secondary equilibrium effect is still thought to closely approximate an upper limit on the value of the secondary kinetic effect even in the presence of special transition-state effects.[23] However, this conclusion is based primarily on cases (S_N2 reactions) in which the reaction coordinate motion is dominated by heavy atoms (carbon and halogen). It seems reasonable to question whether the situation might be different when the masses of the isotopic and leaving atoms are comparable and ask whether it is possible that the bending motion of the α-H of NADH is part of the reaction coordinate motion. Perhaps the observed α effect is in some sense a primary one.

Such a suggestion appears more plausible when we consider earlier proposals[24,25] that hydride ion transfer reactions are likely to have "nonlinear" or triangular transition states. Such an activated-complex geometry would be particularly likely in the present case (Figure 1) because it would allow a favorable electrostatic interaction between the developing positive charge in the dihydropyridine ring and the developing negative charge in the nitrobenzene ring. The reaction coordinate would then correspond to a wagging of the $CH_2(D)$ at the 4 position of the dihydropyridine ring so that the α-H(D) moves into the plane as the hydride ion is transferred to the nitrobenzene ring.

Acknowledgment. This work was supported in part by U.S. Public Health Service Grant AM 13332 and by National Institutes of Health Grant RR00954 from the Division of Research Resources. We are grateful for the fruitful discussions provided by Professors George Drysdale, Judith P. Klinman, A. J. Kresge, Joseph L. Kurz, and V. J. Shiner, Jr. Dr. Michael K. Hoffman provided essential assistance with the mass spectrometry work.

References and Notes

(1) Sigman, D. S.; Hajdu, J.; Creighton, D. J. In "Bioorganic Chemistry", van Tamelen, E. E., Ed.; Academic Press: New York, 1978; Vol. IV, p 385, and references cited therein.
(2) van Eikeren, P.; Grier, D. L. *J. Am. Chem. Soc.* **1975**, *97*, 8057–8060.
(3) Kurz, L. C.; Frieden, C. *J. Am. Chem. Soc.* **1975**, *97*, 677–679.
(4) Kurz, L. C.; Frieden, C. *Biochemistry* **1977**, *16*, 5207–5216.
(5) Abbreviations used: 4-CN-DNBS, 4-cyano-2,6-dinitrobenzenesulfonate; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; [4,A-^{2}H]NADH, NADH stereospecifically labeled with deuterium in the A (*pro-R*) position; [4,B-^{2}H]NADH, NADH stereospecifically labeled with deuterium in the B (*pro-S*) position; [4,4-^{2}H]NADD, NADH labeled with deuterium in both positions; YADH, yeast alcohol dehydrogenase (E.C. 1.1.1.1); LDH, lactate dehydrogenase (E.C. 1.1.1.27); 3PGDH, glycerol 3-phosphate dehydrogenase (E.C. 1.1.18); NADase, NAD nucleosidase (E.C. 3.2.2.5) from *N. crassa;* GDH, glutamate dehydrogenase (E.C. 1.4.1.2); Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Bicine, *N,N*-bis(2-hydroxyethyl)-glycine.
(6) Holmes, W. F.; Holland, W. H.; Shore, B. L.; Bier, D. M.; Sherman, W. R. *Anal. Chem.* **1973**, *45*, 2063–2071.
(7) Elliot, W. H.; Waller, G. R. In "Biochemical Applications of Mass Spectrometry", Waller, G. R., Ed.; Wiley-Interscience: New York, 1972; p 499.
(8) Prosser, A. R.; Sheppard, A. J. *J. Pharm. Sci.* **1968**, *57*, 1004–1006.
(9) San Pietro, A.; Kaplan, N. O.; Colowick, S. P. *J. Biol. Chem.* **1955**, *212*, 941–952. Velick, S. F. *Ibid.* **1958**, *223*, 1455–1467. Oppenheimer, N. J.; Arnold, L. J.; Kaplan, N. O. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 3200–3205.
(10) Snodgrass, P. J.; Vallee, B. L.; Hoch, F. L. *J. Biol. Chem.* **1960**, *235*, 504–508.
(11) Initially in an attempt to ensure that no readjustment of the equilibrium took place upon chromatographic separation, the samples were rapidly shell frozen in liquid N_2 and the volatile reaction constituents removed by lyophilization. This procedure scrambled the deuterium label. Synthetic reaction mixtures containing isotopically pure [4,B-^{2}H]NADH and [4-^{2}H]-NAD^+ (but no enzyme) typically lost between 20–30% of the deuterium in the [4-^{2}H]NAD^+ through nonenzymatic transhydrogenation. This extent would be typical of nucleotide concentrations 1000-fold greater and temperatures 20 °C higher than those actually used in the experiments. Either the shell freezing was not sufficiently rapid to prevent concentration of the solution or the sublimation temperature was not low enough to prevent the formation of micropockets of concentrated solution. This latter phenomenon has been invoked to explain why the partly second-order degradation of NADH proceeds at a faster rate in frozen solutions at −20 °C than in liquid solutions at 4 °C.
(12) Klinman, J. P. *Biochemistry* **1976**, *15*, 2018–2026.
(13) Hartshorn, S. R.; Shiner, Jr., V. S. *J. Am. Chem. Soc.* **1972**, *94*, 9002–9012.
(14) Neumann, T. E.; Shiner, Jr., V. J., personal communication, 1979: ϕCH_2DOH = 1.446, $\phi C_6H_5(D)$ = 1.368.
(15) Cook, P. F.; Cleland, W. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1977**, *36*, 665.
(16) Buddenbaum, W. E.; Shiner, Jr., V. J. In "Isotope Effects on Enzyme Catalyzed Reactions", Cleland, W. W., O'Leary, M. H., Northrop, D. B., Eds.; University Park Press: Baltimore, 1977; p 11.
(17) Kirichenko, I. N.; Yurzhenko, A. I.; Vel'shanskii, V. A. *Zh. Fiz. Khim.* **1971**, *45*, 1233–1235.
(18) Kirichenko, I. N.; Yurzhenko, A. I.; Vil'shamskii, V. A. *Zh. Fiz. Khim.* **1972**, *46*, 799–801.
(19) For the specific mechanism proposed in (2) and for an alternative mechanism which is also consistent with detailed balance (and making the steady-state approximation), it can be shown that the ratio of secondary kinetic effects determined in the two directions is equal to the equilibrium effect.
(20) Brown, A.; Fisher, H. F. *J. Am. Chem. Soc.* **1976**, *98*, 5682–5688.
(21) Kresge, A. J., personal communication, 1979.
(22) Shiner, Jr., V. J. In "Isotope Effects in Chemical Reactions", Collins, C. J., Bowman, N. S., Eds.; Van Nostrand-Reinhold: Princeton, N.J., 1970; p 90.
(23) Shiner, Jr., V. J. In ref 16, p 32.
(24) Lewis, E. S.; Symons, M. C. R. *Q. Rev., Chem. Soc.* **1958**, *12*, 230–249.
(25) More O'Ferrall, R. A. *J. Chem. Soc. B* **1970**, 785–790.

