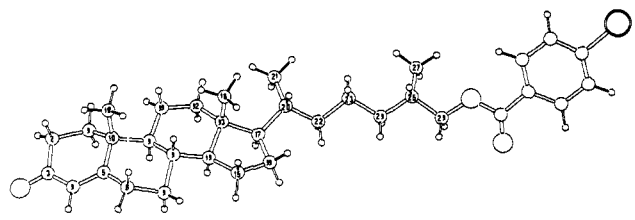
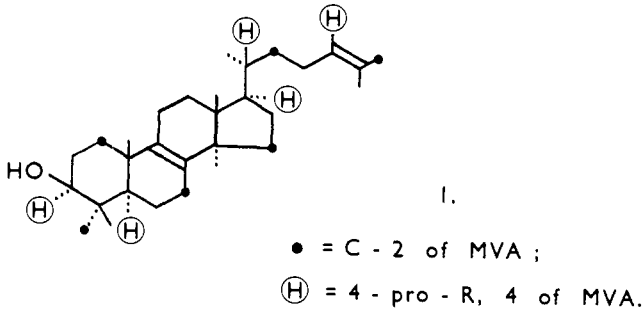
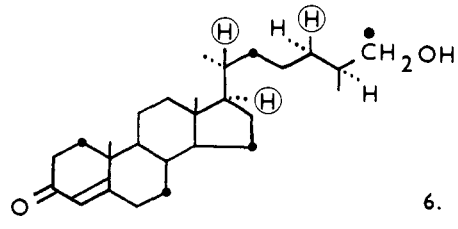


Figure 1. Drawing of 26-hydroxycholestenone *p*-bromobenzoate from X-ray results. The molecule is shown in projection down the *b* axis of the crystal.

with $a = 10.80$, $b = 9.86$, and $c = 14.52$ Å, and $\beta = 91.0°$. Three-dimensional X-ray diffraction intensity data (3274 reflections) were gathered on a computer-controlled diffractometer using nickel-filtered Cu Kα radiation. The data were corrected for systematic errors including absorption.[10] A trial structure was determined by the heavy atom method. The asymmetric unit of a three-dimensional electron density map, phased using the Br position found by Patterson analysis, contained images of two superimposed molecules, as expected. In this analysis, however, separation of the images was quite straightforward. Atomic positions and first isotropic, then anisotropic, thermal parameters were refined by least-squares techniques. Hydrogen positions were checked by difference Fourier techniques. The agreement factor R $(=\Sigma||F_o| - |F_c||/\Sigma F_o)$ is 0.069.

A drawing of the molecule prepared directly by computer from current crystallographic positions[11] is shown in Figure 1. The configuration is clearly 25*S* and not as previously inferred[6] 25*R*. The reason for the abnormality of the specific rotations (Table I) is under investigation.





(10) W. R. Busing and H. A. Levy, *Acta Crystallogr.*, **10**, 180 (1957).

(11) A listing of atomic coordinates will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Reprint Department, ACS Publications, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to author, title of article, volume, and page number. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

We have proven that in the 25*S*-26-hydroxycholest-4-en-3-one obtained by microbial oxidation of cholesterol the carbon derived from C-2 of MVA bears the oxygen function.[6] Consequently the absolute configuration at C-24 and -25 is as in **6**. Since the geometry at Δ^{24} of lanosterol is as in **1**, it follows that the reduction of this olefinic bond in the biosynthesis of cholesterol in the S-10 fraction of rat livers entails a cis addition of hydrogens at C-24 and -25 as indicated in **6** and is *not* a trans reduction.[6]

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Carbon-13 Fourier Transform Nuclear Magnetic Resonance Spectroscopy. II.[1] The Pattern of Biosynthetic Incorporation of [1-^{13}C]- and [2-^{13}C]Acetate into Prodigiosin[2]

Sir:

We wish to report the first carbon-13 Fourier transform (FT) nmr study of the detailed labeling pattern in a metabolite biosynthetically enriched with ^{13}C. Spectra were determined using a computer controlled FT system for ^{13}C.[1]

No detailed pattern of incorporation of biosynthetic substrates into prodigiosin (I) has yet been reported. In part, this reflects the difficulties involved in isolating substantial yields of fragments in the chemical degradation of the prodigiosin molecule. Use of ^{13}C nmr represents a potent method which helps circumvent the need for chemical degradation. Recent studies have employed both ^{13}C–^{1}H satellite nmr spectroscopy[3] and ^{13}C cw-nmr[4] to assign biosynthetic labeling patterns. Fourier transform nmr, as used in this study, provides substantially greater sensitivity, which has allowed us to use ^{13}C single resonance spectra as an aid to assignment, and to work with small samples.

