Structure of Rubradirin

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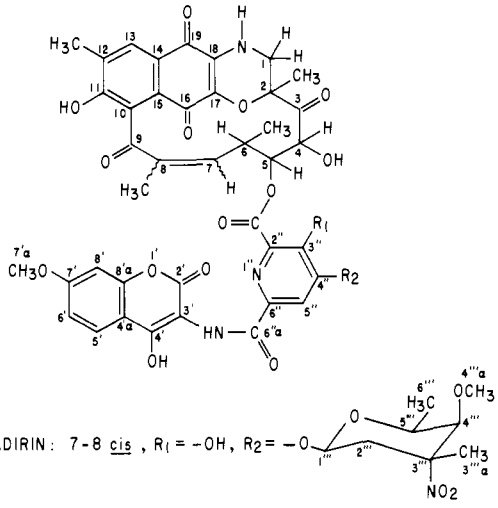
Abstract: The antibiotic rubradirin is shown to consist of a central moiety, 3,4-dihydroxydipicolinic acid, of which the 2-carboxyl group is esterified by a large ansamycin-like moiety while the 6-carboxyl forms an amide with 3-amino-4-hydroxy-7-methoxycoumarin, a compound of the type found in the novobiocins. Position 4 is glycosylated with a nitro sugar, rubranitrose, which is epimeric with evernitrose, found in a third class of antibiotics, the everninomicins. Rubradirins B and C are members of the rubradirin complex which lack rubranitrose and also have slight modifications elsewhere.

Introduction

Such diverse antibiotics as achromoviromycin,[1] sarcidin,[2] streptozotocin,[3] the tomaymycins,[4] and the rubradirins[5] are secondary metabolites of various strains of *Streptomyces achromogenes*. Preliminary reports[6] have indicated that the structures of rubradirin and rubradirin B are **1** and **2a**, respectively.[7] We



1 RUBRADIRIN: 7-8 cis, $R_1 = -OH$, $R_2 = -O$-(rubranitrose)

2a RUBRADIRIN B: 7-8 trans, $R_1 = -OH$, $R_2 = -H$
ABS. CONFIGURATION: 2S, 4S, 5R, 6S

2b RUBRADIRIN C: 7-8 trans, $R_1 = -H$, $R_2 = -H$

now wish to elaborate the details of these determinations, and also disclose the isolation and structure of rubradirin C, **2b**, a third component of the complex.

Rubradirin is the most important member of this complex in terms of the relative quantities produced in the fermentation as well as specific antibiotic activities. It is highly active against a variety of Gram-positive bacteria and *Haemophilus influenza*, and further investigations into its biological properties are continuing.[8] The antibacterial activity of rubradirin B is 25% or less than that of rubradirin, and rubradirin C shows considerably less activity.

Preliminary Considerations

The analytical quantities used in the original characterization by Meyer[9] were accumulated from 200 transfer countercurrent distributions. For the current studies rubradirin was purified chromatographically over silica gel buffered at pH 5.8, developed with chloroform–methanol mixtures. This system also afforded quantities of the other two components. Molecular formulas were established by elemental analyses and field desorption mass spectra: $C_{48}H_{46}N_4O_{20}$ for rubradirin, $C_{40}H_{33}N_3O_{15}$ for rubradirin B, and $C_{40}H_{33}N_3O_{14}$ for rubradirin C. Crystals with suitable characteristics for a crystallographic structure analysis were not found in any of our samples. The ultraviolet and visible spectra indicated that the chromophoric systems were essentially the same for the three antibiotics. The most intense maximum, appearing at 323 nm in ethanolic acid, shifts to 303 nm in base. The acidic solutions have a magenta hue with a maximum at 508 nm, changing to a green color in base with a maximum at 588 nm. These color shifts are unique among all the known antibiotics. Evidence for independent systems of chomophores was drawn from the presence of several shoulders on the curves.

Nine absorptions are found between 1727 and 1504 cm^{-1} of the infrared spectra of rubradirin. Eight of these appear in the spectra of rubradirins B and C, that at 1504 cm^{-1} being absent. The latter also show an additional absorption near 1680 cm^{-1}. The ^1H and ^{13}C NMR spectra permitted only some general observations. The three antibiotics had four methyls, one methoxyl, and one methylene in common. Singlets below 140 ppm suggested that there were 14 carbonyl or hydroxylated vinyl/aromatic carbons in rubradirin, 13 in rubradirin B, and 12 in rubradirin C. Upfield, from 140 to 100 ppm, were 16 signals in the spectra of each antibiotic, indicating vinyl/aromatic carbons. Four of the eight additional carbons in rubradirin were accounted for by two methyls, one methoxy, and one methylene group.

There was evidence for a quinonoid or related structure. The red color of acidic solutions of rubradirin was rapidly discharged by catalytic reduction or by carbonyl reducing agents, and rapidly restored by air oxidation after removal of the reducing conditions. After uptake of 1 mol of hydrogen under platinum catalysis in deuteriochloroform, ^{13}C NMR spectra showed a loss of two singlets at 176 and 178 ppm, along with coalescence of absorptions at 14 and 16 ppm, and slight shifts of absorptions in the aromatic region and those at 202 and 207 ppm. Air oxidation restored the original spectrum of rubradirin, and the isolated product displayed the antibacterial activity and TLC behavior expected for rubradirin.

Degradation Reactions

Various solvolysis procedures selectively cleaved rubradirin at the three obvious sites, affording the individual moieties as well as various combinations of them. Mild acid, such as 70% acetic acid fortified to pH 2 with HCl, hydrolyzed the glycoside, yielding

(1) H. Umezawa, T. Takeuchi, Y. Okami, K. Oikama, and T. Tazaki, *J. Antibiot. Sec. A* **6**, 38 (1953).
(2) T. Takeuchi, K. Nitta, and H. Umezawa, *J. Antibiot., Ser. A* **6**, 31 (1953).
(3) J. J. Vavra. C. DeBoer, A. Dietz, L. J. Hanka, and W. T. Sokolski, *Antibiot. Annu.*, 230 (1959/60).
(4) K. Arima, M. Kohsaka, G. Tamura, H. Imanaka, and H. Sakai, *J. Antibiot.*, **25**, 437 (1972).
(5) B. K. Bhuyan, S. P. Owen, and A. Dietz. *Antimicrob. Agents Chemother. 1964*, 91 (1965); H. Hoeksema, C. Lewis, S. A. Mizsak, J. A. Shiley, D. R. Wait, H. A. Whaley, and G. E. Zurenko, *J. Antibiot.*, **31**, 945 (1978).
(6) H. Hoeksema, C. Chidester, S. A. Mizsak, and L. Baczynskyj, *J. Antibiot.*, **31**, 1067 (1978); S. A. Mizsak, H. Hoeksema, and L. M. Pschigoda, *ibid.* **32**, 771 (1979); H. Hoeksema, S. A. Mizsak, and L. Baczynskyj, *ibid.*, **32**, 773 (1979).
(7) The numbering of the positions in **1** is inconsistent among the various moieties, but reflect those systems in vogue for the various classes of compounds present.
(8) F. Reusser, *Biochemistry*, **12**, 1136, (1973).
(9) C. E. Meyer, *Antimicrob. Agents Chemother.*, **1964**, *97* (1965).

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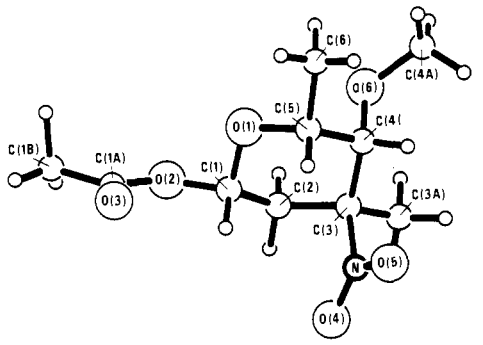
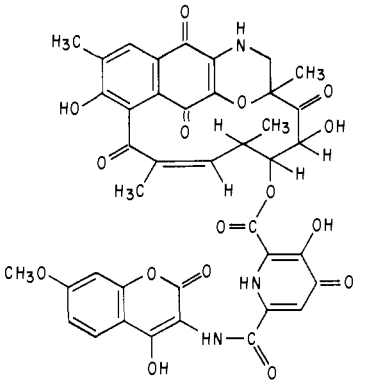
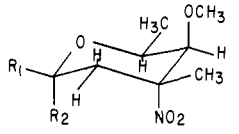


Figure 1. Computer drawing of 1-acetylrubranitrose.