Structure of Natural Antibiotic CP-47,444

W. D. Celmer, G. N. Chmurny, C. E. Moppett, R. S. Ware, P. C. Watts, and E. B. Whipple*

Contribution from Pfizer Inc., Groton, Connecticut 06340. *Received December 6, 1979*

Abstract: The structure of the first member of a new class of natural antibiotics is deduced by the systematic application of high-resolution NMR techniques.

Antibiotic CP-47,444, isolated from *Nocardia argentinensis* Huang sp. nov., has been recently described in the patent literature.[1] This paper describes the systematic application of conventional NMR methods to deduce its chemical structure, which is of novel class.[2] The argument proceeds through distinct stages which are common to most problems of this nature.

1. Molecular Formula

The highest peak observed in the mass spectrum of CP-47,444 is at *m/e* 515.2493 corresponding within 2.6 ppm to the molecular formula $C_{28}H_{37}NO_8$. This agrees reasonably with chemical analysis, which yields 64.3% carbon (~65.2%), 7.6% hydrogen (~7.2%), and 2.6% nitrogen (~2.7%). The ^{13}C

0002-7863/80/1502-4203$01.00/0

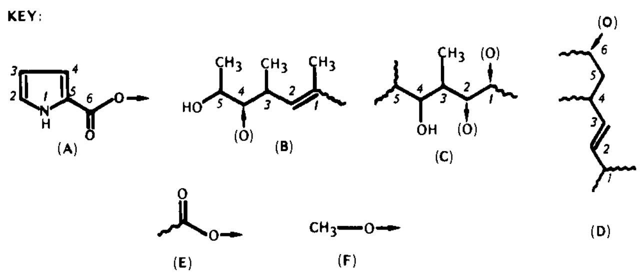
Table I. ^{13}C NMR Summary

line	δ_C[a]	fine structure: no. of H's	$^1J_{CH}$	^1H NMR	hyperfine structure[b]: hfs (J)	^1H MMR	D_2O $\Delta\delta$	assignment[c]: unit	str
1	172.4	0			mu	IIf, IVe, IIIb†		E	13
2	160.4	0			su	IIf		A6	
3	134.1	0			mo	IVd		B1	18
4	132.5	1	160	IIe	mu				(9)
5	131.1	1	156	IIe	mo				(17)
6	127.8	1	165	IId	su			D2	8
7	123.5	1	186	IIa	→t (8)			A2	
8	121.9	0			→q (7.3)			A5	
9	115.3	1	174	IIb	→dd (6.7, 4.5)			A4	
10	110.0	1	173	IIc	→dd (6, 4)			A3	
11	88.9	0			mu				1
12	83.1	1	148	IIIb	mu			D6	12
13	81.0	1	156	IIIa	su			C1	6
14	78.8	1	149	IIf					15
15	75.7	1	144	IIIb	mo		−0.13	C4	3
16	73.6	1	150	IIf	su	IVg			5
17	66.1	1	144	IIIa			−0.10	B5	15′
18	57.4	3	142	IIIc	d (3.0)	IIIb		F	12′
19	49.0	1	139	IVa					(7)
20	42.8	1	126	IVb					(10)
21	38.9	1	139	IVa			−0.05		(2)
22	34.6	1	127	IVb			−0.05		(4)
23	34.3	2	131					D5	11
24	32.6	1	126	IIId				B3	16
25	20.5	3	125	IVf					(15″)
26	16.8	3	128	IVd	d (8.6)	IIe		B1′	18′
27	15.4	3	122	IVf					(16′)
28	12.8	3	123	IVg				C3′	4′

[a] Measured from the solvent in 0.4 M $CDCl_3$ solution at 25.2 MHz and corrected to Me_4Si by a solvent shift of 76.90 ppm. [b] s = singlet, d = doublet, t = triplet, q = quartet, etc.; m = multiplet, u = unresolved, o = obscured by overlap, and → implies the result of D_2O exchange. Compound notation, line splittings in hertz, and coupled band codes are in order of decreasing magnitude from left to right. [c] Parentheses enclose immaterial assignments not following immediately from data in the table. Unit assignments refer to Table II and the structure code is given in Section 5.