Prodigiosin (I), isolated from the bacterium *Serratia marcescens*, is one of a series of bacterial secondary metabolites having in common an unusual system

(1) Part I; R. J. Cushley, D. R. Anderson, and S. R. Lipsky, *Anal. Chem.*, **43**, 1281 (1971).

(2) Biosynthesis of Prodigiosin. I.

(3) (a) M. Tanabe and G. Detre, *J. Amer. Chem. Soc.*, **88**, 4515 (1966); (b) D. Desaty, A. G. McInnes, D. G. Smith, and L. C. Vining, *Can. J. Biochem.*, **46**, 1293 (1968).

(4) (a) M. Tanabe, T. Hamasaki, H. Seto, and L. F. Johnson, *Chem. Commun.*, 1539 (1970); (b) N. Neuss, C. H. Nash, P. A. Lemke, and J. B. Grutzner, *J. Amer. Chem. Soc.*, **93**, 2337 (1971); (c) A. G. McInnes, D. G. Smith, L. C. Vining, and L. F. Johnson, *Chem. Commun.*, 325 (1971).

incorporating the methoxybipyrrole unit in a dipyrromethene structure.[5] There is special interest in the biosynthesis of the prodigiosins since it has been shown that they are formed by a route unrelated to porphyrin biosynthesis.[6]

Studies with mutants of *Serratia marcescens* have established the terminal steps in the biosynthetic pathway,[7] while ^{14}C tracer studies have shown that acetate, glycine, proline, and methionine are prime precursors.[6,8]

The complete ^{13}C assignments of prodigiosin hydrochloride (II) are shown in the ^{1}H noise-decoupled FT spectrum in Figure 1A and Table I. Nineteen peaks

Table I. Carbon-13 Chemical Shifts of Prodigiosin Hydrochloride in $CHCl_3$

Position of ^{13}C[a]	Chemical shift, ppm[b] Nat abun	[1-^{13}C]-Acetate	[2-^{13}C]-Acetate
A-2	−44.72		
A-3	−39.75		
A-4	−34.25		
A-5	−49.03		
B-2	−68.78		
B-3	−88.30[c]	−88.40[c]	
B-4	−15.53		−15.64
B-5	−70.23		
Methoxyl	18.72		
Bridgehead (1″)	−38.24		
C-2	−43.31		
C-3	−47.63	−47.78	
C-4 }	−50.76		−51.13
C-5 }		−50.91	
C-2-methyl	65.21		
1′	52.22		51.80
2′	47.79	47.68	
3′	46.01		45.85
4′	55.02	54.97	
5′	63.49		63.32

[a] Numbering of positions given in Figure 1A. [b] Relative to ^{13}C resonance of $CHCl_3$. Chemical-shift data using an 8K data set are accurate to ±0.05 ppm. [c] Slight chemical-shift differences are due to concentration effects in $CHCl_3$ solution.

are shown with the C-4 and C-5 superimposed peaks being resolved by an off-resonance experiment. The magnitudes of the off-resonance residual splittings (J_r)[9] for the A-5, A-4, A-3, B-4, C-4, and 1″ carbons were consistent with the ^{13}C–^{1}H couplings determined in the ^{1}H spectrum.

