. Commun.* **1981**, 239–240.

(28) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T.; Yamasaki, K. *Tetrahedron Lett.* **1977**, 483–486. The same result was reported in the accompanying communication: Seto, H.; Yonehara, H. *Tetrahedron Lett.* **1977**, 487–488.

nuclear couplings due to the incorporation of intact acetate units from sodium [1,2-^{13}C]acetate could be represented as **2** in which the intact units are indicated by the heavy lines in accordance with current practice. If fonsecin **3** were to arise biosynthetically from acetate in a like manner, i.e., with a similar folding pattern for the polyketide chain, then we would expect to observe a homonuclear coupling pattern for fonsecin as in **4** for the ^{13}C NMRDL experiment.

The molecule scytalone **5**, on the other hand, in the ^{13}C NMRDL experiment exhibited two discrete sets of homonuclear couplings in its ^{13}C NMR when biosynthesized from sodium [1,2-^{13}C]acetate.[30] This result is summarized in formulas **6** and **7**. The result suggests the intermediacy of a symmetrical metabolite en route to scytalone, which could give rise to the "scrambling" effect on the labels. Although we did not consider it likely a priori, it was conceivable that such a preformed naphthalenic system could interact biosynthetically with a linear four-carbon unit to form the fonsecin carbon skeleton. This would then be detectable in fonsecin by a set of couplings similar to that observed for scytalone **5** if the ^{13}C NMRDL experiment were carried out, i.e., one set of couplings as in **4** and one set as in **8**.

Finally, we noted that a chain folding for fonsecin which corresponded to **8** alone had not been observed in the naphthalenic series.

Peak Assignments in the Carbon Magnetic Resonance Spectrum of Fonsecin. As a prerequisite to our studies, an unambiguous assignment of the carbon resonances needed to be made. For ease of reference, we assigned a letter arbitrarily to each of the carbon magnetic resonance peaks, starting with the peak farthest downfield, (δ_C 195.6), called A, and proceeding upfield until the highest field peak in the spectrum, called O (δ_C 25.5). The δ_C and letter designations are summarized partly in Table I and completely in Table II. (See paragraph at the end of paper about supplementary material.)

Several peak assignments were obvious from an inspection of the multiplicities in the off-resonance decoupled spectrum (ORD) due to heteronuclear ^{13}C–^{1}H couplings. These were analyzed in conjunction with the δ_C values expected. We observed that no other peaks were close to the carbonyl singlet at δ 195.6. The aliphatic methyl quartet at δ 25.5 and the methoxyl quartet at δ 53.5 were also easily detected. The methylene triplet at δ 45.7 was unique. The peak at δ 98.1, while in the same region as the aromatic doublets from carbons 9, 11, and 13,[31] was easily assigned based on its lack of splitting in the ORD. Unfortunately, none of these carbons were involved in the pivotal ring positions necessary for chain folding determination. The results of the initial assignments are listed in Table I.

For analysis, the resonances could be grouped together as follows, based on the ORD results: peak A, carbonyl singlet; B–E, phenolic and anisolic singlets; F–H, nonphenolic singlets; I–L, aromatic doublet region containing aromatic doublets I, J, and L, as well as singlet K, carbon 2 of fonsecin; M, methoxyl quartet; N, methylene triplet; and O, methyl quartet.

In order to assign the aromatic ring carbons, we made use of empirical data accumulated for mono- and disubstituted naphthalenes.[32-34] Inherent in the application of chemical shift parameters to our system were several difficulties. (1) There were no less than five groups attached to the naphthalene system, so that our empirical calculations for the expected carbon magnetic resonance of each carbon atom of fonsecin was the sum total of five different chemical shift effects based on the type and position of each functional group added to a base value for the type of naphthalenic carbon (whether α, β, or γ). (2) One of our main concerns in the application of empirical chemical shift calculations was that the model compounds for which the chemical shift increments were calculated were either mono- or disubstituted naphthalenes,[35] in which the rotation of the functional groups attached was not restricted. On the other hand, the group attached to carbon 14 of fonsecin was part of a pyrone ring, and was necessarily restricted, so that the chemical shift parameter would have to be different from our approximation. A similar objection could apply to carbon 6 of fonsecin, which, although it bears a simple phenolic group, will necessarily be affected by the hydrogen bonding of this group to the carbonyl oxygen at position 4. The carbonyl is, of course, restricted by the pyrone ring, affecting its chemical shift increment for position 5. Also, the methoxyl group on carbon 8 may also experience some restriction due to the proximity of the phenolic oxygen at position 6. (3) A significant solvent effect on the chemical shift values could be expected because of the use of deuterioacetone rather than deuteriochloroform in which fonsecin was insoluble. Moreover, the values would be further changed by the presence of water and sodium dithionite, necessary for protection of the sensitive β-naphthol function against oxidation. (4) Although Me_4Si was used as an internal standard, we routinely recorded spectra with the deuterioacetone peak set at δ_C 206.000.[36a]

In order to determine whether any peaks were clearly assignable on the basis of empirical chemical shift calculations in conjunction with heteronuclear couplings observable via multiplicities in off-resonance decoupled spectra, we constructed a profile of the δ_C values calculated on the basis of the mono- as well as disubstituted naphthalenes[37] run in deuteriochloroform and compared this to the actually observed profile (i.e., the set of peaks ranked

(29) Simpson, T. J.; Stenzel, D. J. *J. Chem. Soc., Chem. Commun.* **1981**, 239–240.

(30) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T.; Yamasaki, K. *Tetrahedron Lett.* **1977**, 483–486. The same result was reported in the accompanying communication: Seto, H.; Yonehara, H. *Tetrahedron Lett.* **1977**, 487–488.

