Determination of the Solution Conformation of Rifamycin S and Derivatives by Nuclear Magnetic Resonance

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Abstract: A detailed study of the conformation of rifamycin S was carried out. It is shown that the conformation is similar to that deduced by single crystal X-ray crystallographic techniques and that, in aqueous solution, the ansa bridge undergoes a conformational change best referred to as an inward buckling.

We report here an investigation into the proton NMR spectrum of rifamycin S and a number of its derivatives (Figure 1). The purpose of this was severalfold. (1) The rifamycins, as inhibitors of DNA dependent RNA polymerase[1] and RNA dependent DNA polymerase,[2] are molecules of considerably biological significance. (2) The structure and conformation of several of these[3,4] have been determined by X-ray crystallography; one would like to know to what extent their solution conformations, as evidenced by their NMR spectra, parallel their solid state conformation. (3) As a structural class, the ansamycins are related by the presence of an ansa bridge, conveying a doughnut-like shape to these molecules.[3] It is intriguing to speculate that it is indeed the doughnut nature of the ansamycins that is responsible for their close similarity of function in the presence of otherwise widely diverse structural features. In particular, there is the idea that formation of inclusion complexes similar to those of the cyclodextrans is an important facet of the energetics of the ansamycin-DNA dependent RNA polymerase interaction. This proposal is consistent with the known structure-activity modification work on these compounds[6] and the fact that all substituents on the ansa bridge are directed outside of rather than into the ring. We report below some experiments designed to test this idea. (4) To the extent that one wishes to correlate structural changes and modifications with biological activity, it is important to be able to ascertain the effect of structural changes on the conformation of the molecule as a whole. It is not obvious in molecules of the complexity of the ansamycins that local structural modifications will not induce "global" conformation changes. Examples of this type of long-range conformational change are seen in the 3-substituted rifamycins wherein bulky substituents induce a conformational change in a remote region of the ansa bridge.[7]

Our conclusions are contained in the detailed proton assignment of rifamycin S and in statements that: (1) the solution conformation of rifamycin S closely parallels that determined by X-ray crystallographic methods; (2) the ring current of the naphthalene increases on going from the naphthoquinone to naphthoquinol form; (3) solvent effects suggest that, on going to more and more aqueous solvents, the ansa bridge partially collapses into its hydrophobic interior. After this work was completed, a similar study of rifamycin S appeared[8] wherein the same conclusion as 1 above was reached. There have also been published several earlier papers involving partial assignment of the spectra of rifamycin derivatives that we acknowledge.[9]

Experimental Section

NMR Spectra. Proton NMR spectra were recorded on either a Varian XL-100-15 spectrometer, operating at a probe temperature, unless otherwise specified, of 28° or on a Bruker WH-270 spectrometer operating at a probe temperature of ca. 21°. The former is equipped for both frequency sweep and pulsed Fourier transform (FT) operation, while the latter is a dedicated FT machine. In all cases (XL-100) except the spectra in deuterium oxide, the CW mode was used. The homonuclear mode of the XL-100 Gyrocode decoupler was used for decoupling experiments (≈1 W of rf power). Chemical shifts are reported relative to Me_4Si, and their accuracy is ±0.01 ppm. Concentrations were in the 2–6 w/v % range except for two deuterium oxide samples which were 10^{-4} *M*. The deuterium oxide (Wilmad) used was 100 atom % deuterated. All samples were vacuum degassed and sealed.

Materials. Rifamycin SV (Calbiochem) was oxidized to rifamycin S and recrystallized from methanol or benzene–hexane, mp 179–182° (lit.[10] 180–181°). Thio derivatives **1b** and **1c** were prepared by addition of the appropriate mercaptan to rifamycin S in dioxane in the presence of triethylamine. They were isolated as glasses by chromatography, were pure by TLC, and characterized by their uv and NMR spectra. Quinones **1d** and **1e** were prepared by oxidation of **1b** and **1c**, respectively, by potassium ferricyanide as described for rifamycin SV.

Results and Discussion

A number of attempts at using lanthanide shift reagents were unsuccessful, leading to either no effect or chemical decomposition of the sample. We note that no virtual coupling effects were seen in the present work, nor was any expected, because of the alternating high field–low field arrangement of protons in polyketides such as the rifamycin S ansa bridge. Of the many solvents tried, the best solvent for use in assigning the proton spectrum of rifamycin S is benzene-d_6 because overlap among the 45 protons is minimal. A 100-MHz spectrum at 28° in benzene-d_6 is shown in Figure 2A. A 270-MHz spectrum of rifamycin S in deuteriochloroform is shown in Figure 2C. The nine methyl groups all appear distinct as five singlets and four doublets. Of the remaining 18 protons, 14 are visible, although there is some overlap among them at 100 MHz. The four protons not directly observable are the H-C-CH_3 protons (protons 26, 24, 22, and 20) of the ansa bridge, and their method of assignment is described below. The complete proton assignment is given in Table I and shown for benzene-d_6 in Figure 3.