The resonance frequencies of the seven tertiary carbons were confirmed by progressive saturation experiments while resonance frequencies for A-5, A-4, A-3, B-4, C-4, 1″, B-3-O*Me*, 5″, and C-2-*Me* were assigned by indor spectroscopy utilizing the ^{13}C–^{1}H satellites in the previously assigned ^{1}H spectrum of II.[10] The remaining methylene carbons were assigned in the following manner. Strong cw irradiation in the proton region at the H-2′, H-3′, and H-4′ resonances gave a ^{13}C spectrum showing a triplet ($J_r \sim 3$ Hz) at 52.22 ppm. This carbon was assigned to 1′ since H-1′ occurs ~1 ppm downfield of the decoupling frequency. Carbon 4′ was assigned by analogy with carbon-13 shifts of *n*-alkanes.[11] Although the 2′ and 3′ absorptions were too close to be differentiated on the basis of chemical-shift data alone, an assignment was made from the spectra of ^{13}C-enriched II shown in Figures 1B and 1C. In neither spectrum is there evidence of ^{13}C–^{13}C spin coupling. Thus, 2′ must lie upfield of 3′.

Of the seven tertiary centers, B-3 was assigned by comparison with 2-carbethoxy-3-methoxy-5-methylpyr-

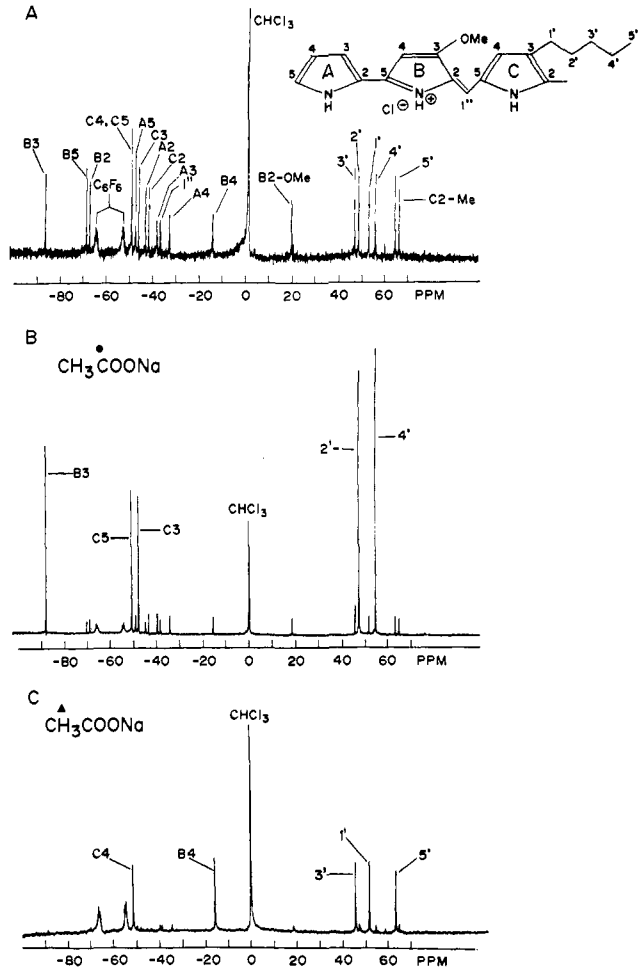


Figure 1. (A) Proton noise-decoupled carbon-13 FT spectrum of approximately 1 *M* prodigiosin hydrochloride (II) in $CHCl_3$; data set = 8K; pulse width = 40 μsec; receiver skip = 100 μsec; digitizing rate = 10kHz; scan time = 2.3 hr. (B) Proton noise-decoupled carbon-13 FT spectrum of a 0.5 *M* solution of II in $CHCl_3$ biogenetically enriched with ^{13}C from [1-^{13}C]acetate; scan time = 20 min. (C) Proton noise-decoupled carbon-13 FT spectrum of a 0.05 *M* solution of II in $CHCl_3$ biogenetically enriched with ^{13}C from [2-^{13}C]acetate; scan time = 6.7 hr.