(31) Since the objective of our studies was determination of the chain folding pattern, an arbitrariy numbering system has been given for fonsecin. See formula **3**.

(32) The most useful compilation of empirical constants for naphthalenes is the following reference, in which a large number of different mono- and disubstituted naphthalenes were studied, along with deuterated derivatives. Also of interest is ref 34, which made use of strategically placed methyl groups on naphthalene derivatives. A useful tabulation of δ_C values for naphthalenes, including polysubstituted naphthalenes is given in ref 33 (see Tables I–V).

(33) Hansen, P. E. *Org. Mag. Res.* **1979**, *12*, 109–142.

(34) Wells, P. R.; Arnold, D. P.; Doddrell, D. *J. Chem. Soc. Perkin Trans. 2* **1974**, 1745–1749.

(35) Hansen, P. E. *Org. Mag. Res.* **1979**, *12*, 109–142.

(36) (a) Routine use of Me_4Si or other more traditional reference compounds was precluded due to the low solubility of fonsecin (e.g., use of Me_4Si in the calibration samples required 2–3 times as much acquisition time to achieve an acceptable signal to noise level for unenriched fonsecin as did later samples which did not contain Me_4Si). (b) The δ_C values given in the preliminary report of this work (ref 2) differ from those given in the present paper by ~2.5 ppm, since a literature value for $\delta_{C=O}$ for *protic* acetone was used earlier. Subsequently, we became aware of the sensitivity of this resonance to experimental parameters. Under the conditions of our experiments, a Me_4Si resonance within acceptable limits (0.01 ppm) was obtained when $\delta_{C=O}$ for acetone-d_6 was set at 206.000 ppm. After the initial calibration runs, this solvent carbonyl resonance was used as a secondary standard with Me_4Si absent. (c) For a detailed discussion of conditions such as temperature, solvent, hydrogen bonding, and concentration, see: De Jeu, W. H. *J. Phys. Chem.* **1970**, *74*, 822–830. Nakashima, T. T.; Traficante, D. D.; Maciel, G. E. *J. Phys. Chem.* **1974**, *78*, 124–129.

(37) See ref 33.

in decreasing δ_C value in the ^{13}C NMR). Note that most of the carbon numbers given in Table II do not represent assignments. Tentative assignments are only possible for those peaks indicated specifically. As discussed above, the peak farthest downfield (δ_C 195.6, a singlet in ORD) was readily assignable to the carbonyl carbon, carbon 4. The successive peaks B, C, D, and E (δ_C 162.9, 160.0, 159.1, and 151.8, respectively) were due to the phenolic and anisolic functions. Of this group, one peak (151.8) was well upfield of the others in the observed spectrum. In the calculated profile, the value calculated for carbon 6 (149.5) was also well upfield of the others in the phenolic/anisolic region (by 3.2 ppm), thus, a likely assignment for the peak at 151.8 was to carbon 6.[38]

In the region of non-phenolic singlets (δ_C 141.7–100.9), one peak (F, 141.7) was far removed from both its upfield and its downfield neighbors in both the observed spectrum and calculated profile, so that it could be assigned with some confidence to carbon 12, although the calculated value (134.4) did not agree well with the observed. It was separated from its downfield and upfield neighbors by more than 15 and 26 ppm, respectively. The succeeding non-phenolic singlets (G and H, 104.1 and 100.9 ppm, respectively), while well separated in the experimental, were not clearly discernible via calculation for carbons 5 and 7 in the theoretical profile.

As described above, the region (δ_C 100.0–94.6) contained the easily assignable quaternary alicyclic carbon resonance δ_C 98.1, peak K, as well as the peaks due to the aromatic carbons which appeared as doublets in the ORD. Of the aromatic doublets (δ_C values 100.0, 99.6, and 94.6, respectively) the latter (94.6) was well upfield of the others in the observed spectrum. In the calculated profile, carbon 9 (93.9) was also well upfield of the values calculated for carbons 11 and 13. Thus we tentatively assigned the peak at 94.6 to carbon 9.

In summary, except for the tentative assignments noted specifically, peak assignment by calculation was plagued by both closeness of values obtained by empirical methods, e.g., carbons 10 and 14 or carbons 5 and 7, or where calculated values were divergent, experimentally indistinguishable chemical shifts, e.g., peaks C and D or peak I and J. The latter set were only 0.4 ppm apart. Tentatively assignable peaks were thus peak F (δ 141.7), assigned to carbon 12, peak E (δ 151.8), assigned to carbon 6, and peak L (δ 94.6), assigned to carbon 9. These specific assignments are indicated in Table II.

Since it was not possible to make a complete assignment of the carbon resonances on the basis of the simple empirical calculations described above, we investigated the wealth of data on heteronuclear ^{13}C–^{1}H couplings across the naphthalene ring obtainable from a gated-decoupled experiment. Data for the parent structure naphthalene had been reported in which a number of two-, three-, and four-bond heteronuclear ^{13}C–^{1}H couplings were observed.[39]

A consideration of the phenolic–anisolic region (150–165 ppm) was particularly instructive. As a working hypothesis, we assumed that the peak at 151.8 ppm was due to carbon 6. If this were so, then we ought to be able to observe a four-bond heteronuclear ^{13}C–^{1}H coupling to the hydrogen on carbon 13. Published data suggested that the magnitude of this would be ca. 0.6–1.0 Hz.[40] Since a four-bond coupling which would correspond to carbon 6 with the hydrogen on carbon 11 was not reported in the previous studies,[41] we expected the peak at δ 151.8 to be a doublet in the gated-decoupled spectrum. Making similar use of these same heteronuclear long-range couplings reported for naphthalene we would expect the couplings for the phenolic–anisolic region to be as given in Table III.