The most useful method for assignment of the various protons was: (1) double irradiation experiments for all coupled protons (this includes the 5 methyl groups and 13 tertiary hydrogens of the ansa bridge); (2) D_2O exchange for the four acidic protons; (3) chemical shift considerations for the five methyl singlets and H-3 of the naphthoquinone ring.

Assignment of all protons by the above techniques proceeded in a straightforward manner with several exceptions. The four H-C-CH_3 protons (H-20, H-22, H-24, and H-26) of the ansa bridge are not directly visible. Moreover, because of the Freeman–Anderson effect, their assignment by decoupling experiments depended on the nucleus observed; for example, the chemical shift of H-26 varied from 1.85 to

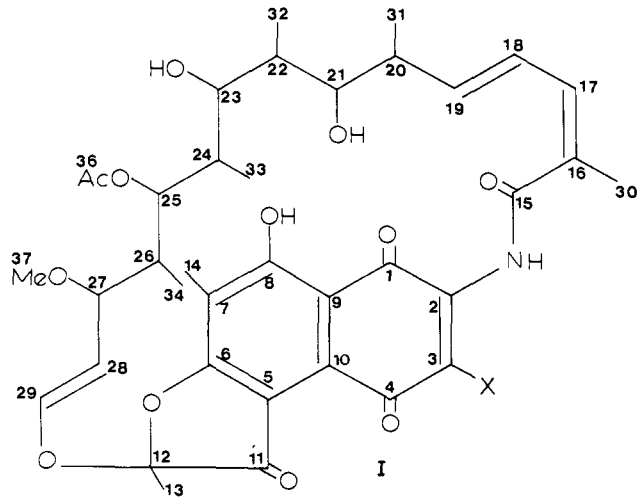

Figure 1. Structures and numberings conventions used for rifamycin derivatives. (Ia, X = H; Ib, X = $SCH_2CH_2C_6H_5OCH_3$, hydroquinone; Ic, X = $S(CH_2)_4C_6H_5OCH_3$, hydroquinone; Id, X = $SCH_2CH_2C_6H_4OCH_3$; Ie, X = $S(CH_2)_4C_6H_4OCH_3$.

2.00 ppm when determined in this manner. Application of the equation[11-14]

$$W_2 = W_x + \frac{\gamma^2 H_2^2}{W_A - W_x}$$

where W_2 is the irradiating frequency, W_x is the true frequency of proton X, γH_2 is the decoupling power, and $W_A - W_x$ is the true chemical shift difference between the observed and irradiated proton, with careful determination of W_2 for maximum decoupling in deuteriochloroform and benzene-d_6, allowed us to calculate three W_A values for each H-C-CH_3. In three of the four cases, the W_A values calculated agreed to within ±0.02 ppm. In the fourth case (H-22), the values agreed to within ±0.05 ppm.

The four vinyl protons H-17, H-18, H-19, and H-29 overlap badly in benzene-d_6 at 28° (Figure 2A). At 100° one can pick out the splittings. These are confirmed by inspecting the spectra of other 3-substituted rifamycins (Figure 4B). Assignment of methyls 13, 14, and 36 required a knowledge of the corresponding carbon chemical shifts.[16-18]

The four acidic protons appear as separate resonances. Irradiation of the phenol proton did not cause saturation of the alcohol protons. They thus exchange rather slowly at room temperature,[15] which is interesting considering that the phenol and hydroxyl groups are cis relative to the ansa macrocycle.

Since the coupling constant between protons had been determined for every bond along the ansa bridge, we were able to use these values to predict approximate dihedral angles between ansa bridge protons.[19] Alternatively, the dihedral angles determined from the X-ray structure of a rifamycin B derivative[3] can be used to predict coupling constants. If the conformation in solution and in the solid state are similar, the observed and predicted coupling constants should agree qualitatively. This is the case as shown in Table II. Only qualitative agreement can be expected principally because not only dihedral angle but also substituents affect coupling constants, and substituent effects cannot be accurately calculated.[20]

Temperature and Solvent Effects

A striking increase in resolution was observed in the rifamycin S spectrum when the sample was heated to 100° (Figure 2B). A corresponding loss of resolution was observed at −30°. While we observe considerable sharpening of spectra at high temperature, we have been unable to "freeze out" any distinct species at low temperature. The compound precipitates from methanol solution at ca. −50°. It accordingly appears that the temperature sensitivity is merely a reflection of molecular tumbling on T_2 and not of conformational interconversion processes.