(5) (a) H. H. Wasserman, J. E. McKeon, L. A. Smith, and P. Forgione, *J. Amer. Chem. Soc.*, 82, 506 (1960), and earlier references; (b) H. H. Wasserman, G. C. Rodgers, and D. D. Keith, *Chem. Commun.*, 825 (1966); (c) R. P. Williams and W. R. Hearn in "Antibiotics," Vol. II, D. Gottlieb and P.D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967; (d) Yu. M. Khokhlova, L. N. Sergeeva, N. S. Vulfson, V. I. Zaretzkii, V. I. Sheichenko, V. G. Zaikin, and A. S. Khokhlov, *Khim. Prir. Soedin*, 4, 307 (1968); (e) H. H. Wasserman, G. C. Rogers, and D. D. Keith, *J. Amer. Chem. Soc.*, 91, 1263 (1969); (f) N. N. Gerber, *Appl. Microbiol.*, 18, 1 (1969); (g) N. N. Gerber, *Tetrahedron Lett.*, 809 (1970); (h) for synthesis see H. Rapoport and K. G. Holden, *J. Amer. Chem. Soc.*, 84, 635 (1962).

(6) D. M. Shrimpton, G. S. Marks, and L. Bogorad, *Biochim. Biophys. Acta*, 71, 408 (1963).

(7) (a) M. T. M. Rizki, *Proc. Nat. Acad. Sci. U. S.*, 40, 1057 (1954); (b) H. H. Wasserman, J. E. McKeon, and U. V. Santer, *Biochem. Biophys. Res. Commun.*, 3, 146 (1960); (c) W. R. Hearn, R. E. Worthington, R. C. Burgus, and R. P. Williams, *ibid.*, 17, 517 (1964); (d) J. Medina-Castro, Ph.D. Thesis, Iowa State University, 1969.

(8) (a) R. Hubbard and C. Rimington, *Biochim. J.*, 46, 220 (1950); (b) L. B. DeMedina, Ph.D. Thesis, Iowa State University, 1969; (c) S. M. Qadri and R. P. Williams, *Biochim. Biophys. Acta*, 230, 181 (1971).

(9) R. R. Ernst, *J. Chem. Phys.*, 45, 3845 (1966).

(10) L. A. Smith, Ph.D. Thesis, Yale University, 1962, p 20.

(11) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, 86, 2984 (1964).

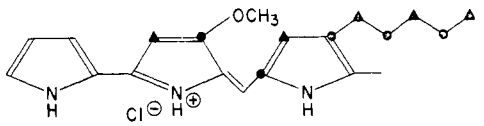


Figure 2. Labeling pattern of prodigiosin: ●, [1-^{13}C]acetate; ▲, [2-^{13}C]acetate.

role and the mutant-produced 3-methoxy-(5,2′)-bipyrrole-2-carboxaldehyde (III).[7b]

A sample of II, enriched with ^{13}C from CH_3–^{13}COONa, showed three tertiary ring carbons bearing label, including B-3. Degradation of II gave 2-methyl-3-amylpyrrole (IV)[12] enriched at positions 3 and 5 thus assigning C-3 and C-5 in II. A single resonance spectrum of *N*,*N*′,*N*″-trideuterio II showed the upfield absorption of this pair as a doublet ($J \sim 4$ Hz) due to long-range coupling[13] with the C-4 proton; hence it was assigned to C-3.

Two further doublets ($J \sim 7$ Hz) were assigned to B-5 and C-2 on the basis of their long-range couplings. C-2 was assigned to the higher field absorption since it was noted that the chemical shifts of C-3, C-4, and C-5 were close to the region of typical pyrrole chemical shifts. The strongly deshielded absorption of the pair was assigned to B-5.

The two remaining tertiary carbons were assigned in the single resonance spectrum of perdeuterio II (ring positions A-3, A-5, B-4, and C-4 deuterated). The absorption at −44.72 ppm is a sharp doublet ($J \sim 7$ Hz) and is assigned to A-2 while the absorption at −68.78 ppm was a broad peak (B-2) due to coupling with 1″-*H*.

Comparison of the B-ring ^{13}C chemical shifts with pyrrole[13-15] indicate large chemical shifts compared to the delocalized pyrrole structure. The α carbons B-2 and B-5 are shifted −28.54 and −29.99 ppm downfield from the α carbons in pyrrole while the free β position, B-4, undergoes a large diamagnetic shift (+14.57 ppm) due to the ortho methoxyl substituent.

