THE CHEMISTRY OF RUBRADIRIN. III THE RUBRADIRIC ACIDS AND THE STRUCTURE OF RUBRADIRIN

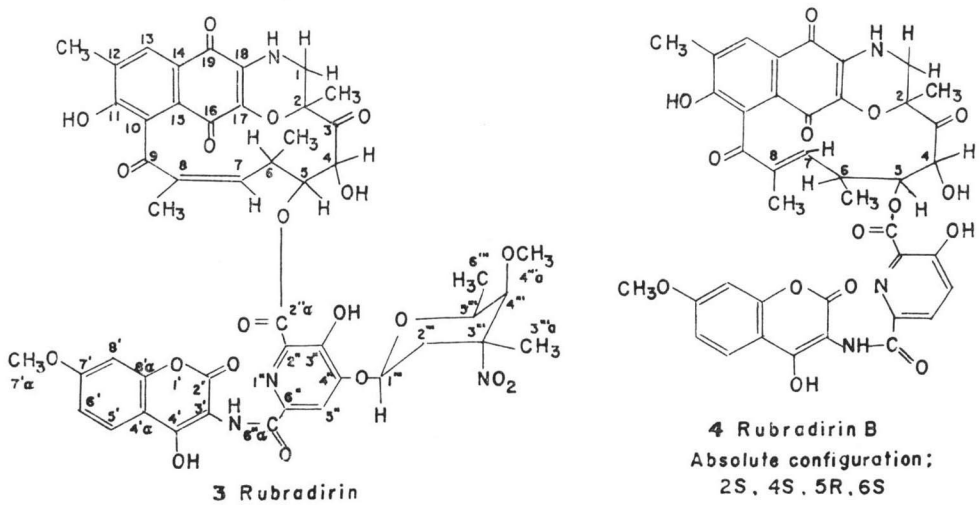
Sir:

Basic hydrolysis of rubradirin[1,2,3] and rubradirin B[4] affords in addition to the previously described rubransarols A and B[5], the respective acidic portions, rubradiric acid A, $C_{25}H_{25}N_3O_{13}$*, and rubradiric acid B, $C_{17}H_{12}N_2O_8$. We here record the considerations which lead us to assign structures **1a** and **2a** to these acids. These acids were reported to be ester-linked to position **5** of their respective rubransarols[5]. Thus rubradirin has structure **3** and rubradirin B has structure **4**.

The rubradiric acids were most conveniently prepared as amides, methyl amides, and ethyl amides. Titration of rubradiric acid A methylamide **1b**, in 83% aqueous dimethyl formamide showed that it had two acidic functions, pKa' 5.3 and 7.8. Its NMR spectra suggested an aromatic structure with three protons. Two of them, δ 7.00 and δ 7.83, displayed *ortho* coupling, J=9Hz, and the former was also coupled with a *meta* proton, δ 6.97, J=*ca* 1. Furthermore, in the off-resonance proton-decoupled ^{13}C NMR spectrum (Table 1) there was a notable upfield doublet at δ 101.2 ascribable to di*ortho* substitution by oxygen[6]. Major ultraviolet bands at 310 nm (acid) and 303 nm (base) were reminiscent of the spectra of the novobiocins and some of their analogs. Since this information was consistent with a 3-amido-4-hydroxy coumarin such as is found in the novobiocin family, an acetic anhydride transacylation reaction was selected to rupture the amide bond of **1a**, affording (as in the case of novobiocin)[7] an oxazole, **5**. The other fragment from this reaction was not isolated here because additional decomposition occurred in the sugar portion. The structural assignment for **5** was concluded from the analytical, NMR, and MS data, and particularly by a direct UV comparison with 3-amino-4-hydroxy-7-noviosyloxy-8-methylcoumarin obtained from novobiocin. It was clear from the ^1H- and ^{13}C-NMR data that this coumarin was also present in **2a** and rubradirin B. An unusual aspect of this structure is the presence of the 7'-methoxyl function rather than that of the 7'-rubranitrosyl function which might be expected from novobiocin-coumermycin analogy. It was clear from the acidity of **1b** (pKa'=5.3 in 83% DMF) and from its UV absorptions in acidic and basic solutions that the enolic hydroxyl in the coumarin was not substituted. Thus the only remaining moiety for glycosylation was the dipicolinic acid described in the following paragraphs.