The chemical shifts of the ansa bridge protons were found to be slightly temperature dependent. (The chemical shifts of the acidic protons were, of course, more solvent dependent.) Within our limits of measurement (≈±0.5 Hz), the coupling constants were unaffected by temperature changes.

Solvent polarity variation has a greater effect on proton chemical shifts than temperature variation. As can be seen from Table I, methyl groups 33 and 34, the visible tertiary hydrogens on the ansa bridge, and H-3 on the naphthoquinone ring all show considerable solvent dependence.[21] Coupling constants, as with temperature variation, were not detectably affected by solvent changes.

These findings, that chemical shifts but not coupling constants are affected by solvent polarity, are consistent with the results obtained by Gallo et al.[8] It seems clear that there is one major conformer for rifamycin S in solution. Although one can explain solvent effects on the basis of specific interactions between the ansa bridge functional groups and solvent molecules in most cases, it would appear that there is a change in the mean conformation of the ansa bridge on going to polar solvents, in particular deuterium oxide. Although the low solubility of rifamycin S in D_2O precluded a detailed analysis of its spectrum in this solvent, there is a marked upfield shift of CH_3-34, from δ 0.15 in acetone-d_6 to δ 0.30 in pure deuterium oxide. Similarly CH_3-33 moves from δ 0.67 to 0.20 on going from acetone-d_6 to deuterium oxide. In both of these cases, the upfield shift is not seen in 1:3 D_2O-acetone-d_6; here the chemical shift of these methyls is essentially the same (±3 Hz) as it is in acetone-d_6 itself. Moreover CH_3-31 and CH_3-32, are essentially solvent independent from acetone-d_6 to deuterium oxide. We propose that the molecule undergoes an inward buckling of the ansa bridge in aqueous solution, this leading to an enhanced ring current effect on the methyl peaks. Methyl-34 is at an anomalously high field in all solvents, as expected from X-ray studies which show this methyl to be sitting over the benzeneoid ring. A striking feature of the rifamycin structure is the positioning of all ansa functionality on one side of the ansa bridge, the same side as the 5-hydroxynaphthoquinone part structure, and the lining of the interior of the ansa bridge exclusively with C-H's. Traditional concepts[22] of hydrophobic interactions would suggest that, conformational changes permitted, the rather substantial hole defined by the ansa bridge should collapse into itself as the medium changes to water. This, we are confident, is the cause of the pronounced upfield shifts of the two methyl groups CH_3-33 and CH_3-34 on changing the solvent to water. Clearly these two methyls, and to a lesser extent CH_3-31, move toward the aromatic π system as one goes to water solvent.

We unfortunately cannot make any quantitative statement as to the degree of conformational change involved in this solvent dependent shift. At water concentrations wherein there is sufficient solubility of rifamycin to measure coupling constants (up to 25% D_2O), one is clearly seeing only a small conformational change by chemical shift changes and coupling constants are unchanged. In pure deuterium oxide, we can measure the chemical shifts of only the methyls. Since we are dealing with the ring current of a naphthoquinone and since the variation of a proton's chemical shift with its position relative to the ring becomes very sensitive