Table II. Structure Units and Fragments

KEY:



unit	no.	fragments: A	B	C	D	E	F	residual units
-OH	2		1	1				
$-CH_3$	5		3	1			1	
>C=O	2	1				1		
$-CH_2-$	1				1			
-NH-	1	1						
-O-	4	1				1	1	1
>CH-	11		3	5	3			
>C<	1							1
-CH=	6	3	1		2			
>C=	2	1	1					
total	35	7	9	7	6	2	2	2

NMR spectrum shows 28 resolved lines of nominally equal intensity, and its multiplet fine structure[3] shows a total of 34 protons attached to carbon, distributed as indicated in column 3 of Table I. The ^1H NMR spectrum shows a total of 37 hydrogens, three of which undergo isotopic exchange with D_2O.[4] Since the fine structure in the ^{13}C spectrum is not altered by D_2O exchange, the three exchangeable protons must be attached to heteroatoms. All initial evidence thus implies a structure derived from the molecular formula $C_{28}H_{37}NO_8$, which has a double-bond equivalent of 11.

2. Structure Units

The reduction of the atom set to structure units consists of linking all atoms connected to only one other atom in the structure to their respective framework atoms, and subdividing the resulting set according to framework bond multiplicity. In the present instance, this is a straightforward exercise once the single nitrogen unit is assigned. Its presence as an -NH- unit was quickly apparent from the slow isotopic exchange rate, corresponding to a half-life of the order of days, of one of the three protons on heteroatoms. Two hydroxyl units follow immediately from the presence of two additional, rapidly exchangeable protons.

Carbon-13 chemical shifts above 160 ppm can now be considered characteristic of carbonyl units; those in the range 110–135 ppm can be assigned to olefinic carbons, and those below 90 ppm assigned to saturated carbons.[5] Fine-structure multiplicities of the carbon-13 bands (column 3, Table I) then serve to complete the specification of all carbon units in Table II. The four remaining ether oxygen units are inferred by difference between the molecular formula and assigned oxygen (hydroxyl and carbonyl units). A structure derived from the resulting unit set listed in column 1 of Table II must contain five rings.

3. Fragments

The primary fragment set is constructed by linking sequences of structure units principally on the basis of reciprocal hyperfine splittings between ^1H NMR bands, since the spin couplings responsible have, as a fairly general rule, magnitudes in excess of ~2 Hz over a maximum of three (single) bonds.[6] To extract this information requires an extensive, if not com-

Figure 1. 270-MHz ^{1}H NMR spectrum of CP-47,444 in 0.1 M $CDCl_3$ solution. Band designations refer to Table III. Band I at 9.6 ppm is not displayed.

Table III. ^{1}H NMR Summary[a]

band	δ	no. of H's	band structure[b]	D_2O[c]	coupled bands	assignment	
						unit	str
I	9.6	1	mu	e(s)	IIa, IIc, IIb	A1	
IIa	7.03	1	td (2.7, 1.5)	J(s)	I, IIc, IIb	A2	
IIb	6.93	1	ddd (3.7, 2.4, 1.5)	J(s)	IIc, I, IIa	A4	
IIc	6.33	1	dt (3.6, 2.6)	J(s)	IIb, I, IIa	A3	
IId	5.91	1	ddd (9.2, 7.0, 1.4)		IIe$_1$, IVa$_2$, IVb$_1$	D2	8
IIe$_1$	5.62	1	dd (9, 2.7)		IId, IVb$_1$	D3	9
IIe$_2$	5.6	1	dq (7.3, 0.6)		IIId, IVd	B2	17
IIf$_1$	5.20	1	t (7)		IIIa$_2$, IIId	B4	15
IIf$_2$	5.18	1	t (4.6)		IIIa$_1$, IVb$_2$	C2	5
IIIa$_1$	4.28	1	d (4.9)		IIf$_2$	C1	6
IIIa$_2$	4.17	1	~qn (6.5)	sh	IIf$_1$, IVf$_1$	B5	15′
IIIb$_1$	3.75	1	dd (11.7, 3.7)		IVa$_1$, IVe	D6	12
IIIb$_2$	3.72	1	→dd (10.8, 2.5)	sh	IVb$_2$, IVa$_3$	C4	3
IIIc	3.37	3	s			F	12′
IIId	3.22	1	~sx (7)		IIe$_2$, IIf$_1$, IVf$_2$	B3	16
IVa$_1$	2.59	1	ddd (15.1, 11.7, 3.7)		IVe, IIIb$_1$, IVb$_1$	D5$_a$	11
IVa$_2$	2.57	1	d (7)		IId	D1	7
IVa$_3$	2.58	1	du (2)		IIIb$_2$	C5	2
IVb$_1$	2.4	1	mu		IVa$_1$, IVe, IIe$_1$, IId	D4	10
IVb$_2$	2.4	1	dqd (10.8, 6.8, 5.0)		IIIb$_2$, IVg, IIf$_2$	C3	4
IVc	(2.0)	2	s	e		(OH)	
IVd	1.84	3	su		IIe$_2$	B1′	18′
IVe	1.47	1	dt (15.1, 3.4)		IVa$_1$, IVb$_1$, IIIb$_1$	D5b	11
IVf$_1$	1.38	3	d (6.0)		IIIa$_2$	B5′	15″
IVf$_2$	1.31	3	d (6.8)		IIId	B3′	16′
IVg	1.02	3	d (6.8)		IVb$_2$	C3′	4′