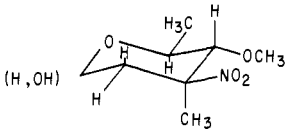
rubradirin aglycon (**3**) and a mixture of rubranitrose anomers (**4a** and **4b**).



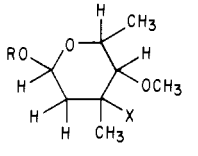
3 RUBRADIRIN AGLYCONE



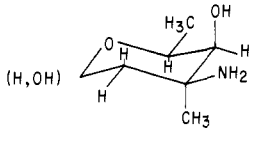
	R_1	R_2
4a	OH	H
4b	H	OH
4c	OAc	H
4d	H	OAc



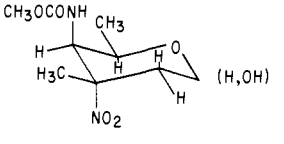
4f



4e R = H or OAc

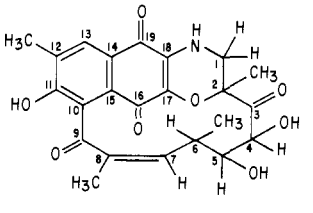


4g

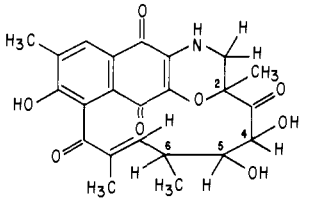


4h

An efficient hydrolysis of the ester function was accomplished in aqueous base at pH 10, affording the ansa moiety, rubransarol A (**5**) from rubradirin, and rubransarol B (**6**) from rubradirins

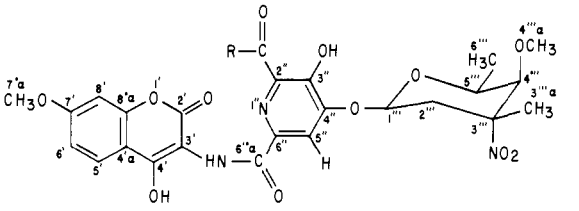


5 RUBRANSAROL A



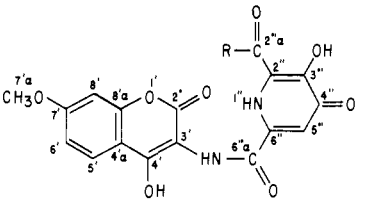
6 RUBRANSAROL B
ABS. CONF. 2S,4S,5R,6S

B and C. Solubility characteristics hindered purification of the acid portions of the esters, rubradiric acids A, B, and C, as the free acids, but amides, obtained by aminolysis of the respective antibiotics, were readily recrystallized. *N*-Methyl- and *N*-ethylrubradiramide *A* (**7a** and **7b**), *N*-ethylrubradiramide B (**9**)



7a R = CH_3NH
7b R = CH_3CH_2NH
7c R = OH

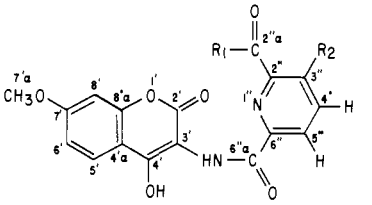
N-ALKYLRUBRADIRAMIDES A AND RUBRADIRIC ACID A



8a R = NH_2
8b R = $NHCH_3$

N-ALKYLRUBRADIRAMIDE A AGLYCONE

N-methylrubradiramide C (**10**), rubradiramide A aglycon, (**8a**),

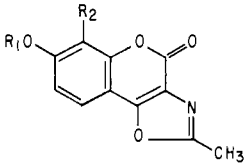


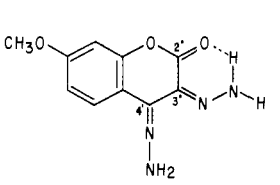
9 R_1 = NHEt ; R_2 = OH
10 R_1 = NHMe ; R_2 = H

N-ALKYLRUBRADIRAMIDE B (9) AND C (10)

and *N*-methylrubradiramide A aglycon (**8b**), were so obtained from their respective antibiotics.

The rupture of the amide function of rubradirin required more vigorous conditions. The combined information from the ultraviolet spectra and the ^1H and ^{13}C NMR spectra of rubradiric acid A (**7c**) suggested to us similarities to the coumarin found in novobiocin. We therefore successfully employed an acetic anhydride transacylation procedure which had been used with novobiocin.[10] The isolated product was 7-methoxy-2-methyl-4*H*-[1]benzopyrano[3,4-*d*]oxazo-4-one (**11a**) along with traces





11a R_1 = methyl, R_2 = H
11b R_1 = acetyl; R_2 = methyl
11c R_1 = noviosyl; R_2 = methyl

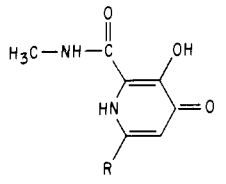
14

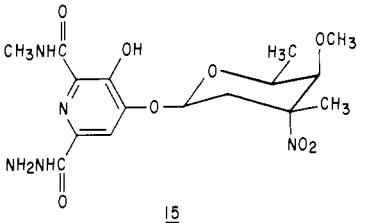
(10) J. W. Hinman, E. L. Caron, and H. Hoeksema, *J. Am. Chem. Soc.*, **79**, 3789 (1957).

Table I. ^{1}H NMR Spectra of Rubranitrose Compounds

C no.	no. of protons	shifts (ppm) and couplings (Hz) 4b	4c	4d
1	1	5.28 dd, J_{2ax} 2.5 J_{2eq} 3.5	5.73 dd, J_{2ax} 9.8 J_{2eq} 2.4	6.1 dd, J_{2ax} ~3.5 J_{2eq} ~2
1a	3		2.09 s	1.94 s
2 ax	1	2.05 dd, J_{2eq} 14.5 J_1 2.5	1.97 dd, J_{2eq} 14.5 J_1 9.8	2.14 dd, J_{2eq} 14.5 J_1 ~3.5
2 eq	1	2.67 dd, J_{2ax} 14.5 J_1 3.5	2.52 ddd, J_{2ax} 14.5 J_1 2.4 J_4 1.5	2.73 dd. J_{2ax} 14.5 J_1 ~2 J_4 ?
3a	3	1.67 s	1.69 s	1.64 s
4	1	3.71 br s, J_5 <1	3.63 m	3.74 br s, J_5 <1
4a	3	3.62 s	3.65 s	3.60 s
5	1	4.40 br q, J_6 6.5 J_4 <1	3.63 m	4.24 br q, J_6 6.5 J_4 <1
6	3	1.33 d, J_5 6.5	1.33 d, J_5 6.5	1.33 d, J_5 6.5

of the *N*- and *O*-acetates of the 3-amino-4-hydroxy-7-methoxycoumarin. While these vigorous reaction conditions usually degraded the remainder of the treated molecules, small amounts of 6-carboxy-3,4-dihydroxy-2-(methyaminocarbonyl)pyridine (**12**)





15

12 R = $-CO_2H$

13 R = $-H$

16 R = $-CO-NH-NH_2$

and its decarboxylation product (**13**) were obtained from this treatment of *N*-methylrubradiramide A aglycon.

Hydrazinolysis of the coumarin amide bond was found to occur slowly in neat solutions of *N*-methylrubradiramide A in hydrazine hydrate at ambient temperatures. This reaction does not occur under those conditions with novobiocin.[11] Most of the coumarin fragment was recovered as the 3,4-osazone **14**, as might be anticipated from the work of Hassner and Catsoulacos in a steroid series.[12] From the acidic side of the amide **7a**, the expected hydrazide, **15**, was obtained, and this was further hydrolyzed in acid into rubranitrose and the hydrazide **16** of *N*-methylrubradiramide A aglycon.

Rubranitrose

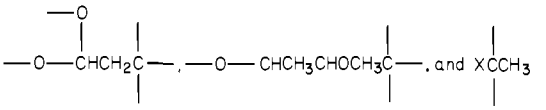
The two anomers of the sugar moiety were distinguished by signals in the ^{1}H NMR spectra at 4.75 (**4a**) and 5.28 ppm (**4b**). Only **4b** was isolated from the anomeric mixture in a pure crystalline form. Acetylation of the mixture afforded anomeric 1-acetylrubranitroses (**4c** and **4d**) from which only the former was obtained as a pure crystalline entity. In **4c** a 9.8-Hz coupling between signals at 5.73 and 1.97 ppm showed that this anomer was epimeric at C-1 with the crystalline free sugar anomer **4b** where the corresponding coupling was 2.5 Hz.