Table III. Expected Multiplicites[a] for Peaks in the Phenolic–Anisolic Region (150–160 ppm) of Fonsecin 3[a]

C	coupled to H on C[b]	expected J, Hz	appearance
8	9 (two-bond)	0.6–10	doublet of
8	11 (four-bond)	0.6–1.0	doublets
10	9 (two-bond)	0.6–10	doublet of doublets
10	11 (two-bond)	0.6–10	or triplet[c]
14	13 (two-bond)	0.6–10	doublet

[a] Based on long-range heteronuclear couplings given in reference. [b] Not all four-bond couplings possible are observed. See reference. The prediction here is based on those reported. The number of bonds over which coupling occurs is given in parentheses. [c] $^{2}J_1 \approx {}^{2}J_2$.

Table IV. Summary of Peak Assignments for the Phenolic–Anisolic Region (150–160 ppm) for Fonsecin 3

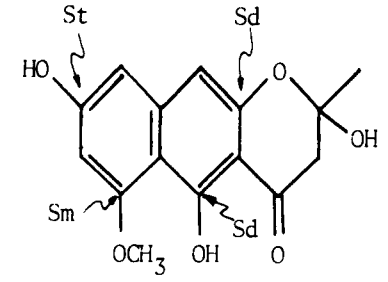
peak	δ_c obsd	multiplicity[a]	J, Hz	δ_c calcd (C)[b]	$\Delta\delta_c$ obsd – calcd
B	162.9	Sd	4.50	157.2 (14)	+5.7
C	160.0	Sm	3.40, 2.72[c]	152.7 (8)	+7.3
D	159.1	St	3.04	157.4 (10)	+1.7
E	151.8	Sd	3.71	149.5 (6)	+2.3

[a] Primary couplings ($^{1}J_{C-H}$) are given as capitals, and long-range heteronuclear couplings (^{2}J, ^{3}J, ^{4}J) as lower case. [b] Carbon number assignments for fonsecin 3. [c] This peak was not well resolved.

In the experimentally observed gated-decoupled spectrum, in addition to the predicted long-range coupling which split the peak at δ 151.8 into a doublet, the peaks at 162.9, 160.0, and 159.1 ppm were found to be a doublet, multiplet, and triplet, respectively. If our original assumption that the peak at δ 151.8 was carbon 6 was correct, then the peaks at δ 162.9, 160.0, and 159.1 could be assigned to carbons 14, 8, and 10, respectively. Although the peaks at δ 162.9 and 151.8 both appeared as doublets, these were well separated. The empirical calculations predicted carbon 6 to be 7.7 ppm upfield from carbon 14, compared to the observed difference of 11.1 ppm. These data are summarized in Table IV, and graphically in formula **9**, where the primary (one-bond) couplings are designated by capital letters and the secondary (two, three, and four-bond couplings) by the lower case.

The 95–100 ppm region contained the peak at 98.1 ppm ascribed to carbon 2 of fonsecin as well as aromatic ring carbons which each bear a hydrogen atom and which appear as doublets in the ORD spectrum. Empirical calculations suggested that since the peak at δ 94.6 was well upfield of the others (5 ppm removed from its nearest aromatic neighbor, peak J at 99.6 ppm) we might use as a working assumption that this peak was due to carbon 9 of fonsecin. If this were so, then we would expect to see long range coupling in the gated-decoupled spectrum due to the 3-bond heteronuclear coupling with the hydrogen on carbon 11 which would be expected to be in the range 4–9 Hz. Experimentally, the peak at δ 94.6 exhibited long-range heteronuclear coupling in the gated decoupled spectrum in which each of the peaks due to the primary one-bond coupling of 159.3 Hz due to the directly attached hydrogen was further split into doublets, each with a second J value of 5.21 Hz. Proceeding on this assumption, we would expect the remaining peak due to carbon 11 to

(38) See note 31.
(39) Long-range heteronuclear couplings for naphthalene are given in Table XIV, page 128, of ref 33.
(40) See the preceding note.
(41) See note 39.



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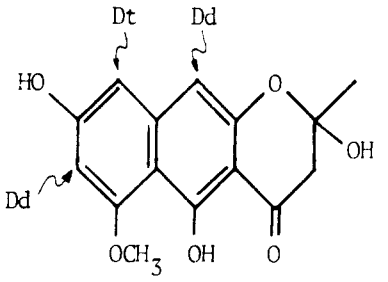

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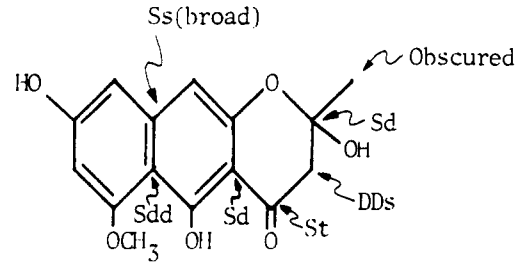

11

show a three-bond coupling with the hydrogen on carbon 9 in the range 4–9 Hz, as well as a three-bond coupling to the hydrogen on carbon 13. Thus, carbon 11 would give a doublet of doublets, or if the J values were nearly the same value, a degenerate system in which an apparent triplet would be observed. On the other hand, carbon 13 would only be expected to show a three-bond coupling to the hydrogen on carbon 11. These predicted splittings are summarized in Table V.