[a] Measured at 270 MHz in ~0.1 M $CDCl_3$ solution. [b] All abbreviations are the same as those in Table I. [c] e = exchangeable, (s) = slow, sh = sharpens, and J implies a change in multiplet structure only.

plete, analysis of the ^{1}H spectrum which is shown in Figure 1 and described in Table III. The process, aided by isotopic exchange and homonuclear spin decoupling experiments, is routine in principle and the results of greatest bearing are contained in columns 4–6 of Table III. The construction and/or assignments of fragments are usually extended by three

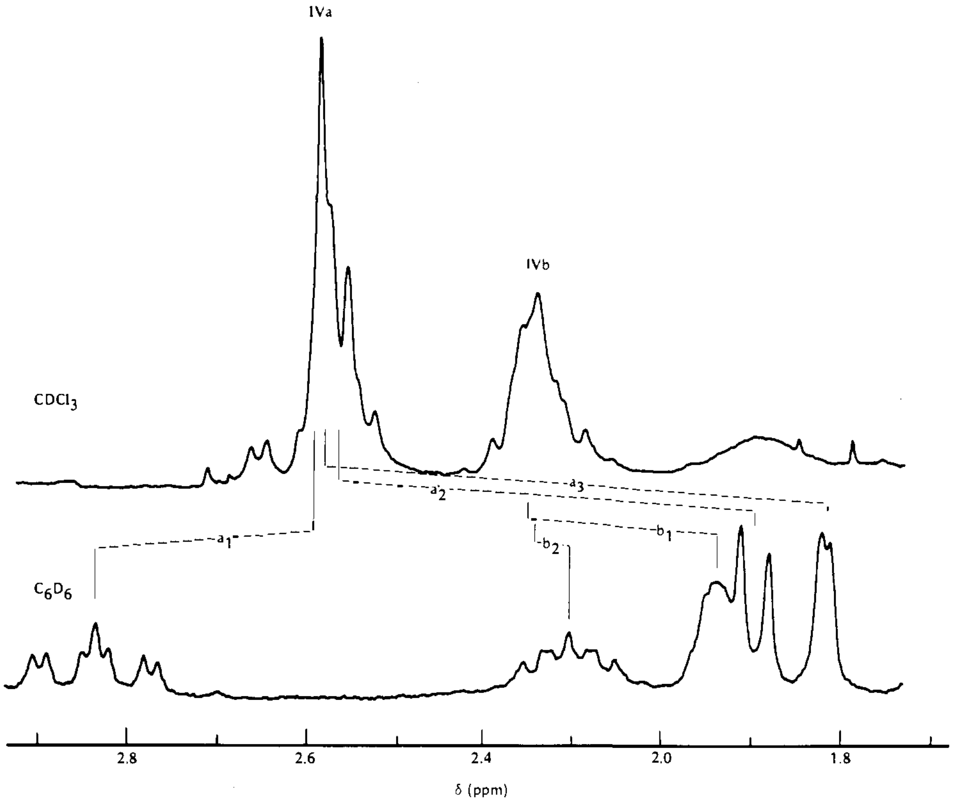


Figure 2. ^1H NMR spectrum expansions of bands IVa and IVb from Figure 1 in $CDCl_3$ and C_6D_6 solutions.

additional sources of information. Resonances of carbon and directly bonded protons can be correlated by interpolation of residual splittings in a series of variable offset, coherent heteronuclear spin decoupling experiments,[7] whose results are summarized in column 5 of Table I. Since these experiments are accurate only to ~0.1 ppm, individual protons within a single band could not be separately assigned. Directly bonded coupling constants (column 4, Table I), whose relative values are also available from offset decoupling experiments, were obtained in most cases here directly from the coupled ^{13}C spectrum. The third source of added information is the geminal isotope shift usually observed[8] on replacement of an exchangeable proton by deuterium (column 8, Table I).

The fragments derived from the various experiments above are depicted in Table II, and the NMR assignments within these fragments are listed in the next to last columns on the right of Tables I and III.

Fragment A. Fragment A in Table II was rather quickly recognized following the observation that slow isotopic exchange of ^1H NMR I[9] reduces the hyperfine structure in three vinyl proton bands. Analysis of the reciprocal hyperfine structure in these bands (^1H NMR IIa–IIc) before and after D_2O exchange yields coupling constants characteristic of an α-substituted pyrrole ring, which also serves to explain the slow exchange rate of the -NH- proton. The base peak in the mass spectrum at *m/e* 94 corresponds under accurate mass measurement to the elemental composition $C_5H_4NO^+$, suggesting that the substituent on the ring is a carbonyl unit, and an additional ester oxygen is required to account for the chemical shift of either observed carbonyl carbon. Fragment A is confirmed by the close correspondence of its NMR spectra to that of the model structure, 2,-carbomethoxypyrrole.

With the nitrogen atom thus eliminated from further consideration, one can make a detailed accounting of the oxygen substituents. Four of the eight oxygens in the structure must be in carboxyl groups, one of which is in fragment A, and there are two hydroxyl groups. While both hydroxyl protons exchange readily with D_2O, their mutual exchange is not fast enough to average out all vestiges of spin coupling to neighboring protons. As a consequence, one observes immediate sharpening, as opposed to the slow changes resulting from NH exchange, in the multiplet patterns of two other proton bands, ^1H NMR $IIIa_2$ and ^1H NMR $IIIb_2$, on addition of D_2O. As an accompanying effect, isotope shifts of −0.1 ppm are observed in two carbon-13 lines, ^{13}C NMR 15 and ^{13}C NMR 17, both of which have one directly attached proton. These observations serve to establish that both hydroxyl units are secondary alcohol functions, and remove all ambiguity in correlating individual protons in bands IIIa and IIIb with carbon-13 resonances.