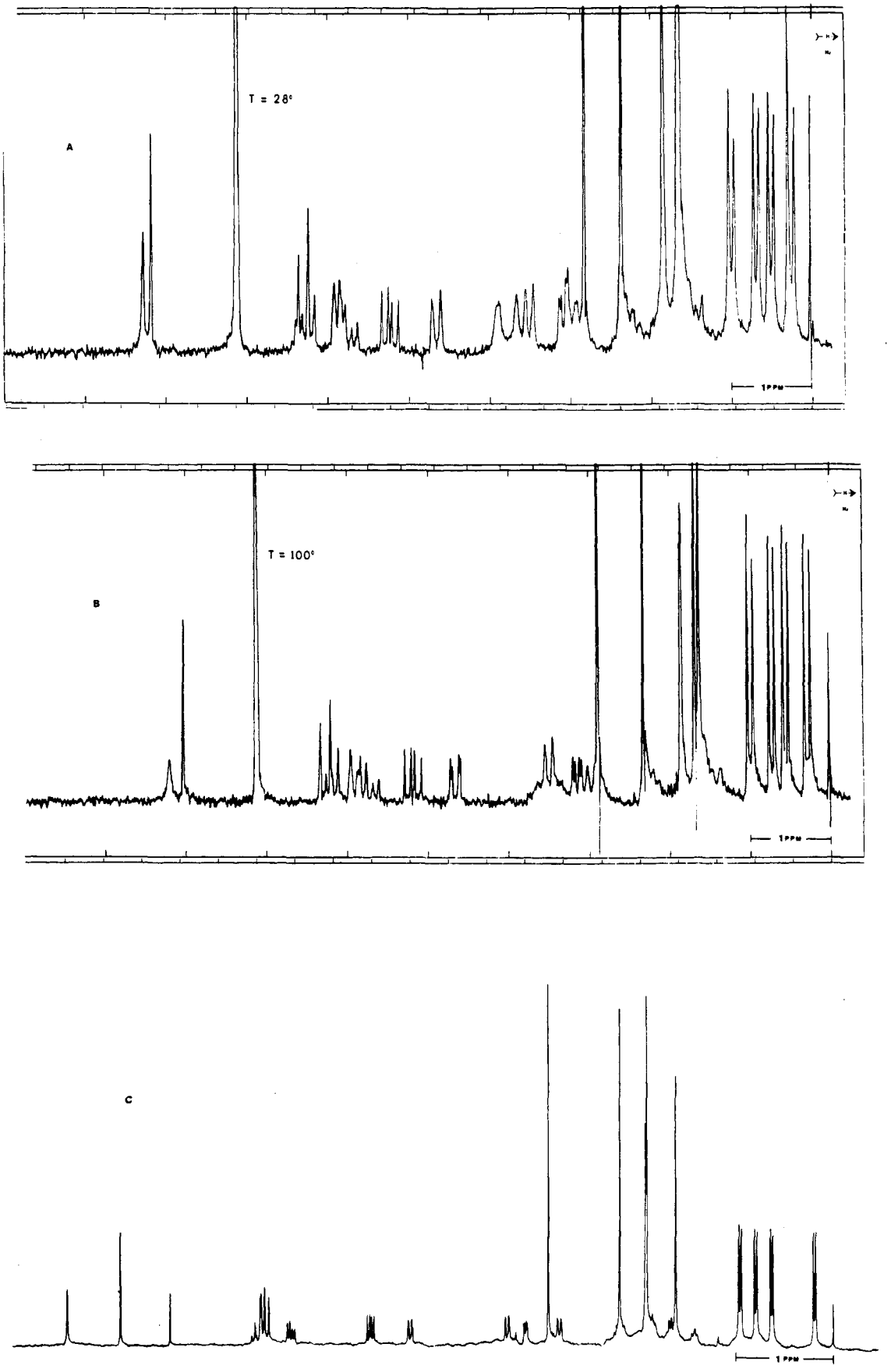

Figure 2. Spectra of rifamycin S in: (A) benzene-d_6 at 28°, 100 MHz; (B) benzene-d_6 at 100°, 100 MHz; (C) chloroform-d at 25°, 270 MHz.

to the position of the proton when it is near the ring,[23] we can only note the trend.

We have examined the NMR spectra of several rifamycin SV (rifamycin S hydroquinone) derivatives and find that CH_3-34 (in chloroform-d) lies in all cases in the range δ −0.3 to −0.45. This is presumably a reflection of the en-

Table I. Rifamycin S Chemical Shifts and Their Solvent Polarity Dependence

Solvent (dielectric constant)	CH_3-34	CH_3-33	CH_3-32	CH_3-31	H-29	H-28	H-27	H-26	H-25	H-24
Benzene-d_6 (2.28)	0.23	0.48	0.99	0.69	6.37	5.27	3.12	1.92[a]	4.70	1.53[a]
$CDCl_3$ (4.80)	0.15	0.67	0.97	0.87	6.25	5.09	3.40	1.93[a]	4.65	1.41[a]
Phenol-d_6 (9.78)	0.20	0.60	0.98	0.76	~6.30	5.32	3.36	–	5.03	–
Acetone-d_6 (20.7)	0.15	0.67	0.97	0.87	6.22	5.22	3.44	~1.90[b]	4.86	~1.56[b]
90% Acetone-d_6–10% D_2O	0.13	0.70	0.99	0.83	6.23	5.25	3.45	–	4.96	–
75% Acetone-d_6–25% D_2O	0.12	0.68	1.00	0.88	6.25	5.28	3.47	–	4.99	–
D_2O (77.9)	–0.30	0.20	0.94	0.72	–	–	–	–	–	–