The structure of 1-acetylrubranitrose (**4c**) was determined by a single-crystal X-ray diffraction study for which details are found in the Experimental Section. The computer-generated drawing found in Figure 1 shows the numbering, conformation, and relative configuration for this compound.

The L-configuration was determined by directly comparing the circular dichroism of **4c** with that of evernitrose (**4f**), which differs in relative configuration at two chiral centers C-3 and C-4.[13,14] Evernitrose occurs in everninomicins B and C[15] and was the first nitro sugar found to be elaborated by a microorganism. A crystallographic analysis of the derivative methyl 3-acetamido-2,3,6-trideoxy-3-*C*, 4-O-dimethyl-L-*xylo*-hexopyranoside by Ganguly et al.[13] showed that it was in the L series. Our studies showed that **4c** and **4f** had oppositely signed Cotton curves in the vicinity of the 280-nm nitro group electronic transition,[16] +2500 for **4c** and −1200 for **4f** at 285 nm. Assuming that the chirality at C-3‴ should determine the sign at this wavelength, **4c** and **4f** have opposite configurations at this position. Because the relative configurations for the two compounds differ at C-3‴ but are the same at C-5‴, **4c** appears to belong in the L-series with evernitrose.

This assignment has recently been brought into question by Mallams et al.[17] who assigned a D configuration to a related third nitro sugar, kijanose (**4h**), found in the new antibiotic kijanimicin. This assignment was made by the application of Hudson's rules. The CD curve for kijanose was very similar to that for rubranitrose. An absolute configuration based on an X-ray structure determination with a heavy atom containing compound of rubranitrose would be desirable, but our current limited supply of rubranitrose precludes such a study.

The other physical and analytical data were in full accord with the X-ray findings. These data were in fact used to arrive at the identical structure independently. Elemental analyses, FD mass spectra, and NMR data were satisfied by molecular formulas of $C_8H_{15}NO_5$ for **4b** and $C_{10}H_{17}NO_6$ for **4c**. An analysis of the proton spectra (Table I) of these compounds, using spin decouling, accounted for all of the carbons and hydrogens in three overlapping segments:



These fragments were then combined into a pyranose ring, **4e**. The magnitude of the coupling constants, particularly the 9.8-Hz coupling of the C-1, C-2 protons in **4c**, required the six-membered ring rather than the alternative furanose. The remaining unassigned atoms, one nitrogen and two oxygens, were indicated as a nitro group (X) since only one unused carbon valence was available. Infrared bands at 1550 and 1310 cm^{-1} supported this assumption, as did evoluation of NO_2 from sugar-containing compounds undergoing reactions at elevated temperatures, e.g., transacylation in refluxing pyridine. We failed, however, to observe an (M − NO_2) fragment in our mass spectra as had been reported with evernitrose.[15]

A comparative analysis of the ^{1}H NMR spectra of **4c** and **4d**, using decoupling where appropriate, seemed to favor the indicated

(11) L. A. Dolak, personal communication.
(12) A. Hassner and P. Catsoulacos, *Tetrahedron Lett.* 489 (1967).
(13) A. K. Ganguly, O. Z. Sarre, and H. Reimann, *J. Am. Chem. Soc.*, **90**, 7129 (1968).
(14) A. K. Ganguly, O. Z. Sarre, A. T. McPhail, and K. D. Onan, *J. Chem. Soc., Chem. Commun.*, 313 (1977).
(15) We are grateful to Dr. A. K. Ganguly for the sample of evernitrose used in this study.
(16) D. Crabbe, "ORD and CD in Chemistry and Biochemistry", Academic Press, New York and London, 1972, pp 73, 79, 80.
(17) A. K. Mallams, M. S. Puar, and R. R. Rossman, *J. Am. Chem. Soc.* **103**, 3938 (1981).

Table II. NMR Spectra of Rubransarols A and B in $CDCl_3$

	^{13}C NMR[a] chemical shifts (ppm)		^{1}H NMR[b] chemical shifts (ppm) and coupling constants (Hz)	
	rubransarol		rubransarol	
position	A	B	A	B
NH			d, 1 H, 5.55, J_1 4	d, 1 H, 5.56, J_1 4
1	t, 46.5	t, 47.2	d, 1 H, 3.25, J_1 −13 dd, 1 H, 4.16, J_1 −13 J_{NH} = 4	d, 1 H, 3.24, J_1 −13 dd, 1 H, 4.16, J_1 −13 J_{NH} = 4
2	s, 81	s, 82		
2α	q, 11.7	q, 13.3	s, 3 H, 1.72	s, 3 H, 1.73
3	s, 211	s, 212		
4	d, 64.8	d, 65.2	s, 1 H, 4.59, J_5 0 (J_{OH} = 10.0 in Me_2SO)	s, 1 H, 4.33, J_5 0 (also by OH in Me_2SO)
5	d, 71.8	d, 74	d, 1 H, 3.52, J_6 2, J_4 0 (broad singlet in Me_2SO)	d, 3.22, J_6 5, J_4 0
6	d, 42	d, 35.5	m, 1 H, 2.68	m, 1 H, 2.73
6α	q, 21	q, 22.4	d, 3 H, 1.34, J_6 7	d, 3 H, 0.91, J_6 7
7	d, 134.8	d, 136	dd, 1 H, 5.31, J_6 4, $J_{8\alpha}$ <1	dd, 1 H, 4.99, J_6 11, $J_{8\alpha}$ <1
8	s, 118.5	s, 119		
8α	q, 13	q, 15.8	d, 3 H, 2.2, J_7 <1	d, 3 H, 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		
12α	q, 15.4	q, 16.2	s(D), 3 H, 2.28, J_{13} <1	s(D), 3 H, 2.31, J_{13} <1
13	d, 130.8	d, 131.2	s(D), 1 H, 7.77, $J_{12\alpha}$ <1	s, 1 H, 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: s = singlet, d = doublet, t = triplet, q = quartet. [b] Multiplicity.

relative stereochemistry, if the probable 1-C conformation of the pyranose was assumed. The proton on carbon 1 in **4c** is axial as assured by its 9.8-Hz coupling with H_{ax} on C-2. The equatorial C-2 proton was coupled not only with protons at C-1 and C-2_{ax}, but also with that at C-4. The four-bond coplanar ("W") relationship required for this long-range coupling is only possible if the C-4 proton is equatorial and if the pyranose is in the 1-C conformation, validating the original assumption.

Contrary to the usual occurrence,[18] the chemical shift for the protons of the axial acetyl group in **4d** is significantly upfield from those of the corresponding equatorial function in **4c**. The electronic shielding provided by an axial nitro group at C-3 could account for this effect. Molecular models show that an equatorial nitro group would exert an equivalent influence on either an axial or equatorial acetyl substitutent. This 1,3-diaxial interaction appears to affect other positions in the molecule as well, particularly the C-5 proton where there is a 0.6-ppm difference in chemical shifts between the two anomers, suggesting that this proton is axial.

This second natural occurrence of a nitro sugar takes place in a new family of secondary metabolites elaborated by a different genus of microorganism. Since the oxidations of amino groups to nitro groups by both *Streptomyces* sp.[19] and *Penicillium atrovenetum*[20] have been documented, it seems plausible that such could be the origin of this group in these nitro sugars. The skeleton of the carbon, nitrogen, and oxygen atoms in these sugars is similar to that of vancosamine (**4g**)[21] from vancomycin, although vancosamine is epimeric to rubranitrose at C-3. A vancosamine epimer could well be an immediate biogenetic antecedent of rubranitrose.

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Rubransarols

Rubransarols A and B, $C_{23}H_{23}NO_8$ for each compound, were acids, $pK_a' = 7.8$, the pH at which the characteristic color change occurs. They could be distinguished by their TLC mobilities and by a few slight differences in their ^{13}C and ^{1}H NMR spectra (vide infra).

Both compounds were eventually available in crystalline form, but the crystal dimensions of rubransarol B were suitable only for an X-ray diffraction structure determination. This work, for which the details will be reported elsewhere,[22] established structure **6** with the indicated absolute configuration for rubransarol B.

The relationship of **6** to rubransarol A, was established by NMR studies (see Table II). Shifts for all of the protons of rubransarols A and B were assigned in the ^{1}H NMR spectra with the aid of spin decoupling and deuterium exchange. Only the coupling between the C-4 and C-5 protons could not be established because of poor resolution in the critical region of the spectra.