Experimentally, in addition to the anticipated splitting of the peak at 94.6 ppm into a doublet by long-range coupling, the other two aromatic peaks bearing hydrogens, 100.0 and 99.6, exhibited long range couplings which manifested themselves as triplet and doublet subsplittings imposed on the large one-bond couplings due to the directly attached hydrogens. On this basis we would assign the peaks at 100.0 and 99.6 to carbons 11 and 13 of fonsecin, respectively. While the peaks at 99.6 and 94.6 both existed as double doublets (Dd) they were well separated (5.2 ppm). Empirical calculations suggested values of δ 97.4 and 93.9 for these, also well separated (3.5 ppm). In summary, the peaks at δ 100.0, 99.6, and 94.6 were assigned to carbons 11, 13, and 9, respectively, based on the assumptions noted above. These results are summarized in Table VI and formula **10**.

The remaining non-phenolic singlets (ORD) in the 100–105 ppm range are due to carbons 5 and 7. For these, we would anticipate the long-range heteronuclear couplings

Table V. Predicted Multiplicities[a] for the Aromatic Doublet Region (94–100 ppm) for Fonsecin 3

C	coupled to H on C[b]	expected J,[b] Hz	appearance
11	9 (three-bond)	4–9	doublet of doublets
11	13 (three-bond)	4–9	or triplet[c]
13	11 (three-bond)	4–9	doublet

[a] See note a, Table III. [b] See note b, Table III. [c] See note c, Table III.

Table VI. Peak Assignments for the Aromatic Doublet Region of Fonsecin (94–100 ppm)

peak	δ_c obsd	multiplicity[a]	J, Hz	δ_c calcd (C)[b]	$\Delta\delta_c$ obsd − calcd
I	100.0	Dt	161.4, 4.79	100.8 (11)	−0.8
J	99.6	Dd	162.8, 5.80	97.4 (13)	2.2
L	94.6	Dd	159.3, 5.21	93.9 (9)	0.7

[a] See note a, Table IV. [b] See note b, Table IV.

Table VII. Predicted Multiplicities[a] for Remaining Non-Phenolic Singlets in the 100–105 ppm Region for Fonsecin 3

C	coupled to H on C[b]	anticipated J	appearance
5	13 (three-bond)	4–9	
5			doublet
7	13 (three-bond)	4–9	
7	11 (three-bond)	4–9	
7	9 (three-bond)	4–9	
7			multiplet

[a] See note a, Table III. [b] See note b, Table III.

Table VIII. Observed Multiplicities and J Values for the Remaining Carbons of Fonsecin 3

peak	δ_c	multiplicity[a] (C)	J, Hz
A	195.6	St	5.9
F	141.7	S(s)[b] (12)	
G	104.1	S(d)[c] (5)	not digitized
H	100.9	Sdd[d] (7)	6.2, 5.4
K	98.1	Sd[e] (2)	4.7
M	53.5	Qs (15)	144.8
N	45.7	Dds (3)	131.6, 129.6[f]
O	25.5	(1)	obscured

[a] See note a, Table IV. The assigned carbon of fonsecin is given in parentheses. [b] Broadened but not resolved. [c] By visual inspection. [d] Peak is of low intensity, and splitting may be more complex than it appears. [e] Altogether, five $^2J_{C-H}$ are possible, involving H on carbons 1 and 3. [f] These are quite similar. The peak appears as a triplet in the ORD.

Table IX. Pigment Production by *Aspergillus carbonarius*

growth, h	pigment, mg[a]	growth, h	pigment, mg[a]
24	0	168	140
48	0	192	190
72	7	216	188
96	75	264	163
120	102	312	220
144	134	408	188

[a] Per 500-mL flask culture containing 100 mL of medium.

effects shown in Table VII. The experimentally observed peak at 104.1 appeared as a doublet (visually); however, the signal was too close to the baseline noise to allow digitization. The peak at 100.9 appeared as a doublet of doublets. Both peaks were relatively small, though the one at 100.9 definitely appeared to have a more complex splitting, allowing a tentative assignment of peaks at δ 104.1 and 100.9 to carbons 5 and 7 of fonsecin, respectively.

The long range couplings for the alicyclic carbons are summarized in Table VIII and formula 11. The peak at

Table X. Effects of Flask Size, Replacement Volumes, and Culture Components on Pigment Production in *A. carbonarius*

flask size, mL	volume,[a] mL	replacement volume,[b] mL	NaOAc, g[c]	malonic acid, g[c]	sucrose, g[c]	pigment, mg[c]
500	100	20	0.1	0	0.3	91
500	100	20	0.1	0	0.15	81
500	100	20	0.1	0	0.03	72
500	100	20	0.1	0	0.003	73
500	100	20	0.1	0.76		424
500	100	20	0.1	0.38		216
500	100	20	0.05	0.38		int[d]
			Effect of Using Smaller Flask			
250	50	20	0.1	0.76		146
250	50	20	0.1	0.38		57
250	50	20	0.05	0.38		int[e]
			Smaller Flask and Smaller Replacement Volume			
125	25	10	0.025	0.19		98
125	25	10	0.025	0.095		32
125	25	10	0.0125	0.095		60

[a] Standard culture medium, mL, initial growth period 84 h. [b] Three replacements at 24-h intervals. [c] Per flask. [d,e] Intermediate value by visual estimate. Not measured.

δ 98.1, ascribed to carbon 2 would have been expected to have two-bond couplings with each of the hydrogens on carbons 1 and 3.[42] Experimentally, we observed only a doublet. The peak at δ 141.7 ascribed to carbon 12 would have been expected to have two-bond couplings to hydrogens on both carbons 11 and 13, as well as a four-bond coupling to the hydrogen on carbon 9. In the gated-decoupled spectrum this peak was broadened but not resolved. Long-range couplings for the aliphatic methyl group (25.5 ppm) were obscured by the deuterioacetone solvent peaks. The remaining results from the gated decoupled spectrum were unexceptional, and are summarized in Table VIII and formula 11. (See paragraph at the end of paper about supplementary material.)