The stoichiometry and NMR data (Table 1) on compounds **1** and **2**, required that this final moiety would be a nitrogen heterocycle with two carboxyl functions. In rubradiric acid B ethyl amide, **2b**, there were two protons, at δ 7.2

Fig. 1. Structures of rubradirin and rubradirin B.



* Analytical values are consistent with the molecular formulas shown.

and 8.0, and one vinyl hydroxyl. The 8-Hz coupling of the protons established a 6-membered ring. The shifts of the two doublets in the off-resonance ^{13}C NMR spectrum[8] (δ 131.2 and 127.5) along with their 1-bond coupling constants[9] (166 Hz each) virtually precluded nitrogen adjacency for these protons, locating them at positions 4″ and either 3″ or 5″. The carbons linked to the carbonyl groups had relatively equivalent values and therefore were assigned to positions 2″ and 6″. This was supported by 3-bond couplings (J=8.2 and 4.9 Hz) of these carbons with the proton at 4″. Such couplings were also observed for compound **6**. These assignments left positions 5″ or 3″ for the hydroxyl group. This identified the final moiety in rubradirin B as 3-hydroxydipicolinic acid, **6**. We confirmed these assignments by comparison with the NMR spectra of the synthetic material (Table 1) prepared according to BOJARSKA-DAHLIG and SWIRSKA[10] from 3-hydroxypicolinic acid.

The corresponding moiety in rubradirin was similar according to analytical and spectral data for rubradiric acid A (**1a**, **1b**) and also for **8**. It had one vinyl proton, δ 7.6, but an additional oxygen which we located at position 4″ from the consideration of a series of gated decoupled carbon spectra. The spectra for compounds which have a proton at C-4″, *i.e.* **2** and **6**, showed 3-bond coupling between this proton and both C-6″ (J=4.9 Hz) and C-2″ (J=8.3 Hz). This coupling was not seen in the corresponding spectra for **1** indicating that this site was the site of oxygenation. It is an unverified assumption that 4″ is the position of glycosylation, but rubradirin B, lacking this oxygen, has no sugar. The diaxial coupling ($J_{2'''ax}=9$) of the C-1‴ proton at δ 5.58 in the ^1H NMR of **1b** established the β-configuration of the glycoside.

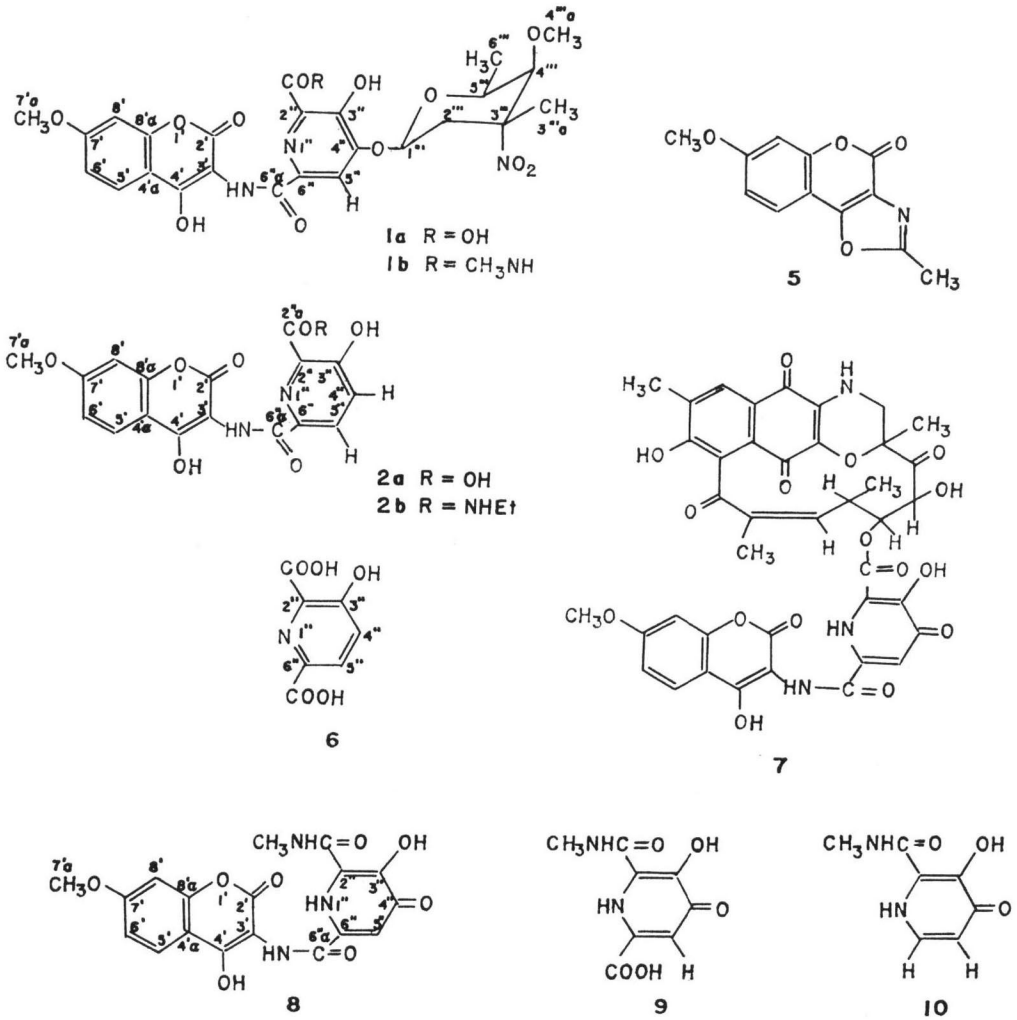
It remained to assign the ester and amide groups in the rubradirins to their specific carbonyls on the nonsymmetrical hydroxydipicolinic