Solvent	H-23	H-22	H-21	H-20	H-19	H-18	H-17	CH_3-14	CH_3-13	CH_3-30
Benzene-d_6	3.00	1.67[a]	3.52	2.27[a]	5.80	6.35	5.89	2.35	1.66	1.86
$CDCl_3$	3.01	1.68[a]	3.58	2.28[a]	5.92	~6.34	~6.34	2.34	1.73	2.07
Phenol-d_6	3.12	–	3.80	–	~5.95	–	–	2.32	1.76	1.92
Acetone-d_6	3.15	~1.75[b]	3.75	~2.24[b]	5.90	~6.37	~5.99	2.30	1.70	–
90% Acetone-d_6–10% D_2O	3.20	–	3.74	–	–	–	–	2.30	1.74	–
75% Acetone-d_6	3.22	–	3.79	–	–	–	–	2.32	1.74	–
D_2O	–	–	–	–	–	–	–	2.22	1.76	–

Solvent	CH_3-36	CH_3-37	H-3	Phenol proton	Amide proton	C-23–OH	C-21–OH
Benzene-d_6	1.70	2.86	8.23	12.76	8.36	3.92	3.67
$CDCl_3$	2.05	3.13	7.82	12.50	8.40	~3.68	~3.68
Phenol-d_6	1.92	3.02	7.97	–	–	–	–
Acetone-d_6	–	3.09	7.66	12.72	8.75	4.02	3.62
90% Acetone-d_6–10% D_2O	–	3.10	7.66	–	–	–	–
75% Acetone-d_6–25% D_2O	–	3.12	7.68	–	–	–	–
D_2O	–	3.14	–	–	–	–	–

[a] Chemical shifts calculated from Freeman–Anderson equation. [b] Estimated value. Exact shift not calculated.

hanced ring current of a naphthohydroquinone and supports the general idea that, in the case of rifamycin S, one is seeing a ring current effect that is dependent on solvent composition. Lastly, we note that, of the solvent effects detailed in Table I, clearly the most important one is that distinction between aqueous and nonaqueous solvents.

To test the hypothesis that interaction of rifamycins with DNA dependent RNA polymerase involves inclusion complexes, the spectra of the anisyl derivatives **1b**, **1c**, and **1d** were examined. One could predict that a response of these compounds to being placed in aqueous solution could be adoption of a conformation wherein the anisyl group occupies the ansa bridge hole, i.e.:

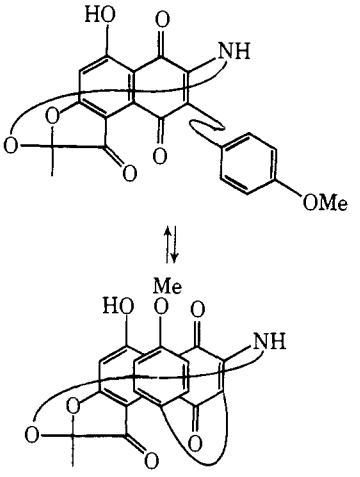

Table II. Observed and Calculated Coupling Constants for Ansa Bridge Protons

Coupled protons	Dihedral[a] angle, deg	Calcd[b] J_{vic}, Hz	Obsd J_{vic}, Hz
28–27	8.3	7.8	8
27–26	65.1	1.4	3
26–25	177.5	10	10
25–24	86.8	0	1.5
24–23	176.3	10	8
23–22	47.1	3.7	1
22–21	118.2	2.2	1
21–20	159.4	9	9
20–19	17.5	7	7

[a] Dihedral angles were calculated from data in ref 3. We thank Professor L. F. Dahl for access to the appropriate program. [b] Calculated using $J = J_0 \cos^2 \theta$; $J_0 = 8$ Hz for $0 \leq \theta \leq 90°$, $J_0 = 10$ Hz for $90 \leq \theta \leq 180°$.

This would constitute a model for interaction with the enzyme wherein a tyrosine residue of the enzyme is inserted into the hole.