Metabolic Studies and Feeding Experiments. The strain of *Aspergillus carbonarius* used in the original studies on the isolaton and structure of fonsecin **3** was obtained from the NRRL.[43] Czapek-Dox medium containing 1% added corn steep liquor was used for culture maintenance and metabolite production.

In our initial experiments with sodium [1-^{13}C]acetate, the labeled acetate was simply added to the growth medium. This technique was not successful in achieving any significant incorporation, as measured by peak enhancement in the ^{13}C NMR spectrum of the isolated fonsecin, for cultures previously grown for either 144 h or for 96 h. An attractive alternative appeared to be the use of replacement media.

In order to determine the most propitious time for administration of labeled precursors, the amount of pigment produced was plotted vs. time of growth to determine at which point pigment production was at a maximum, as our original intent was to administer precursors just prior to this. These data are summarized in Table IX. For a standard 500-mL culture, production increases most rapidly 72–96 h after inoculation.

The effect of replacement of culture medium, at the point of rapid increase of pigment production, by a small amount (20 mL) of solution containing 0.1 g of sodium acetate and varying amounts of sucrose (0.003–0.3 g), was studied with the result that pigment production at the highest sucrose level was 91 mg vs. 73 mg for the lowest sucrose level in standard 500-mL flask cultures. These studies are summarized in Table X. The organism thus appeared to tolerate levels of acetate suitable for labeled precursor administration, without requiring the presence of large amounts of other carbon source. Three replacements at 24-h intervals could be carried out without significant loss of pigment production.

Table XI. Effect of Sodium Acetate Concentration and Culture Age on Sodium Acetate Uptake[a]

age,[b] h	mg[c]	uptakes[d]
84	50	79, 74, 67, 57, 49
84	100	82, 78, 58, 37, 36
84	150	79
84	200	80
108	50	85, 80, 77
108	100	86, 80, 63
132	50	86, 84, 78
132	100	88, 79, 65
156	50	91, 85, 75

[a] On 500-mL flask cultures grown with 100 mL of culture medium for time specified. [b] Hours at first replacement. [c] Of NaOAc per 10 mL of replacement volume. [d] Percentage of sodium acetate taken up, given for each succeeding 24-h period.

Administration of sodium [1-^{13}C]acetate as a replacement medium, as described above, to an 84-h culture of the organism gave fonsecin in which alternating carbon atoms were isotopically enriched, as evidenced by the ^{13}C NMR spectrum. The result is summarized in Table XIII, along with results of subsequent experiments. Repetition of the acetate replacement with sodium [1,2-^{13}C]acetate, however, failed to give an incorporation level satisfactory for the acetate double label experiment. The isolated fonsecin, in the ^{13}C NMR, was poorly isotopically enriched, and only two homonuclear ^{13}C–^{13}C couplings could be discerned. Because of the poor enrichment in the acetate double label experiment, we undertook studies to determine what the level of acetate uptake was, as well as what factors could improve pigment production, and finally, what factors influenced the incorporation of acetate into fonsecin **3**.

The acetate uptake level was conveniently studied by the administration of replacement solutions containing varying amounts of sodium acetate plus a standard amount of sodium [1-^{14}C]acetate to cultures which had been grown normally under standard conditions for varying lengths of time. The percentage uptake was measured by taking an aliquot of the replacement solution at the beginning and end of each 24-h replacement. By measuring the percentage of original radioactivity remaining, that taken up

(42) Long-range couplings in aliphatic and alicyclic systems do not appear to be well documented. A possible model for our system would be a similar quaternary carbon in averufin (ref 11). Although long-range multiplicity was reported, no coupling constants were given.

(43) See note 3.

Table XII. Incorporation of Acetate vs. Acetate and Malonic Acid Concentrations[a]

sodium acetate, g[b]	malonic acid, g[c]	% incorporation[d]
0.200	1.5200	
0.100	0.7600	
0.050	0.380	1.66
0.025	0.190	0.85
0.0125	0.095	0.67
0.100	0.380	0.17
0.050	0.570	0.16
0.050	0.760	0.16

[a] 250-mL flask cultures, 50 mL of growth volume, grown for 84 h. [b,c] Per 20 mL of replacement volume, using three replacements at 24-h intervals. [d] Based on mixed pigments.

was calculated by difference. These studies showed that, for an 84-h old 500-mL flask culture with a 10-mL replacement volume, three 100-mg portions of sodium acetate could be taken up in the extent of 82% the first day, 78% the second day, and 58% the third day. Thereafter, the uptake dropped dramatically, possibly as a result of toxicity of acetate to the organism. The results for the series of studies are summarized in Table XI. Use of cultures grown for a longer time before replacement improved the acetate uptake only slightly. In any case, uptake of the acetate under the original replacement conditions did not appear to be a problem.

Since we were interested in factors which might favor secondary over primary metabolism, we conducted studies on relative pigment production with respect to the use of different replacement media. Malonic acid significantly stimulated pigment production when added to the acetate replacement solutions, giving up to a 4-fold increase in mixed pigments per culture. The results of these experiments are summarized in Table X. The optimum concentrations of sodium acetate and malonic acid were found to be 5 and 38 g L^{-1}, respectively. With the improved pigment production, smaller cultures were satisfactory to produce an adequate NMR sample of fonsecin. The effect of varying flask size and replacement culture volumes are also summarized in Table X.

