Communications to the editor

THE CHEMISTRY OF THE
RUBRADIRINS. I
THE STRUCTURES OF
RUBRANSAROLS A AND B

Sir:

Rubradirin,[1,2,3] $C_{48}H_{46}N_4O_{20}$,* and rubradirin B (to be published), $C_{40}H_{33}N_3O_{15}$, are the most active components of a family of related antibiotics. In this paper we discuss the structures of rubransarols A and B, the ansamycin moieties of the two rubradirins.

The rubradirins were isolated as non-crystalline powders which were acids with pKa's of 6.0 (1 group) and 8.4 (2~3 groups). The colors of their solutions change from red to green around pH 7 to 8. Their ultraviolet spectra have identical maxima, the λ_{nm} (ε) values[†] in 0.01 N ethanolic HCl being 510 (1,711), 312 (24,950), 270 (19,960) and 222 (47,904). The ^{13}C NMR spectra showed that 40 of the 48 carbons of rubradirin were similar to those in rubradirin B. Thus the two antibiotics appear to differ by an additional $C_8H_{13}NO_5$ moiety in rubradirin. Rubradirin was reduced rapidly by a platinum-catalyzed hydrogenation, with decolorization and also the disappearance in the ^{13}C NMR spectra of signals at δ 176 and δ 178. Rubradirin was regenerated upon air oxidation. When considered with the ultraviolet data, this suggested a quinonoid system.
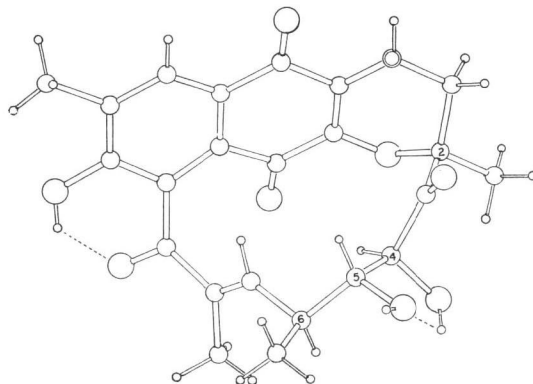
Under various basic conditions, each rubradirin was cleaved into two fragments. In each case, a compound with the red-green chromophore, sharing the molecular formula of $C_{23}H_{23}NO_8$, and a pKa' of 7.8, was isolated. These moieties, noncrystalline rubransarol A (**1**) from rubradirin, and crystalline rubransarol B (**2**) from rubradirin B, differed slightly in their ^1H NMR spectra and their TLC behavior. The second fragment isolated from these hydrolyses accounts in each case for the remainder of the

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* New physical and analytical data now support this formula. The other molecular formulas presented herein were supported by elemental analyses and mass spectrometric determinations.

† The extinction coefficients shown are for rubradirin, but the values found for rubradirin B were the same within experimental error.

antibiotic. That from rubradirin, crystallized as the methylamide, was $C_{26}H_{28}N_4O_{12}$, while that obtained from rubradirin B as the primary amide was $C_{17}H_{13}N_3O_7$. The nature of these molecules will be discussed in a future communication.

Crystal data for rubransarol B were as follows: orthorhombic; space group $P_{2_12_12}$, with 8 molecules of rubransarol B, $C_{23}H_{23}NO_8$, and 6 molecules of chloroform-d in the unit cell; $a=$ 22.25 (1), $b=28.77$ (2), $c=7.477$ (7); 4690 reflections. Intensity data were measured at low temperature ($-155°C$), on a Syntex PĪ diffractometer controlled by an IBM 1800 computer using graphite monochromatized CuK$_\alpha$ radiation ($\lambda=1.5418$ Å). The data were corrected for systematic errors, including absorption.[4] The asymmetric unit of the cell contains 2 molecules of rubransarol B and 1.5 chloroform molecules; one of the chloroforms is located on a 2-fold axis. Coordinates and anisotropic thermal parameters of heavier atoms were refined by multiple matrix least squares; hydrogen atoms were located in difference maps but not refined. The absolute configuration was determined by anomolous dispersion measurements on the 19 reflections most affected by a change in enantiomer, using the method of BIJVOET.[5] All 19 reflections indicated the enantiomer reported.