In the case of **1b** and **1c**, the signal ascribable to CH_3-34 appears in the region above tetramethylsilane. This high-field shift appears to be characteristic of rifamycin SV (hydroquinone) derivatives and, as commented above, is apparently a reflection of the difference in ring currents of naphthoquinones and naphthoquinols. The insolubility of **1b**, **1c**, and **1d** in deuterium oxide ($<10^{-5}$ *M*) precluded observation of the important resonances (e.g., CH_3-34 and CH_3-33) in heavily aqueous solvents. Inspection of Tables

Table III. Solvent Dependence of Chemical Shifts of Hydroquinone **1b**

Solvent	Ar–H	$ArOCH_3$	CH_3-37	CH_3-14	CH_3-36	CH_3-30	CH_3-13	CH_3-32	CH_3-31	CH_3-33	CH_3-34
$CDCl_3$	6.92	3.77	3.05	2.23	2.07	2.03	1.79	1.01	0.88	0.71	−0.28
CD_3OD	6.89	3.76	3.03	2.22	2.05	2.05	1.78	0.98	0.88	0.73	−0.40
70:30 $CD_3OD:D_2O$	6.95	3.8	3.06	2.15	2.09	2.09	1.80	1.00	0.88	0.68	−0.34
50:50 $CD_3OD:D_2O$	~7.00	3.79	3.03	2.07	2.07	2.07	1.77	–	–	–	–
30:70 $CD_3OD:D_2O$		~3.81[a]	~3.04[a]	~2.08	~2.08	~2.08	~1.78	–	–	–	–

[a] Signals broad.

Table IV. Solvent Dependence of Chemical Shift of Hydroquinone **1c**

Solvent	Ar–H	$ArOCH_3$	CH_3-37	CH_3-14	CH_3-36	CH_3-30	CH_3-13	CH_3-32	CH_3-32	CH_3-33	CH_3-34
$CDCl_3$	6.84	3.72	3.03	2.22	2.06	2.06	1.78	1.03	0.91	0.74	−0.26
50:50 $CD_3COCD_3:D_2O$	6.97	3.80	3.06	2.12	~2.10	~2.10	1.80	1.03	0.93	0.75	−0.30
30:70 $CD_3COCD_3:D_2O$	~7.05[a]	3.81	3.07	~2.10[a]	~2.10[a]	~2.10[a]	1.78	1.01	0.92	0.74	−0.22
CD_3OD	6.88	3.73	3.02	2.21	2.05	2.05	1.77	1.00	0.88	0.71	−0.41
65:35 $CD_3OD:D_2O$	6.97	3.78	3.04	~2.13[a]	~2.08[a]	~2.08[a]	1.77	1.00	0.90	0.72	−0.32

[a] Signal broad.

Table V. Solvent Dependence of Chemical Shifts of Quinone **1d**

Solvent	Ar–H	$ArOCH_3$	CH_3-37	CH_3-14	CH_3-36	CH_3-30	CH_3-13	CH_3-32	CH_3-31	CH_3-33	CH_3-34
$CDCl_3$	6.96	3.73	3.09	2.26	2.06	2.06	1.74	1.00	0.81	0.66	0.28
70:30 $CD_3COCD_3:D_2O$	7.00	3.81	3.12	2.30	2.11	2.06	1.80	1.02	0.83	0.68	0.18
35:65 $CD_3COCD_3:D_2O$	~7.06[a]	~3.83[a]	~3.10[a]	~2.38[a]	~2.10[a]	~2.10[a]	~1.74[a]	–	–	–	–

[a] Signal broad.

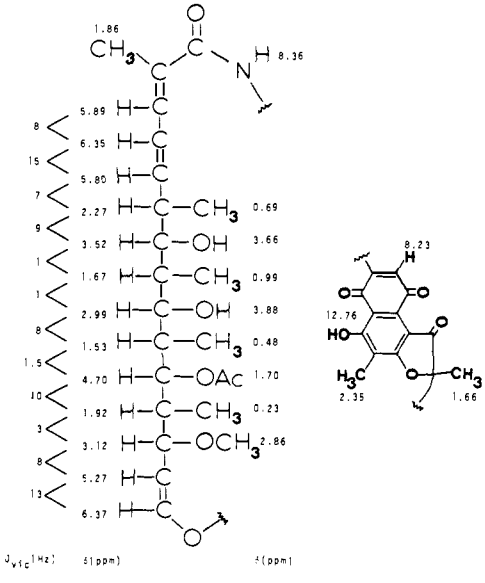

Figure 3. Assignments of the various protons in rifamycin S in benzene-d_6. Chemical shifts and vicinal coupling constants are as shown. This figure is not meant to convey configurational information.