Subsequent examination of Dreiding models indicated zero coupling due to dihedral angles approximating 90°. The significant differences in the ^{1}H NMR spectra between the two rubransarols were a 7-Hz spread in coupling constants between positions 6 and 7, and a difference of 0.32 ppm in the chemical shifts at C-7. In the ^{13}C NMR spectra there was a variation of 6.5 ppm in the resonances assigned to C-6. A spin–lattice relaxation time (T_1) study showed short relaxation periods for all nonmethyl carbons, locating them in rings, and ruling out an open ring form for rubransarol A. The possibility of atropisomerism, observed in the related streptovaricins by Rinehart et al.,[23] was ruled out by the similarity in sign and magnitude of the large specific rotations of the two isomers. An epimeric change at C-6 would result in appropriate changes in the dihedral angles of the proton bonds

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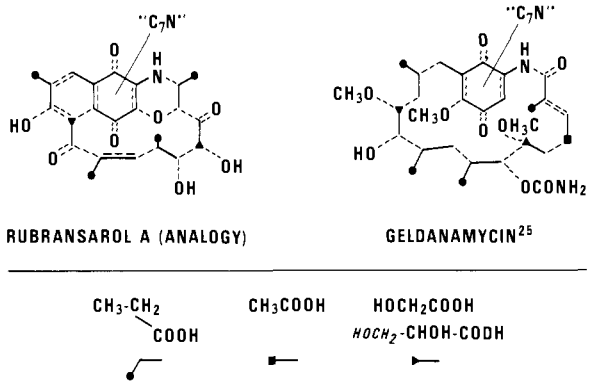

Figure 2. Comparison of rubransarol A and geldanamycin from a biosynthetic viewpoint.

accounting for the observed differences in coupling constants, but would not satisfactorily explain the variation in chemical shifts in the ^{13}C spectra. Geometrical isomerism at Δ^7 would account for both of these differences, however, so that cis–trans isomerism became the most viable option. Thus rubransarol A was assigned as the cis isomer, a choice fortified by long-range coupling of its proton at C-7 with the methyl protons at C-8a as signaled by a doublet, $J = 1$ Hz. In *trans*-rubransarol B, where the coupling would be expected to be much smaller,[24] no coupling was found.

The absence of an amide function in the ansa ring of the rubransarols distinguishes them from all of the other ansamycin antibiotics although the nitrogen found in the usual amide position is present as an amine. The resulting fused heterocyclic ring is also unique, making the ansa ring the smallest one found in the class of substances. The total carbon skeleton of the rubransarols is smaller than that of geldanamycin by but two carbons, and it is further observed that the biosynthetic scheme proposed by Rinehart et al.[25] for the latter may be applicable to the rubransarols with the omission of one acetate unit (see Figure 2).

Coumarin Moiety

Preliminary spectroscopic data (NMR and UV) presented the possibility that the rubradiric acids contained a 3-amino-4-hydroxycoumarin of the type found in the novobiocins. The ultraviolet spectra in acid solutions displayed a broad band, 310–320 nm, with a blue shift to 303 nm in base, associated with the ionization of an acidic function, $pK_a' = 5.3$. The 7′-O substituent in the aromatic ring was predicted when the aromatic region of the carbon spectra of **7a** showed three doublets: 126.5, 112.4, and 101.2 ppm. The latter extreme upfield shift was ascribable to diortho substitution by oxygen.[26] This indication was further supported by the ^1H NMR spectra. A doublet at 7.83 ppm, coupled (9 Hz) with a resonance at 7.00 ppm, satisfied the requirements for protons at C-5′ and C-6′. Meta coupling of the latter (ca. 1 Hz) was also observed for the proton at C-8′ to which was assigned a doublet at 6.97 ppm.

Confirmation for the coumarin amide was furnished by its isolation as the two derivatives, **11a** and **14**. The former was compared directly with the corresponding compounds **11b** and **11c** from novobiocin. The distinctively complex ultraviolet spectra for **11a** and **11b** are virtually superimposable. While a solubility problem dictated that the NMR comparison be made with **11b** rather than **11c**, all of the shifts were comparable, except that for the acetate **11b** there is a slight downfield displacement of the C-6′ proton to 7.35 ppm as would be expected for the acetate.

The hydrazinolysis product was a bright orange compound, **14**, $C_{10}H_{10}N_4O_3$, the elemental formula of which was established by elemental analysis and high resolution mass spectroscopy. This constitution precluded opening of the lactone ring with azide formation, requiring instead replacement of both the enamine and enol groups by hydrazone groups, with oxidation.

Although the same type of compound could not be obtained by similar treatment of novobiocin, it was afforded by novenamine[27] under comparable conditions. The ^1H NMR spectra of these compounds show unique interhydrogen nuclear spin coupling in one of the hydrazone groups, where only two bonds separate the nuclei. Of the four proton signals in the 11–12-ppm region which disappeared on D_2O exchange, two at 11.17 and 11.90 ppm were coupled as doublets, $J = 16$ Hz. This we have attributed to proton differentiation induced by hydrogen bonding of a hydrazone proton at 3′ to the carbonyl oxygen at 2′. We were unable to locate literature examples of mutual coupling of exchangeable protons, although Saika[28] has estimated the total interhydrogen nuclear spin coupling in the ammonia molecule to be 9 Hz. The remaining two exchangeable proton signals, assigned to the 4′-hydrazone group, appear as singlets at 11.02 and 11.88 ppm. In the absence of any apparent restricted rotation of the terminal nitrogen, it seems likely that this differentiation of the protons is due to the presence of a mixture of syn and anti isomers of the hydrazone. Noise-decoupled ^{13}C NMR spectra of **14** provide support for this explanation since a total of five signals is found in the carbonyl region (158.77, 158.96, 159.31, 159.33, and 161.35 ppm) in place of the three which would be expected, were only one form present.

The role of tyrosine in the biosynthesis of 3-amino-4-hydroxycoumarin by a streptomycete has been established.[29]

Dipicolinic Acid

The analytical, NMR, and MS data obtained on the hydrazide (**15**) fragment, originating from the hydrazinolysis of *N*-methylrubradiramide (**7a**), clearly indicated that rubranitrose was present. In addition to the carbon signals corresponding to the rubranitrose part, resonances for the remaining unassigned carbons of rubradirin could be seen: six quaternary resonances at 168.9, 164.3, 153.2, 151.0, 139.8, and 130.3 ppm and a doublet at 111.7 ppm, the amide methyl appearing at 25.9 ppm (see Table III). Acid hydrolysis then gave $C_8H_{10}N_4O_4$, **16**, containing all of the above carbons, and, after discounting the elements of the derivatizing methylamide and hydrazide groups and replacing them with hydroxyls, a moiety of $C_7H_5NO_6$ was indicated. This comprises the hub of the rubradirin molecule to which are attached rubransarol A as an ester, rubranitrose as a glycoside, and the coumarin as an amide. The six oxygen atoms in the formula were accounted for by the two carboxyl functions and the vinyl hydroxyls, which were represented in the ^{13}C NMR spectra by the four downfield resonances listed above. There was a vinyl proton as required by the above doublet, and a corresponding singlet at 7.4 ppm in the ^1H NMR, leaving only a nitrogen atom for accounting. At this point the stoichiometry virtually demanded the postulation of a six-membered heterocyclic having four carbon substituents.

Additional information on this hub moiety was obtained from rubradiric acid B (**9**, $R_1 = R_2 = OH$), where this moiety has two protons seen as doublets with chemical shifts of 8.1 (H-4″) and 7.2 ppm (H-5″). Their coupling constant of 8 Hz confirmed the six-membered heterocycle. The corresponding doublets in the off-resonance ^{13}C NMR spectra were located at 131.2 and 127.5 ppm and each had one-bond coupling constants of 166 Hz. This precluded adjacency to the heteroatom for which the expected coupling would be 175–185 Hz[30] at a shift at least 10 ppm farther downfield.[31] Thus positions 4″ and either 3″ or 5″ were the sites for the vinyl protons. The three downfield singlets in the off-resonance spectrum at 168.0, 169.9, and 174.2 ppm were required for the two carboxyls and the hydroxy vinyl carbon, leaving two