After a desirable flask culture size as well as the optimal concentrations of acetate and malonic acid for pigment production had been determined, a study of the percentage acetate incorporation was performed. This study used varying concentrations of sodium acetate and malonic acid, with added sodium [1-^{14}C]acetate. Fonsecin was isolated in the usual manner and counted. Surprisingly, under conditions of optimal pigment production, the incorporation of acetate was *negligible*! A number of different experiments were run, varying the concentrations of both acetate and malonic acid. We found that the optimum concentrations of acetate and malonate were each about half that required for maximum pigment production, corresponding to 2.5 g L^{-1} and 19.0 g L^{-1}, respectively, where incorporation of activity into the mixed pigments was 1.66%. Earlier experiments, where fonsecin was isolated by TLC and rigorously purified, had indicated that incorporation into fonsecin was 0.66 of the total mixed pigment activity, thus we made a reasonable estimation of the incorporation level by simply counting the mixed pigments and multiplying by 0.66. The activity of total mixed pigments is summarized in Table XII. The maximum incorporation of acetate into fonsecin was thus estimated to be 1.10%.

Using the malonic acid medium for replacement as above, we were able to accomplish a successful double label experiment with sodium [1,2-^{13}C]acetate. The result of this experiment is summarized in Table XIII, along with the single label experiment described above. All homonuclear couplings (^{13}C–^{13}C) due to incorporation of intact acetate units were clearly visible, except for the methyl terminus, which was obscured by solvent peaks. The results were entirely consistent with the simple chain folding 8, which had not been previously demonstrated for the naphthalene series. (See paragraph at the end of paper about supplementary material.)

Table XIII. Results of the Single and Double Label Experiments Using Sodium [1-^{13}C]Acetate and Sodium [1,2-^{13}C]Acetate, Respectively[a]

peak	single label	double label $J_{^{13}C-^{13}C}$, Hz	assign
195.6	*	40	4
162.9	*	66	14
160.0	*	70	8
159.1	*	71	10
151.8	*	59	6
141.7	*	56	12
104.1		59	5
100.9		70	7
100.0		56	11
99.6		66	13
98.1	*	46	2
94.6		72	9
53.5		not coupled	15
45.7		40	3
25.5		not observable	1

[a] Run under conditions of maximum percent incorporation.

Summary

Although the structure of fonsecin **3** is relatively simple, so that carbon resonances in the pyran ring may be assigned with relative ease, the similarity of δ_C values for three different sets of carbons in the naphthalene moiety precluded meaningful peak assignments except in a few cases. When taken in conjunction with δ_C values, analysis of long-range carbon–proton couplings in the naphthalene ring allowed the remaining carbon resonances to be assigned unambiguously.

In spite of the substantial rate of production of fonsecin by *Aspergillus carbonarius*, incorporation of acetate into the metabolite was poor, even when the substrate was administered just prior to maximal pigment production. Use of replacement culture technique gave sufficient incorporation of sodium [1-^{13}C]acetate to allow identification of enriched peaks by ^{13}C NMR; however, extensive metabolic studies, where administration times, substrate levels, and media composition were varied, were required before sodium [1,2-^{13}C]acetate could be incorporated into fonsecin at a level satisfactory for the "double label" experiment. It was found that concurrent administration of malonic acid along with sodium acetate profoundly affected both pigment production and acetate incorporation into fonsecin; however, under optimal production conditions, incorporation of acetate into fonsecin by the organism was negligible. Specifically, a 250-mL flask culture was grown to the point of onset of maximal pigment production under normal culture conditions. Using replacement volumes of 20 mL at 24-h intervals, the optimal concentrations of sodium acetate and malonic acid for pigment production were 5 and 38 g L^{-1}, respectively. These conditions gave negligible acetate incorporation. For maximal *incorporation* of acetate, sodium acetate, and malonic acid concentrations of exactly half those used for maximal pigment production gave the best results.

Under optimal incorporation conditions, administration of sodium [1,2-^{13}C]acetate to the organism gave fonsecin in which homonuclear carbon couplings were each clearly discernible in the ^{13}C NMR. On the basis of the peak

assignment described above, results were consistent with the polyketide chain folding as depicted in 8. While chain folding 8 is both simple and reasonable, the present work constitutes the first experimental proof for its existence.

Experimental Section

Thin-Layer Chromatography. Unless otherwise specified, preparative thin-layer chromatography was carried out by using Analtech 1000 μm silica gel plates with an eluent system of toluene–methanol–acetic acid (8:1:1).

Radiochemical Methods. Radioassays were performed on a Hewlett-Packard liquid scintillation spectrometer, Series 3000, Model 574. Scintillation cocktails were prepared with BBOT scintillator at a concentration of 20 g per gallon of toluene. These were performed in New England Nuclear 20-mm vials of low potassium glass, with foil lined screw tops. Unless otherwise stated, 15 mL of cocktail was added per vial. Quench correction curves were prepared for the external standard, which was used routinely, by using concentrations of up to 1 mL per vial of each of the following: acetone, acetone containing 1 g L^{-1} of fonsecin, and water. Optimization of counter parameters was carried out as described by Wang et al.[44]

Organism Maintenance. *Aspergillus carbonarius* NRRL 8740 strain 0-16-1 (previously described as *Aspergillus fonsecaeus*) was obtained from the Northern Regional Research Laboratories, Peoria, IL, and maintained on 3% agar slants prepared from the modified Czapek-Dox medium consisting of the following: distilled water, 1 L; sucrose, 30 g; $NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; and $FeSO_4$, 0.01 g. Also, 1% (10 mL) of corn steep liquor (obtained from Staley) was added. All solid components were routinely replaced by 35 g of Bacto Czapek-Dox medium. Media were sterilized in a National Sterilizer Model 74-9000-D autoclave with efficiency checked with Steam-Clox indicators.

Isolation of Fonsecin 3. Growth of the organism for both isolation and feeding experiments was routinely carried out in specially modified Erlenmeyer flasks which had a small side arm at the base equipped with a rubber septum which allowed transfer and replacement of culture media under sterile conditions. Unless otherwise specified, these were 500-mL flasks which contained 100 mL of culture medium, prepared as described above. Replacement volumes unless otherwise specified were 20 mL. Incubation temperature was 25 °C.