By elimination, there must also be the two ether units in the molecule and hence a total of eight carbon-oxygen single bonds to units other than carbonyl groups. Eight carbon-13 lines occur in the range 55–90 ppm indicative of oxygen substitution on saturated carbon, and seven of these contain attached protons. A corresponding number of proton resonances fall in the region 3.0–5.5 ppm which is also indicative of oxygen substitution. One can hence conclude that ^{13}C NMR 11–18 and ^1H NMR IIf–IIId are in units singly bonded to one oxygen atom.

Fragment B. The methine units adjacent to methyl doublets ^1H NMR IVf_1 and IVf_2 are identified by decoupling experiments as ^1H NMR $IIIa_2$, which has an adjacent hydroxyl unit, and ^1H NMR IIId. Both of these protons are coupled by ~7 Hz to ^1H NMR IIf_1, a methine unit with triplet hyperfine structure and one attached oxygen atom. ^1H NMR IIId is also coupled (~8 Hz) to a vinyl proton, ^1H NMR IIe_2, whose hyperfine pattern is a reciprocal doublet of quartets (0.6 Hz); the vinyl carbon attached to that containing ^1H NMR IIe_2 therefore contains no proton. Irradiation of methyl singlet (unresolved) ^1H NMR IVd reduces ^1H NMR IIe_2 to a sharp doublet, implying an allylic methyl group and completing the specification and proton assignments in fragment B. The carbon-13 assignments at B2 and B4 each involve twofold ambiguities as the result of nonsingularity in the shifts of their attached protons within the uncertainty of the off-resonance decoupling measurements.

Fragments C and D. Both of these fragments contain overlapping proton resonances in bands IVa (three protons) and IVb (two protons). Successive offset decoupling of the carbon-13 spectrum shows two methine protons in each band, and by elimination of alternatives one can infer that the remaining proton in band IVa must, together with ^{1}H NMR IVe, constitute the single methylene unit. While the protons in band IV were originally (and with great difficulty) assigned to fragments in spite of their overlapping resonances, it was subsequently found that the problem is much simpler in benzene-d_6 solution where most of the overlap is removed (Figure 2). The construction of fragments C and D then follows straightforwardly from spin coupling information, specified in most cases by decoupling experiments, contained in columns 4 and 6 of Table III. Unique carbon-13 assignments could be made at positions C1, C4, D2, D5, and D6 based on off-resonance decoupling experiments augmented by geminal isotope shifts, while assignments at C2 and D3 each involve previously mentioned ambiguities with lines from fragment B. Individual assignment of the four methine carbons whose attached protons occur in band IV are immaterial to the structure proof, and were made after its completion.

Fragments E and F. ^{13}C NMR 18 and ^{1}H NMR IIIc together constitute an apparent methoxyl group, and one additional carboxyl group has already been inferred. Fragments E and F are thus established as separate entities once it is shown that they do not share the same singly bonded oxygen atom. This is apparent from the directly bonded coupling constant, $^1J_{CH}$ = 142 Hz, in the methoxyl unit which is below the characteristic value of ~148 Hz for methyl esters, and an observed hyperfine splitting of 3 Hz in ^{13}C NMR 18 which requires that the methoxyl carbon be attached to a unit containing one proton.

4. Assembly of Structure

The assembly stage in a structure proof is procedurally marked by a shift in strategy from the building of fragments to dealing with alternative structures, and is hence dictated largely by convenience. The primary fragments built up principally from $^3J_{H,H}$ spin coupling data are limited by its availability, and gaps occur either when units are encountered which contain no attached protons or when the protons on adjacent units are located at unfavorable dihedral angles.[6] This unit set may or may not provide a reasonable point of departure, and in the latter instance one might elect to build the fragment set into still larger segments by observing, again with the aid of selective ($\gamma H_2/2\pi$ of the order of the multiplet widths) decoupling experiments, hyperfine splittings of carbon-13 by remote protons since these couplings also occur as a general rule over two or three bonds. Since the experiments take much longer to perform, their planning is more selective.

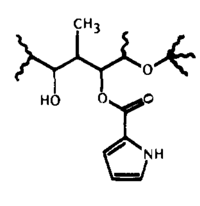
In the present instance, a complete accounting of the proton spectrum has reduced the number of fragments to eight, consisting (Table II) of A through F and two residual units, one of which is a quaternary carbon and the other an ether oxygen. Eleven bonds remain to be closed, five between carbon and oxygen and six between carbon atoms. The five carbon–oxygen bonds occur between the units specified in Table IV. Two of the oxygen atoms are on ester units, which the low-field shifts of the two protons in band IIf would suggest were bonded to B4 and C2. Selective decoupling of ^{1}H NMR IIf confirms this inference by visibly narrowing both carbonyl bands, but leaves an ambiguity concerning which ester unit is attached to which site. Were fragment A attached to B4, however, the result would be a long terminal segment (side chain) which would severely restrict the options for forming the necessary four additional rings; also, the uniformity of methine carbon-13

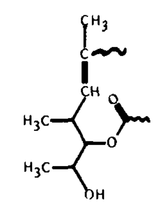
Table IV. Units Comprising Unknown C–O Bonds

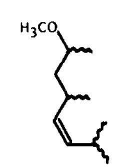
oxygen unit	carbon unit (assignments)
A→	B4 (^{13}C NMR 14/16, ^{1}H NMR IIf$_1$)
E→	C2 (^{13}C NMR 16/14, ^{1}H NMR IIf$_2$)
F→	C1 (^{13}C NMR 13, ^{1}H NMR IIIa$_1$)
←O→	D6 (^{13}C NMR 12, ^{1}H NMR IIIb$_1$)
	—C— (^{13}C NMR 11)

relaxation times throughout the molecule suggest a structure without long side chains. It was accordingly assumed, and later verified by hydrolysis studies described in the next section, that A is attached to C2 and E to B4.