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Table III. Shift Assignments for ^{13}C NMR Spectra[a]

position, mult	chemical shifts in ppm; all solvents deuterated								
	(1) CDCl	(2a) Me_2SO	(2b) Me_2SO	(3) $HCONMe_2$	(5) $CDCl_3$	(6) $CDCl_3$			
1, t	47.1	46.88	46.8	47.8	45.6	47.2			
2, s	81.5	80.7	80.8	81.4	81.0	81.7			
2-CH_3, q	12.1	12.6	12.7	12.9	11.7	13.3			
3, s	207.8	207.8	207.6	207.0	211.3	212.4			
4, d	65.9	65.3	65.5	65.3	64.8	65.2			
5, d	75.2	75.8	75.3	75.1	71.8	73.6			
6, d	41.1	35.2	34.8	41.6	42.1	35.5			
6-CH_3, q	22.3	22.8	22.8	22.6	21.2	22.5			
7, d	134.1	149.9	135.3	136.8	134.8	135.7			
8, s	118.7	121.3	121.3	122.8	118.5	119.2			
8-CH_3, q	14.0	15.3	15.2	13.3	13.2	15.7			
9, s	203.5	195.2	195.3	196.0	202.5	200.7			
10, s	121.6	123.8	124.0	123.9	121.0	121.4			
11, s	160.9	157.2	160.5	159.7	160.5	160.9			
12, s	128.6	133.0		130.7	130.4	130.7			
12-CH_3, q	16.0	16.5	16.6	16.6	15.4	16.1			
13, d	131.4	129.9	129.7	131.3	130.8	131.1			
14, s	130.8	137.7		140.8	136.2	136.7			
15, s	131.6	129.7	129.7	131.3	131.5	131.6			
16, s	177.3	176.2	176.6	177.3	177.0	177.5			
17, s	139.5	139.9	139.7	131.5	136.2	140.0			
18, s	144.9	141.9	140.1	143.1	143.3	143.8			
19, s	178.8	178.6	178.6	179.2	178.0	178.3			
	(1) CDCl	(2a) Me_2SO	(2b) Me_2SO	(3) $HCONMe_2$	(7a) NaOD	(8b) NaOD	(9) NaOD	(14) Me_2SO	(11a) Me_2SO
2', s	162.9	162.7	162.9	156.7+	168.3*	167.0*	166.0*	161.2+	162.9*
3', s	102.3	101.0	100.6	102.6	98.4	98.4	98.4	125.4	
4', s	161.3	160.9	163.1	160.8+	167.0	168.1	168.6	146.8	
4a', s	109.7	109.5	109.3	110.8	115.9	116.0	116.1	108.3	104.3
5', d	125.3	124.8	124.9	125.6	126.5	126.6	126.6	129.3	122.5
6', d	112.9	112.5	112.5	113.3	112.4	112.4	112.45	106.1	113.2
7', s	152.1	152.8	149.3	150.2+	162.8	162.7	162.9	159.2+	162.3*
7'-OCH_3, q	55.8	56.0	56.0	56.4	56.6	56.7	56.7	55.2	56.0
8', d	100.2	100.5	100.6	101.0	101.2	101.2	101.4	101.4	101.7
8a', s	153.4	157.2		154.7	161.2	155.0	155.1	158.8+	
									3a, 154.1; 3b, 13.8
	(1) CDCl	(2a) Me_2SO	(2b) Me_2SO	(3) $HCONMe_2$	(7a) NaOD	(8b) NaOD	(9) NaOD	(15) $CDCl_3$	(18) NaOD
2", s	136.4	135.2	146.7	136.0	134.8	129.4	135.1	130.3	139.5
2"-CO, s	152.1	157.0	153.1	163.7*	169.9*	168.9*	168.0*	164.3	173.8
3", s	162.1	162.9	128.4	153.2+	156.7	159.4	169.9	153.2*	160.5
4", s (d)	153.0	(127.4)	137.4	164.2*	154.9	173.5	(131.2)	151.0*	(128.6)
5", d	112.5	127.6	125.6	113.3	110.1	113.7	127.5	111.7	126.7
6", s	135.6	133.0	142.0	138.3	135.4	140.0	136.0	139.8	142.4
6"-CO, s	167.3	162.9	158.2	164.0*	174.1	174.7	174.7	168.9	175.2
1"', d	95.9				96.4			95.5	
2"', t	34.9				35.4			34.7	
3"', s	90.3				91.6			90.1	
3"'-CH_3, q	24.9				25.2			24.7	
4"', d	79.6				80.0			79.5	
4"'-OCH_3, q	62.2				63.5			62.8	
5"', d	70.4				71.3			70.3	
6"', q	16.7				16.6			16.4	

[a] Super asterisks and pluses indicate assignments could be reversed.

remaining singlets at 135.1 and 136.0 ppm as sites for the carboxyl groups. Being equivalent, and relatively far downfield, these shifts were assigned to carbons 2" and 6", adjacent to the nitrogen. Using gated decoupling, three-bond coupling of H-4" (8.1 ppm) with both C-6" ($J_{H4,C6}$ = 4.9 Hz) and C-2" ($J_{H4,C2}$ = 8.3 Hz) was observed, confirming the two assignments. Thus the final moiety in rubradirin B is 3-hydroxydipicolinic acid, a compound which Bojarska-Dahlig and Swirska[32] had synthesized from 5-hydroxypicolinic acid in 1953 using an iodination, cyanylation, and hydrolysis sequence. By applying this series of reactions to 3-hydroxypicolinic acid we prepared a sample for an NMR comparison which confirmed our assignments.

Similar assignments were made for the corresponding moiety in rubradirin by comparing the NMR spectra of derivatives of rubradiric acids A and B. In the former, the three-bond couplings found for the 3-hydroxydipicolinic acids above could not be seen, indicating an absence of the proton at this C-4", and therefore the additional oxygen substitutent in the rubradirins was assigned to this position.

Sites for the Substituent Moieties on the "Hub"

The coincident absence of an oxygen substituent at C-4" and a rubranitrose moiety in rubradirin B implicated this position as

(32) H. Bojarska-Dahlig and A. Swirska, *Rocz. Chem.*, **27**, 258 (1953).

the site for glycosylation in rubradirin. The ^{13}C NMR spectra of assorted aglycons prepared from rubradirin afforded support for this proposition. Since the 4-hydroxypyridines exist chiefly as the 4-pyridones in water,[33,34] this amide form should be the favored tautomer for the aglycons. On the other hand, the glycosylated compounds are locked in the pyridine form. A significant difference in the chemical shift at C-4″ would be expected for aglycons when compared with the parent glycosylated compounds, if C-4″ indeed is the position in question. The C-4″ resonance in aglycon **8b** is 173.5 ppm (D_2O) and in aglycon **3** it is 164.2 ppm (DMF-d_7). On the other hand, in methylrubradiramide A (**7a**) the corresponding shift is upfield at 154.9 ppm (D_2O), a reasonable resonance for the 4-glycosyloxypyridine structure.

A problem remained: which of the alternate carboxyl groups was esterified with rubransarol and which had the coumarin amide function? In *N*-methylrubradiramide A (**8b**) the methylamide group which marks the rubradirin esterification site stabilizes this carboxyl against decarboxylation, which is known to occur with some facility in dipicolinic acids.[35] Treatment of **8b** with acetic anhydride under our transacylation conditions liberated the pyridone as the hemiacid **12**, in a mixture with its decarboxylation product **13**. The one annular proton in **12** was seen in the NMR spectrum as a singlet at 7.5 ppm. In **13** this proton shifted to 6.75 ppm and was a doublet. It was coupled (J = 6 Hz) to the new proton (7.7 ppm) found at the decarboxylation site. This specified C-6″ (see structure **1**) for the location of the amide carbonyl and C-2″ for the ester. From the above, a similar arrangement of substituents was inferred (with equivocation) for rubradirin B.

Rubradirin C has no ring oxygens on the pyridine part. Ester cleavage with methylamine afforded the usual two fragments, an ansa moiety and a methylrubradiramide. TLC analysis showed that the ansa moiety was rubransarol B. The NMR spectra showed that the coumarin moiety in *N*-methylrubradiramide C was the same as that in the other rubradirins, locating the difference in the dipicolinic acid portion. The ^1H NMR spectra showed a three-proton complex in the region 7.9 to 8.4 ppm, replacing the two pairs of doublets signaling the protons at C-4″ and C-5″ in rubradirin B. The new complex was similar to that observed for diacetylpyridine[36] and related compounds. Structure **2b** was thus indicated for rubradirin C.

The occurrence of dipicolinic acid in bacterial spores[37] and yeasts[38] is documented, and a route from aspartate has been postulated.[39] A microbiological oxidation by a strain of *Achromobacter* has been demonstrated to afford 3-hydroxydipicolinic acid[40] with further oxidation to oxalic acid and carbon dioxide. The 3,4-dioxidized intermediate was not detected.

Experimental Section

Melting points, determined on a Fisher-Johns apparatus, are uncorrected. UV spectra were recorded with a Cary Model 15 spectrophotometer. IR spectra were determined with a Digilab FTS 14D spectrometer in Nujol mulls. Mass spectra were obtained on a Varian MAT CH5 DF instrument. The ^{13}C NMR spectra were obtained with a Varian CFT 20 and the proton spectra were recorded with a Varian XL-100-15 instrument using appropriate deuterated solvents.