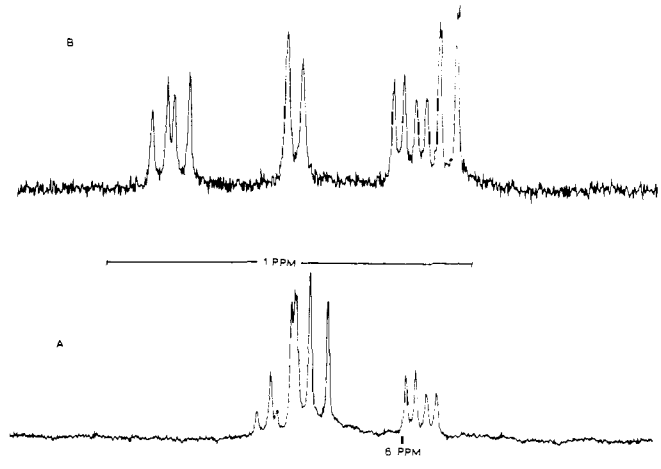

Figure 4. (A) Low-field portion of spectra of rifamycin S and (B) 3-bromorifamycin S in chloroform-*d* at 270 MHz. Vinyl proton H-28 is not shown.

III, IV, and V shows that the two naphthoquinols **1b** and **1c** respond in a very similar manner to solvent change and that they are both similar in their changes to the naphthoquinone **1d.** In all three cases, there is no marked change in chemical shifts in the most aqueous solvent attainable. Quinol **1b** and quinone **1d** which obey the rule of three[24] are very similar to **1c** which does not. Since in the solvents used we did not see the above upfield shift of the ansa methyls, which would seem to be a good measure of the "hydrophilicity" of solvent, we conclude that either the known sensitivity of hydrophobic complexes to solvent surface tension[25] prevents formation of the proposed inclusion complexes in other than pure water or that the hypothesis is deficient.[26]

References and Notes

(1) W. Wehrli, F. Knosel, K. Schmid, and M. Staehlin, *Proc. Nat. Acad. Sci. U.S.A.*, **61**, 667 (1968); W. Wehrli and M. Staehlin, *Bacteriol. Rev.*, **34**, 290 (1971).
(2) F. M. Thompson, A. N. Tischler, J. Adams, and M. Calvin, *Proc. Nat. Acad. Sci. U.S.A.*, **71**, 107 (1974).
(3) M. Bufani, W. Fedeli, G. Giacomello, and A. Vaciago, *Experientia*, **20**, 339 (1964).
(4) J. Leitich, V. Prelog, and P. Sensi, *Experientia*, **23**, 505 (1967).

(5) K. L. Rinehart, Jr., *Acc. Chem. Res.*, **5**, 57 (1972).
(6) W. Wehrli and M. Staehlin, *Biochim. Biophys. Acta*, **182**, 24 (1969).
(7) M. Dampier, unreported results.
(8) G. G. Gallo, E. Martinelli, V. Pagani, and P. Sensi, *Tetrahedron*, **30**, 3093 (1974).
(9) V. Prelog and W. Oppolzer, *Helv. Chim. Acta*, **56**, 2279 (1973); W. Oppolzer and V. Prelog, *ibid.*, **56**, 2287 (1973).
(10) P. Sensi, M. T. Timbal, and G. Maffi, *Experientia*, **16**, 412 (1960).
(11) W. A. Anderson and R. Freeman, *J. Chem. Phys.*, **37**, 85 (1962).
(12) This correction must be applied when the condition $\omega_A - \omega_X \gg 2\pi J_{AX}$ is not satisfied.
(13) For a discussion of the use of the Freeman–Anderson correction, see W. von Phillipsborn, *Angew. Chem., Int. Ed. Engl.*, **10**, 476 (1971).
(14) In acetone-d_6 and acetone-d_6–deuterium oxide mixtures, protons such as H-21 are not defined clearly enough that an accurate measurement of ω_2 for maximum decoupling can be made. Therefore, as noted in Table I, calculated chemical shifts of these hidden protons are approximate in these solvents.
(15) S. Forsén and R. A. Hoffman, *J. Chem. Phys.*, **39**, 2893 (1963).
(16) E. Martinelli, R. J. White, G. G. Gallo, and P. J. Beynon, *Tetrahedron Lett.*, 1367 (1974).
(17) M. L. Casey, unreported results.
(18) In $CDCl_3$, the solvent in which these protons show greatest separation, the shifts in question are 2.34, 2.05, and 1.73 ppm, respectively. A high-field methyl shift is characteristic of methyl groups on ketal-type carbons; thus 1.73 ppm is assigned to CH_3-13. The methyl group on the aromatic ring, CH_3-14, would be expected to come ~2.10–2.40 ppm. A comparison of the following methyl shifts was considered in making this assignment: 2-methylnaphthalene at 2.49 ppm, toluene at 2.34 ppm, 2,6-dimethoxytoluene at 2.10 ppm, and methylphloroglucinol at 2.12 ppm (in D_2O). Acetate methyls usually have chemical shifts between 1.80 and 2.00 ppm. The methyl group assignments were confirmed by single-frequency decoupling of the ^{13}C spectrum. These experiments established that the methyl protons at 2.34, 2.05, and 1.73 ppm were coupled to carbons 14, 36, and 13, respectively.
(19) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963); S. Sternhell, *Q. Rev., Chem. Soc.*, **23**, 236 (1969).
(20) For attempts to calculate substituents effects on coupling constants, see N. Sheppard and P. M. Lynden-Bell, *Proc. Roy. Soc. London, Ser. A*, **269**, 385 (1962); M. L. Huggins, *J. Am. Chem. Soc.*, **75**, 4123 (1953); R. J. Abraham and G. Gatti, *J. Chem. Soc. B*, 961 (1969).
(21) In acetone-d_6–D_2O mixtures, chemical shifts for the four hidden protons could not be accurately measured. (See ref 14.) In D_2O solution, spectra were extremely weak, because of the low solubility of rifamycin S (~10^{-4} *M*) in this solvent. Even using 12-mm NMR tubes and FT NMR (~2000 transients), we were only able to determine chemical shifts for methyl protons in deuterium oxide solution.
(22) W. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969, pp 393–417.
(23) J. S. Waugh and R. W. Fessenden, *J. Am. Chem. Soc.*, **80**, 6697 (1958); C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
(24) J. H. Craig, P. C. Huang, G. Scott, and N. J. Leonard, *J. Am. Chem. Soc.*, **94**, 5872 (1972).
(25) K. A. Connors and S. Sun, *J. Am. Chem. Soc.*, **93**, 7239 (1971).
(26) Partial support of this work by NIH is acknowledged.