In a typical procedure, the mycelial felt of the organism was air dried at ambient temperature overnight. Four 500-mL flask cultures, prepared as described above, yielded, after 144 h growth, 2.7 g of mycelium, which was then extracted with acetone at ambient temperature. Evaporation of the extract yielded a residue, 0.260 g, which was extremely sensitive to aerial oxidation and which was stored under vacuum. For plating out on the preparative TLC system described above, 1 mL of acetone was added for each 0.030 g of residue. The capacity of the silica gel for the pigments was abnormally low, acceptable resolution only being obtained at 0.050 g per 1000 μm plate or less! The plates were scraped while still wet to avoid aerial oxidation, and the bands taken for acetone extraction. Fonsecin, R_f 0.319, rubrofusarin, R_f 0.389, and fonsecin monomethyl ether, R_f 0.4, were obtained. Samples were held at 1 mm pressure for at least 24 h to remove the last traces of acetic acid due to the chromatography procedure.

NMR Sample Preparation. Samples were dissolved in 0.35 mL of deuterioacetone, and the solution shaken with 5 drops of saturated aqueous sodium hydrosulfite. The organic layer was then filtered through a small cotton plug contained in a Pasteur pipette. The sample, now under the reducing conditions necessary to prevent autoxidation and radical formation, was sealed under N_2 prior to NMR study. Carbon magnetic resonance spectra were measured on a Varian XL 100-15 equipped with a Nicolet 1180 pulse package, operating at 25.213 MHz. In initial calibration runs, 5 drops of tetramethylsilane were added prior to the filtration step described above, as an internal NMR standard. A Me_4Si resonance within acceptable limits for these studies (0.01 ppm) was obtained when the deuterioacetone solvent carbonyl was set at δ_C 206.000, therefore we routinely used this setting in all subsequent studies.[45]

Pigment Production Studies. Pigment production by *A. carbonarius* was studied vs. time in the standard 500-mL flask cultures. The weight of mixed pigments obtained vs. time is given in Table IX. The greatest pigment production level was between 72 and 96 h after inoculation.

Effect of Sucrose in Replacement Media Containing Acetate. A second series of studies was undertaken in which standard cultures were grown for 84 h and then subjected to three replacements of culture medium, in which 0.1 g of sodium acetate per flask was fed with varying amounts of sucrose per 20 mL of replacement volume at intervals at 24 h. Decreasing the sucrose level from 0.3 to 0.003 g in the replacement volume gradually dropped the pigment production from 91 mg per flask to 73 mg per flask.

Effect of Added Malonic Acid in Replacement Cultures. A dramatic increase in pigment production was obtained provided there was a high ratio of malonic acid to sodium acetate. The best results were obtained with 0.1 g of NaOAc and 0.76 g of malonic acid per 20 mL of replacement volume, using 3 replacements at 24-h intervals, which gave a 4-fold increase in pigments. The effect of varying acetate and malonic acid concentrations in different size flask cultures was also studied. The results of all these studies are summarized in Table X along with the sucrose concentration studies described above. The optimum concentration of sodium acetate and malonic acid were found to be 5 and 38 g L^{-1}, respectively, in the replacement solutions.

Acetate Uptake Studies. A 500-mL flask with 100 mL of growth solution was inoculated with spores of *A. carbonarius* and growth was allowed to proceed at 25 °C for 84 h. Replacement solutions were prepared from 2 μCi of sodium [1-^{14}C]acetate, 10 mL of water, and varying amounts of unlabeled sodium acetate plus other components as specified. Replacements were carried out at 24-h intervals. Comparison of the original activity of the solution to that 24 h later allowed calculation of the amount of uptake, expressed as a percent loss of the original activity. The results for varying acetate concentration are given for cultures of different ages at time of first replacement. For brevity, the percentage uptakes are listed serially for each 24-h period. For 84-h cultures, incorporation drops significantly after the third to the fourth day at a concentration of 5 g L^{-1} of NaOAc. At twice the acetate concentration, there is a sharp drop on the third day of replacement. Uptake on the first replacement is little effected by acetate levels up to 20 g L^{-1}. There is no dramatic increase in uptake by using older cultures. The results of these studies are summarized in Table XI.

Incorporation Studies. Incorporation studies were all done in 250-mL flasks, 50 mL initial volume, 20 mL replacement volume, and three replacements at 24-h intervals. The culture age at replacement was 84 h. Earlier studies on acetate incorporation where fonsecin was isolated by TLC indicated that activity of mixed pigments was a reasonable indicator of fonsecin activity, assuming fonsecin was 66% of mixed pigment. Incorporation studies in which mixed pigment was counted are summarized in Table XII. At an acetate concentration of 5 g L^{-1} and malonate concentration of 38 g L^{-1}, which gave maximal pigment production for the 250-mL flask cultures, incorporation was too small to warrant measurement. Doubling the concentration of both acetate and malonate also failed to give incorporation. Culture conditions necessary to obtain incorporation of acetate were found to be quite sensitive to the concentration of both acetate and malonic acid. Results of these studies are summarized in Table XII. The optimum concentration of sodium acetate and malonic acid in the experiments which we ran were found to be one-half that required for maximal pigment production, i.e., 2.5 and 19 g L^{-1}, respectively.

The Acetate Single Label Experiment. When sodium [1-^{13}C]acetate was fed to cultures of *A. carbonarius* under the conditions of maximum acetate incorporation, excellent en-

(44) Wang, C. H.; Willits, D. L. "Radiotracer Methodology in Biological, Environmental, and Physical Sciences"; Prentice-Hall: Englewood Cliffs, NJ, 1975; p 184.