The effect of alkyl groups on heterocyclic ring ^{13}C chemical shifts has been described previously.[14,16] However, the corresponding effects on alkyl ^{13}C shifts by the ring have hitherto not been reported. We have, in the course of this work, observed a marked upfield shift of ^{13}C resonance in alkyl groups at positions directly adjacent to the heterocyclic ring. Thus, as shown in Figure 1, ^{13}C resonance in the C-2 methyl group occurs at a substantially higher field (+65.21 ppm) than that of the alkyl methyl group at 5′ (+63.49 ppm) while the 1′ methylene resonance signal is found at a field higher than that of the methylenes 2′ or 3′.

Sodium [1-^{13}C]- and [2-^{13}C]acetate (Prochem Ltd., 62% ^{13}C) were fed in two separate experiments at 10 mmol/l. in a Bacto-peptone and glycerol medium in agar gel. The bacteria were harvested after 3 days and the prodigiosin extracted and purified by a modification of a known method.[17] The ^{13}C FT spectra given in Figures 1B and 1C show the pattern of primary incorporation from [1-^{13}C]- and [2-^{13}C]acetate, respectively. No convolution of the time domain data was performed on the spectra reported in Figure 1. The level of ^{13}C at any labeled center (~8% in Figure 1B and ~11% in Figure 1C) is sufficient to differentiate clearly the labeled positions from Overhauser enhanced signals.

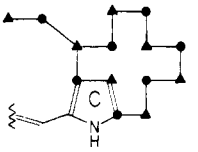
Figure 2 shows the proposed pattern of primary incorporation of acetate into prodigiosin. It is clear that the methylamylpyrrole moiety (ring C) is essentially constituted from an eight-carbon polyacetate chain. The origin of the two remaining carbons in ring C remains to be determined. The other prodigiosin analogs differ only in the nature and location of alkyl substitution in ring C. The above results suggest that these various ring C pyrrole moieties may also incorporate polyacetate chains.[18]

A significant finding is the lack of primary incorporation of acetate into ring A. This indicates a novel route to this pyrrole ring. Carbon-13 FT nmr studies of the biosynthetic origin of this ring and of the remaining carbons in prodigiosin are in progress and will be reported in due course.

Acknowledgments. This work was supported by Grant No. RR 00356 from the Biotechnology Resources Branch of the National Institutes of Health and Grant No. AI-04798 of the National Institutes of Health. R. J. S. and H. H. W. would like to thank R. Holroyd, P. Jensen, P. Peverada, and J. Gage for valuable technical assistance, Dr. M. Bunting and Dr. N. Gerber for helpful discussions, Dr. J. Faller for use of his indor equipment, and (R. J. S.) the Old Dominion Foundation for a Mellon Fellowship at Yale, 1967–1969.

(12) J. E. McKeon, Ph.D. Thesis, Yale University, 1961, p 51.
(13) F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **90**, 3543 (1968).
(14) T. F. Page, T. Alger, and D. M. Grant, *ibid.*, **87**, 5333 (1965).
(15) Noise-decoupled ^{13}C chemical shifts of 4 *M* pyrrole in $CHCl_3$ gives 2,5 = −40.24 and 3,4 = −30.10 ppm downfield from $CHCl_3$.
(16) R. G. Parker and J. D. Roberts, *J. Org. Chem.*, **35**, 996 (1970).
(17) D. F. Friedland, Ph.D. Thesis, Yale University, 1968.

(18) Metacycloprodigiosin[5e] could thus have ring C constituted from a 14-carbon polyacetate chain as shown. This hypothesis is being tested experimentally.



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The Dioxetane-Sensitized Chemiluminescence of Lanthanide Chelates. A Chemical Source of "Monochromatic" Light

Sir:

We wish to report the observation of very narrow band chemiluminescence when trimethyldioxetane (I) is thermally decomposed in the presence of certain lanthanide chelates in solution. In particular, when europium tris(thenoyltrifluoroacetonate)-1,10-phenanthroline (II)[1] was used as the fluorescent acceptor, at

(1) Prepared by the method of Bauer, *et al.* [H. Bauer, J. Blanc, and D. L. Ross, *J. Amer. Chem. Soc.*, **86**, 5125 (1964)]: mp 246–248°; uv (95% ethanol) 340 (log ϵ 4.73), 265 (4.63), 230 nm (4.65). *Anal.* Calcd: C, 43.42; H, 2.02. Found: C, 43.68; H, 2.06.