The Crystal and Molecular Structure of the Unusual Spiro Dihydrofuran Diterpene Nepetaefolin[1]

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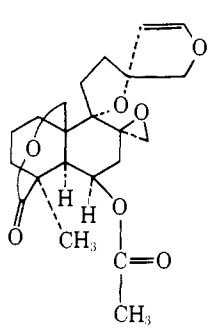
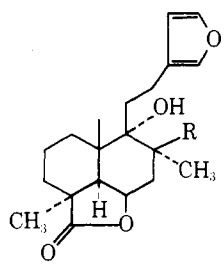
Abstract: The crystal and molecular structure of the spiro dihydrofuran diterpene nepetaefolin (1) has been determined. The structure was solved by direct methods analysis of data from a crystal with space group $P2_12_12_1$ and $a = 11.092 \pm 0.002$ Å, $b = 11.484 \pm 0.002$ Å, $c = 15.379 \pm 0.003$ Å, $Z = 4$ and density $\rho_{calcd} = 1.371$ g/cm^3. An anisotropic large-block least-squares refinement converged to a conventional residual of $R = 0.042$ for 2015 reflections recorded with Cu Kα radiation on an automatic four-circle diffractometer. The effects of the considerable steric interactions in this molecule on the bond lengths are discussed.

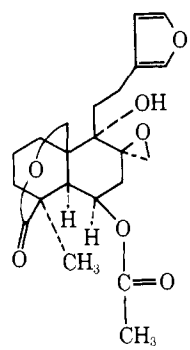
Many species of the wide-spread family Labiatae[2] have been employed in primitive medical treatment of cancer.[2,3] In previous attempts to locate discrete antineoplastic agents produced by Labiatae species, a sample of *Leonotis nepetaefolia* (L.) R. Br. collected in India[4] was investigated, and another sample of this plant, collected in Puerto Rico, was also evaluated.[5] In both cases, ethanol (50%) extracts showed activity in the National Cancer Institute's Walker carcinosarcoma 256 (intramuscular, WM) screening system.[6]



1



2, R = OH
3, R = H



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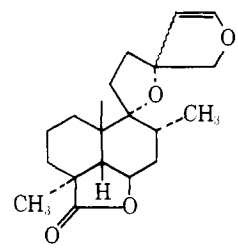

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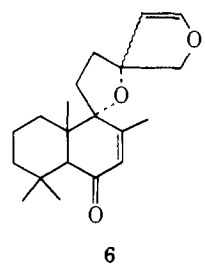

6

One component of *Leonotis nepetaefolia* is the spiro dihydrofuran nepetaefolin. After a very thorough chemical study of nepetaefolin (from *Leonotis nepetaefolia* collected in Trinidad), a structure was proposed by two of us.[7] However, this study did not establish the stereochemistry at the spiro dihydrofuran carbon. Although we have not yet been able to obtain enough nepetaefolin to properly assess its antineoplastic activity, we have undertaken a detailed crys-