The final agreement index R, $(R=\sum\left||F_o|-|F_c|\right|\bigg/\sum|F_o|)$, was 0.116. For the 2,463 reflec-

Fig. 1. The absolute configuration of rubransarol B is shown to be 2S, 4S, 5R, 6S.

Table 1. NMR Spectra of rubransarols A and B in CDCl$_3$

| Position | ^{13}C NMR, [a]Chem. shift, ppm | | ^{1}H NMR, [b]Chem. shift, ppm: coupling constant, Hz | |
| | Rubransarol | | Rubransarol | |
	A	B	A	B
NH	—	—	D, 1H, 5.55, J$_1$=4	D, 1H, 5.56, J$_1$=4
1	T, 46.5	T, 47.2	D, 1H, 3.25, J$_1$=−13	D, 1H, 3.24, J$_1$=−13
			DD, 1H, 4.16, J$_1$=−13, J$_{NH}$=4	DD, 1H, 4.16, J$_1$=−13, J$_{NH}$=4
2	S, 81	S, 82		
2$_\alpha$	Q, 11.7	Q, 13.3	S, 3H, 1.72	S, 3H, 1.73
3	211	212		
4	D, 64.8	D, 65.2	S, 1H, 4.59, J$_5$=0, (J$_{OH}$= 10.0 in DMSO)	S, 1H, 4.33, J$_5$=0, (also by OH in DMSO)
5	D, 71.8	D, 74	D, 1H, 3.52, J$_6$=2, J$_4$=0 (broad singlet in DMSO)	D, 3.22, J$_6$=5, J$_4$=0
6	D, 42	D, 35.5	M, 1H, 2.68	M, 1H, 2.73
6$_\alpha$	Q, 21	Q, 22.4	D, 3H, 1.34, J$_6$=7	D, 3H, 0.91, J$_6$=7
7	D, 134.8	D, 136	DD, 1H, 5.31, J$_6$=4, J$_{8\alpha}$=<1	DD, 1H, 4.99, J$_6$=11, J$_{8\alpha}$<1
8	S, 118.5	S, 119		
8$_\alpha$	Q, 13	Q, 15.8	D, 3H, 2.2, J$_7$=<1	D, 3H, 2.03, J$_7$<1
9	S, 202	S, 201.1		
10	S, 120	S, 121.5		
11	S, 160.5	S, 161.3		
12	S, 130	S, 130.6		
12$_\alpha$	Q, 15.4	Q, 16.2	S(D), 3H, 2.28, J$_{13}$<1	S(D), 3H, 2.31, J$_{13}$<1
13	D, 130.8	D, 131.2	S(D), 1H, 7.77, J$_{12\alpha}$<1	S, 1H, 7.79, J$_{12\alpha}$<1
14	S, 136	S, 137.1		
15	S, 131.5	S, 132.0		
16	S, 177	S, 177.7		
17	S, 136	S, 141		
18	S, 143.3	S, 144		
19	S, 178	S, 178.7		

[a] Multiplicity in off-resonance spectrum or [b] multiplicity;
S=singlet, D=doublet, T=triplet, Q=quartet, DD=doublet of doublets, M=multiplet.

tions with [F$_0$>^{2}3σ(F$_0$^{2})], R was 0.080. The standard deviation of fit was 1.70. Fig. 1 was drawn from final coordinates, and shows the O(11)-H—O(9) and O(4)-H—O(5) intramolecular hydrogen bonds. There also are several inter-molecular hydrogen bonds. All calculations were carried out on an IBM 370 computer using the CRYM system of crystallographic programs.*