For column chromatography, buffered silica gel was used. It was prepared by mixing 1 kg of silica gel, 70–230 mesh, E.M. Laboratories, Inc., with 800 mL of a 0.5 M KH_2PO_4 solution and drying the product at 110 °C for 20 h. Aqueous suspensions of this silica showed a pH of 5.8. Columns were poured in a chloroform slurry and developed with chloroform and mixtures of chloroform containing 1–3% of methanol. Thin-layer chromatographic plates were prepared by gauging (0.7-mm thickness) a slurry of 60 g of silica gel HF (EM) in 140 mL of 0.4 M KH_2PO_4 solution onto glass plates and drying at 130 °C for 20 h. For development chloroform:methanol (98:2 v/v) was used. For a second system, silica gel GF on glass plates (Analtech) developed with ethyl acetate:acetone:water:methanol (8:5:1:0.6, v/v) was employed. Spots were visualized in daylight when possible and in UV light.

Rubradirin (1), Rubradirin B (2a), and Rubradirin C (2b). The three rubradirin components used in these studies were purified from the antibiotic complex by chromatography over buffered silica gel.

Rubradirin (**1**) was eluted with chloroform and isolated as an amorphous solid by precipitation from chloroform–Skellysolve B solutions, $[\alpha]_D$ +571° (*c* 0.0205, acetone). Anal. Calcd for $C_{48}H_{46}N_4O_{20}$: C, 57.72; H, 4.64; N, 5.61. Found: C, 57.75; H, 4.73; N, 5.32. MW (theory) *m/e* 998; $M^{+\cdot}$ (by FD) 998.

Rubradirin B was the first major component eluted with chloroform:methanol (98:2 v/v); it was crystallized from chloroform, mp >300 °C dec. Anal. Calcd for $C_{40}H_{33}N_3O_{15}$: C, 60.38; H, 4.18; N, 5.17. Found: C, 60.32; H, 4.45; N, 5.17. MW (theory) 795; $M^{+\cdot}$ (by FD), 795.

Rubradirin C was the second major component eluted in the above solvent system. After further purification by rechromatography in the same system, the material was crystallized from dimethyl sulfoxide, mp >300 °C dec.

Because of difficulties in analyzing these crystals, this material was analyzed as an amorphous precipitate from water. Anal. Calcd for $C_{40}H_{33}N_3O_{14}\cdot H_2O$: C, 60.23; H, 4.42; N, 5.27; H_2O, 2.25. Found: C, 59.8; H, 4.31; N, 5.18; H_2O, 2.81. MW (theory) *m/e* 779; $M^{+\cdot}$ + 1 (by FD) 780.

Acid Hydrolysis of Rubradirin: Rubradirin Aglycon (3), and Rubranitrose (4a and 4b). A 5-g (5 mmol) quantity of rubradirin was dissolved in 175 mL of glacial acetic acid. To this stirred solution were added 50 mL of water and 25 mL of 2 N HCl; the pH was 2. Stirring was continued for 72 h at room temperature and then 25 mL of 2 N NaOH was added. The mixture was filtered and the filtrate was evaporated to dryness at reduced pressure in a nitrogen stream. The precipitate and the residue were combined and thoroughly leached with a total of 500 mL of chloroform. The chloroform was washed with $NaHCO_3$–Na_2CO_3 buffer, pH 10, to remove residual color and the solvent then removed by evaporation, affording 450 mg (50%) of crude rubranitrose. The residue was further triturated with chloroform and this mixture was filtered, yielding 3.9 g (96%) of partially crystalline rubradirin aglycon. ^{13}C NMR (DMF-d_7 q 12.9, 13.3, 16.6, 22.6, 56.4; t 47.8; d 41.6, 65.3, 75.1, 101.0, two at 113.3, 125.6, 131.3, 136.8; s 81.4, 102.6, 110.8, 122.8, 123.9, 130.7, 131.3, 131.5, 136.0, 138.3, 140.8, 143.1, 150.2, 153.2, 154.7, 156.7, 159.7, 163.7, 164.0, 164.2, 177.3, 179.2, 196.0, 207.0. ^1H NMR (Me_2SO-d_6) 1.65 (d, 3, J = 7 Hz), 1.72 (s, 3), 2.19 (d, 3, J > 1 Hz), 2.37 (br s, 3), 3.04 (m, 1), 3.22 (d, 1, J = 7 Hz), 3.93 (s, 3), 4.16 (dd, 1, J = 7, 2 Hz), 4.91 (s, 1), 4.98 (d, 1, J = 2 Hz), 5.77 (dd, 1, J = 1, 2 Hz), 6.77 (d, 1, J = 2.5 Hz), 7.00 (dd, 1, J = 4.5, 5 Hz), 7.80 (s, 1), 7.84 (s, 1), 7.84 (d, 1, J = 5 hz), 9.76 (s, 1, exch). Anal. Calcd for $C_{40}H_{33}N_3O_{16}$: C, 59.18; H, 4.10; N, 5.18. Found: C, 59.41; H, 4.43; N, 5.14. MW (theory) *m/e* 811; found $M^{+\cdot}$ 811.

The crude rubranitrose from the chloroform soluble fraction (vide supra) was redissolved in chloroform and this solution was evaporated slowly, resulting in the deposition of crystals, mp 150–153 °C, $[\alpha]_D$ +127° → 86°$_{24h}$ (*c* 0.285, EtOH): ^1H NMR ($CDCl_3$) 1.33 (d, 3, J = 6.5 Hz), 1.67 (s, 3), 2.05 (dd, 1, J = 14.5, 2.5 Hz), 2.67 (dd, 1, J = 14.5, 3.5 Hz), 3.62 (s, 3), 3.71 (br s, 1 J < 1 Hz), 4.40 (br q, 1, J = 6.5, <1 Hz), 5.28 (dd, 1, J = 2.5, 3.5 Hz). Anal. Calcd for $C_8H_{15}NO_5$: C, 46.82; H, 7.37; N, 6.83; Found: C, 46.70; H, 7.63; N, 6.67. MW (theory) *m/e* 205; $M^{+\cdot}$ (by FD MS) 205.

1β-Acetylrubranitrose (4c) (Impure 1α-Acetylrubranitrose (4d)). A 300-mg (1.46 mmol) quantity of crude rubranitrose was dissolved in 5 mL of pyridine containing 200 mg (2 mmol) of acetic anhydride. After this solution was kept for 3 days at room temperature, it was added to ice–water which was then extracted with ethyl acetate. The mixture was washed with cold 0.05 N HCl and cold 5% Na_2CO_3 solution, then dried and evaporated, affording 300 mg of white residue. This was chromatographed on 30 g of silica gel G-60 (EM), eluted with chloroform. The fourth of the 80-mL fractions which were collected gave crystalline material (**4c**) on evaporation. This was recrystallized from methanol and water, 40 mg, 11%, mp 74–76 °C: ^1H NMR ($CDCl_3$): 1.33 (d, 3, J = 6.5 Hz), 1.69 (s, 3), 1.97 (dd, 1, J = 9.8, 14.5 Hz), 2.09 (s, 3), 2.52 (ddd, 1, J = 14.5, 2.4, 1.5 Hz), 3.63 (m, 1), 3.63 (m, 1), 3.65 (s, 3), 5.73 (dd, 1, J = 9.8, 2.4 Hz). Anal. Calcd for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67. Found: C, 48.42; H, 7.45; N, 5.78.

Fractions 5–10 from the chromatography gave oily material (**4d**) which also showed the above NMR values, but in addition displayed the following signals: 1.64 (s, 3), 2.14 (dd, 1, J = 14.5, ca. 3.5 Hz), 1.94 (s, 3), 2.73 (dd, 1, J = 14.5, ca. 2 Hz), 4.24 (br q, 1, J = 6.5, <1 Hz), 3.74

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(br s, 1, J = <1 Hz), 3.60 (s, 3), 6.1 (dd, 1, J = ca. 3.5, ca. 2 Hz).

X-ray Crystallography of 4c. A large, colorless monoclinic crystal of 1-acetylrubranitrose (**4c**) was obtained from a methanol and water mixture. The space group of this crystal was $P2_1$, with unit cell parameters: a = 8.528 (0.001), b = 13.133 (0.001), c = 11.741 (0.001) Å, β = 112.38 (0.01)°; volume = 1215.9 (0.1) Å^3. There were four molecules per unit cell.