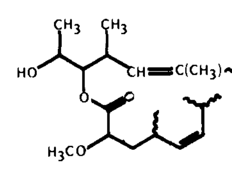
The remaining three pairings in Table IV are established if the location of the methoxyl group is specified. The doublet hyperfine splitting of the methoxyl carbon resonance requires its attachment to either C1 or D6, which each contain a terminal proton, and the choice is made by selective irradiation of ^{1}H NMR IIIb$_1$ = D6 to collapse the hyperfine splitting in ^{13}C NMR 18 = F1, proving that the methoxyl group is attached to D6.[10] The ether oxygen must consequently connect the quaternary carbon atom to C1. With all of the carbon-oxygen bonds thus specified, the assemblages consist of AC′, BE, and DF (shown below). Among a number of experiments tried, the only other clearcut heteronuclear decoupling result


(AC′)


(BE)


(DF)

was an ~50% narrowing of the lactone carbonyl, ^{13}C NMR 1, on segment BE upon irradiation of one of the methylene protons, ^{1}H NMR IVe = D5. A less pronounced, but still apparent narrowing of ^{13}C NMR 1 is also obtained on irradiation of ^{1}H NMR IVa, which contains the other methylene proton. The lactone carbonyl on BE is therefore attached to either D4 or D6. Irradiation of ^{1}H NMR IIIb, which contains the proton on D6, does not visibly narrow ^{13}C NMR 1 but gives an ~33% intensity enhancement (nuclear Overhauser effect[11]), while no change in either band structure or intensity results from irradiation of ^{1}H NMR IVb, which contains the proton on D4. It therefore appears that the carbonyl unit on BE is connected to D6, giving the assemblage BDEF.


(BDEF)

Independent evidence for attachment of the lactone unit to D6 is found in the mass fragmentation pattern of CP-47,444, where a primary $C_3H_5O_3$ loss occurs. Were the attachment to D4, no contiguous sequence of three carbon and oxygen atoms can be constructed except for that attaching the pyrrole ring, which is not concurrently lost. Attachment at D6 provides such a sequence, and leads to a structure in which the fragmentation can be plausibly rationalized (section 5).

Topographic Structures. A structure now results from the closure of five bonds involving six different sites on the two

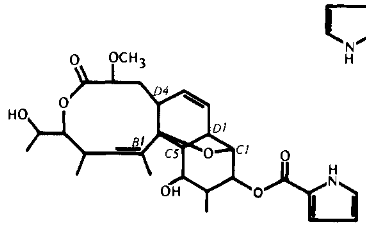
Table V. Topographic Structures and Exclusions

bonds to quaternary carbon	subsequent bonds	basis for exclusion[a]
B1, C1, C5	D1-C5, D4	a, c
B1, C1, D1	C5-D1, D4	a
B1, C5, D4	D1-C5, C1	
B1, D1, D4	C5-D1, C1	d
C1, C5, D4	D1-C5, B1	a
C1, D1, D4	C5-D1, B1	a, d
B1, C5, D1	C5-D1, C1-D4	b
	C5-C1, D1-D4	c
	C5-D4, D1-C1	
C1, C5, D1	C5-D1, B1-D4	a, b
	C5-B1, D1-D4	a, c
	C5-D4, B1-D1	a
C5, D1, D4	C5-D1, B1-C1	b, d
	C5-B1, D1-C1	d
	C5-C1, B1-D1	d

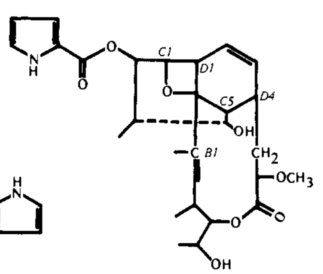
[a] (a) Epoxide ring, (b) cyclopropane ring, (c) cyclobutene ring, (d) cyclopentene ring.

segments BDEF and AC′. The quaternary carbon in AC′ occurs in three pairings, positions C5 and D1 each occur in two, and the remaining positions B1, C1, and D4 all occur once. Each pairing must connect different sites, and be unique. These restrictions permit 15 possible constructions, which are listed in Table V.

Exclusions. Structures containing either of two characteristics can be eliminated on the basis of NMR parameter ranges. These consist of three-membered rings, based on the observed magnitudes of $^1J_{CH}$ = 160 Hz,[12] and incorporation of -CH═CH-units into rings of less than six members, based on the fact that all observed $^3J_{H,H}$ couplings between vinyl protons are in excess of 9 Hz.[6] The exclusion of epoxide rings eliminates seven structures in which the quaternary carbon is attached to C1, and the absence of cyclopropane rings excludes two additional structures (plus one already excluded) in which C5 is bonded to D1 and both are attached to the quaternary carbon. Structures involving D1-D4 closure, of which there are three (one new exclusion), place D2 and D3 in a cyclobutene ring ($^3J_{H,H}$ = 2-4 Hz), and bonding D1 and D4 to the quaternary carbon place D2 and D3 in a cyclopentene ring ($^3J_{H,H}$ = 5-7 Hz), eliminating five structures among which two do not correspond to prior exclusions. In total, one thus excludes 13 of the 15 possible constructions and reduces the structure set to either Ia or Ib.