The assignment of chemical shifts was made for both the ^{13}C NMR and the ^{1}H NMR spectra (Table 1) of 1 and 2. A close similarity of

* The CRYM crystallographic programs were developed by D. J. DUCHAMP, The Upjohn Company, Kalamazoo, Michigan.

rubransarol A to rubransarol B was clear from the fact that the chemical shifts in the former corresponded to those of the latter with only two outstanding exceptions. The first occurs in the ^{1}H NMR spectra where the coupling between positions 6 and 7 in rubransarol B is 11 Hz while the coupling between the corresponding signals for rubransarol A is 4 Hz accompanied by an upfield shift of δ 0.32. Secondly, a doublet at δ 35.5 in the ^{13}C NMR spectrum assigned to carbon 6 in 4 appears at δ 42 in rubransarol A. One possible explanation, atropisomerism which is known to occur in other ansamycins,[6] appeared unlikely in view of the

nearly identical specific rotations for **1** and **2** ($[\alpha]_D$ +103° and +108°, resp., [*c* 0.02, acetone]). Short relaxation times in the ^{13}C NMR spectra for all signals assigned to ansa ring carbons in **3** precluded the possibility of an open ring form. *Cis-trans* isomerism at the double bond of the ansa "handle" with the *cis* arrangement assigned to rubransarol A is, therefore, the most likely source of the variations in the NMR spectra.

The function hydrolyzed in the rubradirins is an ester, as shown by the generation of a new hydroxyl in the rubransarols and a new carboxylic acid or carboxamide in other fragments. The site in the rubransarols is one of the two ansa ring hydroxyls, the phenolic hydroxyl being present in the intact antibiotics as shown by titration and pH-dependent color changes which extend to the rubransarols. The ^{1}H NMR shifts of the protons geminal to these hydroxyls implicate carbon 5. For the C–5 protons the chemical shifts are 4.98 ppm for rubradirin and 3.32 ppm for rubransarol A, while those for rubradirin B and rubransarol B are 4.85 and 3.22 ppm respectively. The chemical shifts for the C–4 protons of rubradirin and rubransarol A are 4.90 and 4.59 ppm, respectively, and those of rubradirin B and rubransarol B are 4.43 and 4.33 ppm. Thus **3** and **4** represent the partial structures of rubradirin and rubradirin B.

1 R = H, Rubransarol A
3 R = –CO–$C_{24}H_{24}N_3O_{12}$, Rubradirin

2 R = H, Rubransarol B
4 R = –CO–$C_{16}H_{11}N_2O_7$, Rubradirin B

Summary

The antibiotic rubradirin, $C_{48}H_{46}N_4O_{20}$ was cleaved at an ester function by aqueous methylamine into rubransarol A, $C_{23}H_{23}NO_8$, and a methyl amide, $C_{26}H_{28}N_4O_{12}$. Rubradirin B, $C_{40}H_{33}N_3O_{15}$, was similarly cleaved in methanolic ammonia into rubransarol B, $C_{23}H_{23}NO_8$, and the primary amide, $C_{17}H_{13}N_3O_7$. The rubransarols are shown to be unique ansamycins which are isomeric at a double bond in the large ring.

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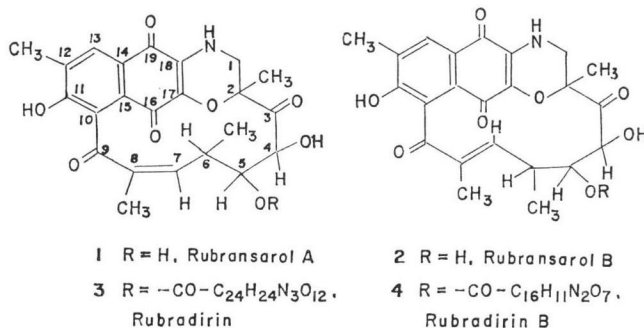
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(Received June 27, 1978)

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