Intensity data on the 2286 reflections with $2\theta \geq 138°$ were measured on a Syntex PI diffractometer at −155 °C, controlled by an IBM 1800 computer,[41] using graphite monochromatized Cu Kα radiation, (λ 1.5418 Å). The step-scan method was used with scan ranges ≤4° in 2θ and a scan rate of 2°/min. Accurate cell parameters were obtained using the precision cell option and 20 selected reflections with $2\theta \leq 118°$. Standard deviations in observed intensities were approximated by $\sigma^2(I) = \sigma^2_{\text{counting statistics}} + (0.01I)^2$, where the coefficient of I in the last term was calculated from those deviations in intensities of 10 monitored reflections not explained by counting statistics. The usual Lorentz correction was made, and a polarization correction for a monochromator with 50% perfect character was applied.[42] An absorption correction was done using the method of Busing and Levy.[43]

The structure was solved by direct methods using DIREC II, a new program written by David J. Duchamp of The Upjohn Co. which uses quartets.[44] The coordinates and anisotropic thermal parameters of heavier atoms were refined by crystallographic least squares in which the function minimized is $\sum w(F_o^2 - F_c^2)^2$. In the final stages of refinement, weights (w), were taken to be the reciprocals of the variances $\sigma^2(F_o^2)$. Hydrogen coordinates were generated using standard tetrahedral or planar geometry, and the hydrogen atoms and temperature factors were then refined. Refinement was considered converged when shifts on coordinates were less than $\sigma/4$. The final agreement index ($R = \sum||F_o| - |F_c||/\sum|F_o|$) was 0.033. The standard deviation of fit, $[\sum w(|F_o|^2 - |F_c|^2)^2/(m-s)]^{1/2}$, was 4.5. All calculations were carried out on an IBM 370 computer using the CRYM crystallographic system developed by D. J. Duchamp. Atomic form factors are from the "International Tables for X-Ray Crystallography",[45] except for hydrogen which was taken from Stewart, Davidson, and Simpson.[46]

The final atomic coordinates and thermal parameters for the nonhydrogen atoms, the bond lengths and angles, as well as the final atomic coordinates for the hydrogen atoms, their respective bond distances, and bond angles can be found as supplementary material.

Ethyl Aminolysis of Rubradirin. Rubransarol A (5) and *N*-Ethylrubradiramide (7b). A 7-g (7 mmol) quantity of rubradirin was dissolved in 100 mL of 33% aqueous ethylamine; after this solution was stored at ambient temperature for 16 h it was evaporated on a rotary evaporator to dryness. A solution of this residue in 150 mL of water was acidified to pH 2 with 6 N HCl and the precipitate which formed was isolated by centrifugation, dried, and leached with chloroform (vide infra). The residue (**7b**) was washed with cold acetone and crystallized from warm acetone yielding 2.85 g, 68%, of **7b**, mp 209 °C dec: UV (0.01 N ethanolic base) 308 nm (ϵ 25.6 × 10^{-3}); (0.01 N ethanolic acid) 312 nm (ϵ 19.5 × 10^{-3}). Anal. Calcd for **7b**, $C_{27}H_{30}N_4O_{12}$: C, 53.82; H, 5.02; N, 9.30. Found: C, 53.32; H, 4.95; N, 9.12. MW (theory) m/e 602; found M^+· 602.

The combined chloroform leachings were evaporated to a residue, 3.0 g, which was chromatographed on buffered silica gel and developed with $CHCl_3$:MeOH (98:2 v/v). Rubransarol A (**5**) was found in the fractions obtained from 1060 to 2000 mL of effluent and isolated by evaporation. This was redissolved in butanone-2 and precipitated with Skellysolve B, 1.13 g, 37% yield. It could be crystallized from evaporating methanol, mp 238–240 °C. Anal. Calcd for $C_{23}H_{23}NO_8$: C, 62.58; H, 5.25; N, 3.17. Found: C, 62.01; H, 5.66; N, 3.16. High resolution MS m/e 441: theory, 441.1424; found, 441.1417.

Ester hydrolyses of Other Members of the Complex: Rubransarol B (6), *N*-Ethylrubradiramide B (9), *N*-Methylrubradiramide A (7a), *N*-Methylrubradiramide C (10), Rubradiramide A Aglycon (8a), and Methylrubradiramide A Aglycon (8b). With slight modifications this procedure was applied to **1**, **2a**, **2b**, and **3** using either (1) an aqueous solution of NaOH maintained for the duration of the reaction at pH 11, (2) 40% aqueous methylamine, or (3) methanol saturated with ammonia. The following products were so obtained:

Rubransarol B (6). Anal. Calcd for $C_{23}H_{23}NO_8 \cdot 0.75\alpha CDCl_3$: C, 53.65; H, D, 4.64; N, 2.63. Found: C, 53.89; H, D, 4.68; N, 2.69. $\alpha CDCl_3$ content is based on X-ray crystallography. High resolution MS, m/e 441: theory 441.1424; found 441.1413. $[\alpha]_D$ +108° (c 0.02, acetone).

***N*-Ethylrubradiramide B (9)**: 90% yield, crystals from dioxane, mp >285 °C. Anal. Calcd for $C_{19}H_{17}N_3O_7$: C, 57.14; H, 4.29; N, 10.52. Found: C, 56.84; H, 4.34; N, 10.49. High resolution MS m/e 399: theory, 399.1066; found, 399.1073.

***N*-Methylrubradiramide A (7a)**: 94% yield, crystals from butanone-2, mp 265 °C dec. Anal. Calcd for $C_{26}H_{28}N_4O_{12}$: C, 53.06; H, 4.80; N, 9.52. Found: C, 53.08; H, 4.88; N, 9.34. MW (theory) m/e 588; M^+· 588.

***N*-Methylrubradiramide C (10)**: 63% yield, crystals from acetonitrile, mp >265 °C. Anal. Calcd for $C_{18}H_{15}N_3O_6$: C, 58.53; H, 4.09; N, 11.38. Found: C, 58.42; H, 4.24; N, 11.55.

Rubradiramide A Aglycone (8a): 90% yield, crystals from hot methanol trituration. Anal. Calcd for $C_{17}H_{13}N_3O_8$: C, 52.72; H, 3.38; N, 10.85. Found: C, 52.63; H 3.55; N, 10.72.

Methylrubradiramide A Aglycone (8b): 86% yield, crystals from dioxane. Anal. Calcd for $C_{18}H_{15}N_3O_8$: C, 53.87; H, 3.77; N, 10.47. Found: C, 53.56; H, 3.83; N, 10.09.

Degradation of Rubradirin with Acetic Anhydride in Pyridine. 7-Methoxy-2-methyl-4*H*-[1]benzopyrano[3,4-*d*]oxazol-4-one (11a). A solution of rubradirin (10 g, 10 mmol) in 125 mL of pyridine and 25 g (250 mmol) of acetic anhydride was refluxed 4 h, then poured into ice and water. The precipitate so formed was collected, dried (1.3 g, 56 mmol, 56% yield), and treated with carbon in hot methanol and recrystallized twice from that solvent. The final yield was 300 mg of white crystals, mp 212–213 °C UV (methanol), nm ($\epsilon \times 10^{-3}$): 315 (18.5), 287 (9.9), 226 (10.3). ^{1}H NMR (Me_2SO-d_6) 7.83 (d, 1, J = 8 Hz), 7.28 (d, 1, J = Ca. 1 Hz), 7.15 (dd, 1, J = 8, ca. 1 Hz), 3.98 (s, 3), 2.7 (s, 3). ^{13}C NMR (Me_2SO-d_6) q 13.8, 56.0; d 101.7, 113.2, 122.5; s 104.3, 154.1, 162.3, 162.9; three singlets not observed. MS: M^+· 231, M^+· − CH_3 216; also 188, 175, 122, 43. Anal. Calcd for $C_{12}H_9NO_4$: C, 62.34; H, 3.92; N, 6.06. Found: C, 62.49; H, 3.78 N, 6.05.