(Ia)



(Ib)

While structure Ib cannot be categorically excluded, the detailed evidence clearly points to Ia as the correct structure. For example, the four methine carbons whose attached protons fall in band IV can be individually assigned if the off-resonance decoupling correlations with ^{1}H NMR IVa or IVb are augmented by the observation of (vicinal) isotope shifts of −0.05 ppm at ^{13}C NMR 21 and ^{13}C NMR 22 which, among the choices available, imply their assignments to units C5 and C3, respectively. The directly bonded coupling constants fall into two sets with $^1J_{CH}$ = 139 Hz thus for C5 and D1 being 10%

Table VI. Derivitization Shifts in CP 47444[a]

position	$\delta_H(I)$	$\Delta = \delta - \delta(I)$ II	III	IV	V
12	3.75				−0.12
12″	3.37			−0.03	−0.08
11a	2.59			−0.73	−0.05
11b	1.47			0.11	−0.14
10	2.4				−0.09
9	5.62			0.02	−0.02
8	5.91				−0.04
7	2.57		0.14		−0.07
6	4.28				−0.03
5	5.18		0.04		−0.04
4	2.4				−0.03
4′	1.02		−0.15	−0.05	−0.06
3	3.72		1.16	−0.11	−0.09
2	2.58		0.06		−0.08
18′	1.84		−0.16	−0.11	−0.11
17	5.6	−0.04	−0.04	0.15	0.03
16	3.22	−0.06	−0.06		−0.61
16′	1.31	−0.11	−0.09	−0.20	−0.20
15	5.20	0.18	0.20	−1.66	−1.80
15′	4.17	1.05	1.05	−0.31	0.87
15″	1.38			−0.23	

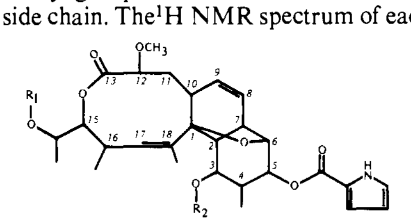
[a] Shifts of <0.02 ppm are omitted.

larger than the values for C3 and D4. This result is an immediate consequence of the ring system (electronegative substituents being absent) in structure Ia, which places C5 and D1 in a doubly fused furan ring ($^1J_{CH}$ = 133 Hz in tetrahydrofuran[12]), while structure Ib provides no internally sufficient rationale.

Structure Ia, or 3-hydroxy-15-(1-hydroxyethyl)12-methoxy-4,16,18-trimethyl-5-(2-pyrroyloxy)-14,19-dioxatetracyclo[8.8.1^{1,6}0.0^{2,7}]nonadec-8,17-dien-13-one is thus derived.

5. Confirming Evidence

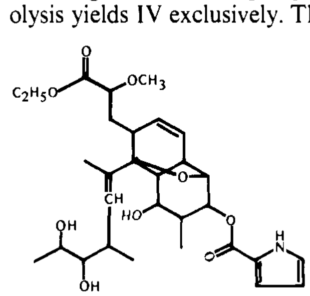
Derivitization. Acetylation yields two derivatives corresponding to reaction at one (structure II) and both (III) hydroxyl groups, the former of which occurs in the hydroxyethyl side chain. The ^{1}H NMR spectrum of each is readily assignable



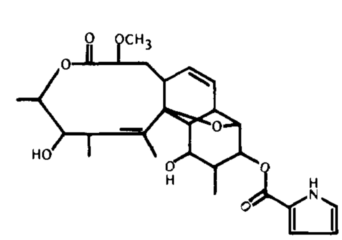
	R_1	R_2
I	-H	-H
II	$-COCH_3$	H
III	$-COCH_3$	$COCH_3$

on the basis of largely predictable substituent shifts, which are listed in Table VI. Both derivatives give strong parent ions in the mass spectrum, and the fragmentation patterns in the high mass region are similar.

Ethanolysis under basic conditions yields two additional derivatives, one of which (IV) corresponds to the expected opening of the lactone ring, and the other to a more surprising rearrangement to a ring-expanded isomer (V). Acidic ethanolysis yields IV exclusively. The ^{1}H NMR assignments in both



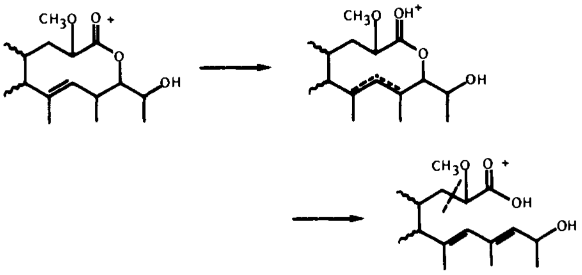
(IV)



(V)

structures are obvious extensions of those for I, and the observed shifts (Table VI) clearly confirm the prior inference that unit A is attached to C2, and E thus to B4. As one would also expect, spin coupling constants between protons on the caged system are essentially the same in I–V, while those involving protons in the macrolide ring vary on going from I–III to IV or V.

Mass Fragmentation. Compounds I–III all give parent ions in the 70-eV EI mass spectrum, and the principal fragments in the region above *m/e* 250 result from (1) loss of the elements of the base peak C_5H_4NO together with those of R_2OH; (2) loss of R_1OH; (3) loss of $C_3H_5O_3$; and (4) a combination of the above, less one proton, to give a peak at *m/e* 297. The ethanolysis product IV gives no detectable parent, but a prominent fragment at *m/e* 444 corresponds to the loss of $C_5H_9O_3$, a homologue of the $C_3H_5O_3$ loss above. This suggests



(1)

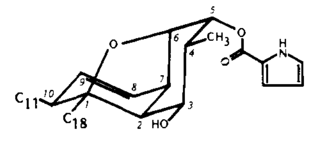
that the parent ion of I might undergo rearrangement 1. It is noteworthy in this connection that no corresponding loss is observed from structure V, where a seven-membered ring would be involved in the transition state for rearrangement.[13]

A loss of C_3H_6O, alone and in combination with (1) and (3) above (the latter as $C_3H_4O_3$), gives another series of CP-47,444 that is not observed in its acetates. Since this is the predominant fragmentation in V, prior rearrangement of I → V may be involved.