(45) See note 36a–c.

hancement of the carbon resonances indicated in Table XIII in the single label column were obtained.

The Acetate Double Label Experiment. Repetition of the optimized acetate feeding with sodium [1,2-^{13}C]acetate gave a sample of fonsecin which exhibited well-defined ^{13}C–^{13}C couplings as indicated in Table XIII. The only coupling not observable was that for carbon 1, at δ_C 25.5, which was obscured by solvent peaks. Several spectra were taken of this sample. It should be noted that not every coupling was observable in every spectrum. A sample spectrum has been provided as supplementary material. (See paragraph at end of paper about supplementary material.)

Acknowledgment. This work was partially supported by National Science Foundation Grant No. CHE-76-05757.

Registry No. Fonsecin, 3748-39-8.

Supplementary Material Available: Natural abundance ^{13}C NMR spectrum of fonsecin **3**, as well as gated-decoupled spectrum in the 150–160 ppm region, ^{13}C NMR spectrum of fonsecin derived from $^{13}CH_3{}^{13}CO_2Na$, with expansions of the congested 150–165 ppm and 93–105 ppm regions of the latter (9 pages). Ordering information is given on any current masthead page.

The Synthesis and Chemistry of Functionalized Furochromones. 2.[1] The Synthesis, Sommelet–Hauser Rearrangement, and Conversion of 4,9-Dimethoxy-7-[(methylthio)methyl]-5*H*-furo[3,2-*g*][1]benzopyran-5-one to Ammiol

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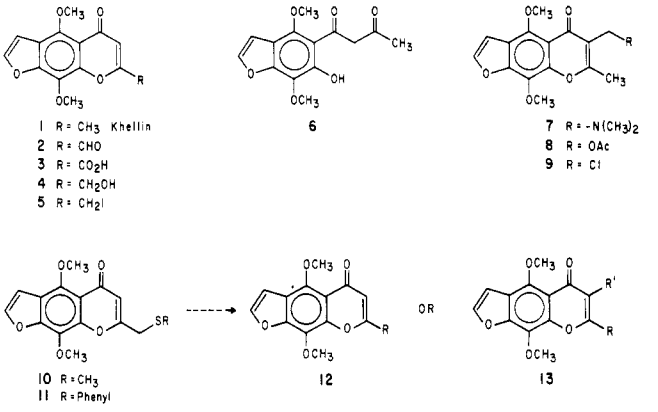
Received March 28, 1984

Condensation (NaH/THF) of khellinone **14** with ethyl 2-(methylthio)acetate followed by acid-catalyzed cyclodehydration in methanolic HCl yielded 4,9-dimethoxy-7-[(methylthio)methyl]-5*H*-furo[3,2-*g*][1]benzopyran-5-one (**10**). Condensation of **14** with ethyl 2-(phenylthio)acetate followed by cyclodehydration yielded the corresponding C-7 (phenylthio)methylene analogue **11**. Sulfide **10** was converted to sulfonium salt **17** which upon treatment with base yielded the rearranged sulfide **18**, 4,9-dimethoxy-6-[(methylthio)methyl]-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one. Desulfurization of **18** yielded the 6,7-dimethylfurochromone **19** while treatment of both **18** and **10** with *N*,*N*-dimethylformamide dimethyl acetal yielded **20** and **21**, respectively. Periodate oxidation of **10** yielded sulfoxide **24** which underwent Pummerer rearrangement to give acetoxy sulfide **25**. Hydrolysis of **25** (to give **2**) and Meerwein–Ponndorf–Verley reduction then yielded ammiol **4**. Treatment of **10** with excess methyl iodide yielded the known allylic iodide **5**. Treatment of **5** with KO_2 or KOAc and then basic hydrolysis of that acetate likewise yielded ammiol. Treatment of **5** with *N*,*N*-dimethylamine afforded the C-7 aminomethylene analogue **27** in 96% yield.

Introduction

As a result of their recently discovered antiatherosclerotic and lipid-altering activity interest in the synthesis and chemistry of furochromones such as khellin (**1**) has increased.[2,3] Crucial to our strategy in exploring the chemistry and structure–activity relationship (SAR) between furochromones and their lipid-altering activity was the identification of certain functionalized furochromones which could accomodate several synthetic objectives. First, these functionalized systems must provide access to "key analogues" necessary in establishing the basis of our analogue program. Secondly, such systems must present the potential for changes in the furochromone system ranging from simple functional group transformations to conversion of the furochromone to other novel heterocyclic compounds.[1] Thus, the key element in our strategy for using functionalized furochromones was to provide a means of combining the synthetic and SAR aspects of as many compounds as possible. For example, each synthesis, in addition to providing a specific target molecule, should, if possible, also present intermediates that might be of general synthetic and/or SAR importance.[4]

Chart I



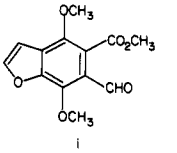
The limited use of *functionalized* furochromones as intermediates in the synthesis of khellin analogues is

(1) For Part 1 of this series see: Gammill, R. B.; Nash, S. A.; Mizsak, S. A. *Tetrahedron Lett.* **1983**, *24*, 3435.

(2) Gammill, R. B.; Day, C. E.; Schurr, P. E. *J. Med. Chem.* **1983**, *26*, 1672 and references therein.

(3) For a recent total synthesis of khellin see: Gammill, R. B.; Hyde, B. R. *J. Org. Chem.* **1983**, *48*, 3863.

(4) An example of this strategy can be found in our total synthesis of khellin (ref 3). In this synthesis, the subtarget i, which was converted to khellin, is also an interesting intermediate for analogue synthesis.



i

0022-3263/84/1949-5035$01.50/0