Reaction of Methylrubradiramide A with Acetic Anhydride in Pyridine: 6-Carboxy-3,4-dihydroxy-2-(methylaminocarbonyl)pyridine (12) and 2-(Methylaminocarbonyl)-4,5-dihydroxypyridine (13). Methylrubradiramide A (4 g, 10 mmol) in 20 mL of pyridine and 5 g (50 mmol) of acetic anhydride was refluxed for 2.5 h. After cooling, 3 mL of methanol was added, and this solution was stored at 4 °C for 15 h, after which 1.8 g, 78%, of **11a** was separated by filtration. The filtrate, concentrated to one-third volume and then diluted to the original volume with ether, afforded 100 mg (5%) of an acid, **12**. Evaporation of the remaining solution to dryness yielded a white residue. Evaporation of the remaining solution to dryness yielded a white residue. This residue was washed with chloroform and ether, and dried (500 mg). When this residue was leached with a small amount of phosphate buffer (pH 7.5), 200 mg (12%) of neutral material (**13**) remained. The soluble portion was recovered by acidification of the filtrate to pH 2 yielding 130 mg (6%) of additional acid (**12**). Compound **12** was recrystallized from water, mp 250–300 °C with sublimation. Anal. Calcd for $C_8H_8N_2O_5$: C, 45.29; H, 3.80; N, 13.20. Found: C, 45.22; H, 3.95; N, 13.18. High resolution MS m/e 212: theory, 212.0433; found, 212.0451. ^{1}H NMR (Me_2SO-d_6) 7.54 (s, 1), 9.46 (d, 1, exch), 2.90 (d, 3, J_{NH} = 5 Hz). Compound **13**, amorphous material: Anal. Calcd for $C_7H_8N_2O_3$: C, 50.00; H, 4.80; N, 16.66. Found: C, 49.73; H, 4.72; N, 16.33. High resolution MS m/e 168: theory, 168.0534; found, 168.0537. Recrystallized (H_2O): mp 225 °C, sublimes. Anal. Calcd for $C_7H_8N_2O_3 \cdot {}^1/_2H_2O$ C, 47.46; H, 5.12; N, 15.81. Found: C, 47.50; H, 4.93; N, 15.69. ^{1}H NMR (Me_2SO-d_6) 9.2 (d, 1 exch), 7.7 (d, 1, J = 6 hZ), 6.75 (d, 1, J = 6 Hz), 2.85 (d, 3, J_{exch} = 5 Hz).

Hydrazinolysis of Rubradiric Acid A. Preparation of 14 and 15. Rubradiric acid A methylamide, (2.7 g, 4.59 mmol) was stored in a solution of 25 mL of hydrazine hydrate for 20 days. The hydrazine was removed by evaporation in a N_2 stream, and the residue was chromatographed on 500 g of buffered (pH 5.8) silica in a 5-cm (diam) column developed with chloroform:methanol (98:2 v:v). Fractions 100–220 (20 mL each) were pooled and concentrated to give 470 mg (44%) of osazone **14**, mp >300 °C. ^{13}C NMR q 55.22, d 101.4, 106.1, 129.3; s 108.3, 125.4, 146.8, 158.8, 159.2, 161.2. ^{1}H (Me_2SO-d_6) 8.09 (d, 1, J = 8 Hz), 6.52 (dd, 1, J = 8, ca. 1 Hz), 6.49 (d, 1, J = ca. 1 Hz), 3.79 (s, 3). Exchangeable protons: 11.90 (d, 1, J = 16 Hz) 11.88 (s, 1), 11.17 (d, 1, J = 16 Hz), 11.02 (s, 1). Anal. Calcd for $C_{10}H_{10}N_4O_3$: C, 51.28; H, 4.15; N, 23.92. Found: C, 50.70; H, 4.15; N, 23.68. MW m/e 234; M^+· 234.

Fractions 225-463 afforded a residue which was rechromatographed on the same support in a 3-cm column, developed with ethtyl acetate:

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methanol (10:1 v:v) and fractions 36–60 (20 mL) afforded 440 mg (23%) of hydrazide **15** on evaporation. Anal. Calcd for $C_{16}H_{23}N_5O_8$: C, 46.48; H, 5.60; N, 16.94. Found: C, 46.90; H, 5.91; N, 16.22. This material was crystallized from ethanolic hydrogen chloride. Anal. Calcd for $C_{16}H_{24}N_5O_8Cl$: C, 42.70; H, 5.37; N, 15.56. Found: C, 42.37; H, 5.77; N, 15.26. $M^{+\cdot}$ 701 (Me_3Si).

Registry No. **1**, 11031-38-2; **2a**, 68833-11-4; **2b**, 69279-50-1; **3**, 69282-19-5; **4a**, 71606-18-3; **4b**, 71606-17-2; **4c**, 71606-19-4; **4d**, 71606-16-1; **4f**, 71839-37-7; **4g**, 82597-20-4; **4h**, 79005-87-1; **5**, 68833-57-8; **6**, 68889-77-0; **7a**, 69282-25-3; **7b**, 69282-24-2; **7c**, 69282-18-4; **8a**, 69282-21-9; **8b**, 71502-33-5; **9**, 72670-48-5; **10**, 82537-35-7; **11a**, 69282-23-1; **11b**, 3780-30-1; **11c**, 82537-36-8; **12** (R = CO_2H), 71502-31-3; **13**, 71502-32-4; **14**, 82537-37-9; **15**, 82537-38-0; **16**, 82537-39-1.

Supplementary Material Available: Tables 1–6 listing final atomic coordinates and thermal parameters for nonhydrogen atoms (1), bond lengths (2), bond angles (3), and final atomic coordinates for hydrogen atoms (4) and their respective bond distances (5) and angles (6) for **4c** (6 pages). Ordering information is given on any current masthead page.

Benzidine Rearrangements. 17. The Concerted Nature of the One-Proton *p*-Semidine Rearrangement of 4-Methoxyhydrazobenzene[1,2]

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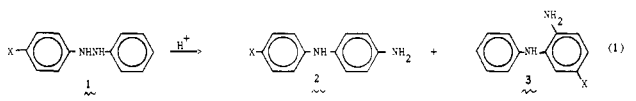
Abstract: The nitrogen kinetic isotope effects (KIE) for the acid-catalyzed rearrangement of 4-methoxyhydrazobenzene (**1b**) into the *p*-semidine (4-methoxy-4′-aminodiphenylamine, **2b**) and *o*-semidine (2-amino-5-methoxydiphenylamine, **3b**) have been measured and are 1.0296 (av) and 1.074 (av), respectively. The carbon KIE for formation of **2b** was found to be 1.039 (av). The results show that *p*-semidine formation is a concerted process. Rearrangements were carried out in 60% aqueous dioxane under conditions of earlier kinetic work. Mixtures of **1b** and [^{15}N,$^{15}N'$]**1b** were used for the nitrogen KIE, measured mass spectrometrically with isolated *N*-benzoyl-**2b**, and mixtures of **1b** and [4′-^{14}C]**1b** for the carbon KIE, measured with scintillation counting on isolated *N*-benzoyl-**2b**. The method of carbon labeling did not allow for a measurement of the KIE for formation of **3b**.

The acid-catalyzed *p*-semidine rearrangement is the rearrangement of a 4-substituted hydrazobenzene (eq 1). The *p*-



(1)

semidine (**2**) is formed from bonding between the nitrogen attached to one ring and the *p*-carbon atom of the second ring. An *o*-semidine (**3**) is also usually formed.

For a number of years in the past the *p*-semidine rearrangement was believed not to be a valid member of the class of acid-catalyzed benzidine rearrangements. This belief came about first because it was difficult for early workers to accept that such a remarkable change in geometry could occur intramolecularly and second from a misreading of the early literature by Hammick and Munro.[4] These workers thought that all of the then known cases of *p*-semidine rearrangements had been achieved by the so-called Jacobson method, namely the reaction of an azobenzene with an acid reducing medium, such as aqueous $SnCl_2$–HCl. In this method the azobenzene is reduced to the hydrazobenzene, which next rearranges in the acid solution. Having failed themselves to bring about a purely acid-catalyzed *p*-semidine rearrangement (of 4-ethoxyhydrazobenzene), Hammick and Munro concluded that this type of rearrangement could be successful only by the Jacobson method because the rearrangement required the presence of a heavy-metal ion and was, in fact, an intermolecular one, assisted, in an unspecified Friedel–Crafts way, by the metal ion. Hammick and Munro (and all other later workers) overlooked some very early reports by Jacobson of rearrangements of 4-substituted hydrazobenzenes in acid solution only, for example, of **1a** (X = Cl).[5,6] Consequently, the *p*-semidine rearrangements attracted particular attention, first with attempts to find if they were really members of the family of benzidine rearrangements and next, after this question had been reanswered affirmatively,[6] with attempts to understand how the rearrangements could occur intramolecularly.

It is now known that *p*-semidine rearrangements are not only acid catalyzed but that they have another characteristic of benzidine rearrangements in that some of them are one-proton (e.g., **1b**, X = MeO)[7] and others (e.g., **1a**) two-proton rearrangements.[8,9] Furthermore, Heesing and Schinke have shown with the use of labeled molecules that the rearrangements of **1a**[10] and **1b**[11] are truly intramolecular.

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