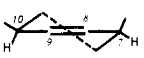
Carbon-13 Relaxation. The relaxation properties of the ring carbons are consistent with isotropic intramolecular dipolar relaxation based on the assigned structure I. In particular, the relaxation times (nT_1) for protonated carbons in the cage system ($n\overline{T}_1 = 0.105 \pm 0.007$ s) or in the macrolide ring ($n\overline{T}_1 = 0.122 \pm 0.003$ s) form two nonoverlapping equal sets, with the latter ~15% longer than the former. This can be simply attributed to greater flexibility in the macrocyclic ring. The placement of ^{13}C NMR 17 = B5 into a pendant unit is also confirmed by its still longer relaxation time of 0.14 s.

6. Relative Configurations and Conformation

CP-47,444 contains 12 asymmetric carbon atoms and two multiply substituted double bonds (excluding the pyrrole ring), which together allow 8 192 isomers in addition to the choice of absolute configuration. The fused ring system fixes, on the basis of geometrical requirements, the relative configurations at four centers (positions 1, 2, 6, and 7) and a *Z* configuration (also evident from $^3J_{H,H}$ = 9 Hz between the vinyl protons) about the 8,9 double bond. The large spin coupling (~11 Hz) between protons on positions 3 and 4 implies a near-trans arrangement, while those on positions 2 and 3 ($^3J_{H,H}$ = 2.5 Hz) and 4 and 5 (4.6 Hz) are gauche; these serve to define the relative configurations at three additional centers as shown in VI.



VII



VIII

The relative configuration at position 10 can also be assigned on the basis of proton coupling constants to be as indicated in VII, thereby completing the cage. All $^3J_{H,H}$ couplings, including the near vanishing ones of H7 to H2 and H6, are in good agreement with dihedral angles calculated for a cage modeled after VI.[14] Lanthanide shift reagents, which appear to complex in the vicinity of the pyrrole ring, shift H3 and H7 about equally, while shifting H6 more and H4 less. Other induced shifts, apart from those in the pyrrole ring, are small in comparison.

The relative configurations in the macrolide ring remain unassigned, the major impediment to solution of this problem being lack of a clear definition of the configuration about the 17,18 double bond.

Acknowledgment. Much of the ^{1}H NMR work was carried out at 270 MHz on the Southern New England High-Field NMR Facility at Yale University, made possible by a grant from the Biotechnology Resources Program at the National Institute of Health. Mr. Peter Denou conducted these experiments.

References and Notes

(1) W. D. Celmer, W. P. Cullen, C. E. Moppett, M. T. Jefferson, L. H. Huang, R. Shibakawa, and J. Tone (to Pfizer Inc., New York), U.S. Patent 4 148 883 (Aug 18, 1977).
(2) The structure suggests a signet ring with a macrolide band, a rigid cage for the stone, and a pyrrole ring for the seal which has so far served to identify additional members of the class. The name Nargenicin A_1 has been proposed.
(3) In describing NMR multiplets, fine structure refers to strong splittings by directly bonded nuclei and hyperfine structure to the smaller splittings by more remote nuclei. Fine structure (carbon-13 satellites) is not ordinarily observed in the proton spectrum.
(4) Under (trifluoroacetic) acid catalysis, one additional proton on carbon (band IIIc, Table II) undergoes slow isotopic exchange.
(5) Many chemical shift correlation charts are available (see, for example, F. W. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 NMR Spectra", Heyden, New York, 1976) which differ somewhat in range limits. The regions cited here for experimentally observed bands are unique on all such charts once the nitrogen unit is known.
(6) L. M. Jackman and S. Sternhell, "Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., Pergamon Press, Elmsford, N.Y., and Oxford, 1969, part 4. Exceptions to the rule are also discussed.
(7) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, **54**, 3367 (1971); B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J. Chem. Soc., Chem. Commun.*, 316 (1972).
(8) R. A. Bell, C. L. Chan, and B. G. Sayer, *J. Chem. Soc., Chem. Commun.*, 67 (1972).
(9) For sake of brevity, the compact notation ^{13}C NMR *n* or ^{1}H NMR *n* refers to Table I or III, with the suffix *n* being the band designation in column 1 of the table. Once assigned, these band designator codes may be followed by a fragment assignment (Table II), separated by an equals sign.
(10) This experiment, rather than the alternative irradiation of ^{1}H NMR $IIIa_1$ = C1 was chosen to avoid complications from a decoupling field near a carbon-13 satellite of the methoxyl proton resonance.
(11) J. H. Noggle and R. F. Schirmer, "The Nuclear Overhauser Effect", Academic Press, New York and London, 1971.
(12) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York and London, 1972.
(13) F. W. McLafferty, "Interpretation of Mass Spectra", 2nd ed., W. A. Benjamin, Reading, Mass., 1973, Chapter 4.
(14) N. L. Allinger et. al., *QCPE*, **11**, 318 (1976). The calculations were performed by Dr. B. W. Dominy, who also provided the systematic nomenclature for CP-47,444.