Table 1. ^{13}C NMR shifts.

Position	Multiplicity	Compound (solvent)				
		1b (NaOD)	**2b** (NaOD)	**6** (NaOD)	**7** (d_7-DMF)	**8** (NaOD)
2′	S	168.3*	166.0*		150.2+	167.0*
3′	S	98.4	98.4		102.6	98.4
4′	S	167.0	168.6		160.8+	168.1
4′α	S	115.9	116.1		110.8	116.0
5′	D	126.5	126.6		125.6	126.6
6′	D	112.4	112.5		113.3	112.4
7′	S	162.8	162.9		156.7+	162.7
7′a	Q	56.6	56.7		56.4	56.7
8′	D	101.2	101.4		101.0	101.2
8′α	S	161.2	155.1		154.7+	155.0
2″	S	134.8	135.1	139.5	136.0	129.4
2″a	S	169.9*	168.0*	173.8	163.7*	168.9*
3″	S	156.7	169.9	160.5	153.2+	159.4
4″	S or (D)	154.9	(D)131.2	(D)128.6	164.2*	173.5
5″	D	110.1	127.5	126.7	113.3	113.7
6″	S	135.4	136.0	142.4	138.3	140.0
6″a	S	174.6	174.7	175.2	164.0*	174.7
1‴	D	96.4				
2‴	T	35.4				
3‴	S	91.6				
3‴a	Q	25.2				
4‴	D	80.0				
4‴a	Q	63.5				
5‴	D	71.3				
6‴	Q	16.6				

*, + Assignments within these groups could be interchanged.

Fig. 2. Rubradirin degradation products.



acids. Rubradirin aglycone, **7**, isolated along with rubranitrose following a mild acidic hydrolysis of rubradirin[11], was treated with aqueous methylamine, affording rubransarol A and the methylamide of rubradiric acid A, aglycone **8**. Treatment of **8** with acetic anhydride in pyridine then gave, in addition to **5**, compounds derived from the dipicolinamide moiety, among which were the hemiacid, **9**, and its decarboxylation[12] product, **10**. The free carboxyl in **9** located the site of the coumarin amide. The ring proton in this compound was recognized in the NMR as a singlet at δ 7.5. Following decarboxylation to **10**, this proton signal shifted upfield to δ 6.75 appearing as a doublet, coupled (J = 6 Hz) to the new proton (δ 7.7) formed at the decarboxylation site. This required that the original proton common to both **9** and **10** be placed at position 5″ rather than 3″.

The fact that the rubradirins borrow structural features from three families of antibiotics as diverse as the ansamycins, novobiocins, and the everninomicins, establishes it as one of the more unique products of secondary metabolism. Biosynthesis of the rubransarol carbon skeleton could parallel that of its nearest kin, geldanamycin[13], exclusive of an acetate unit between carbons 2 and 3. Tyrosine would be a likely precursor for the coumarin group, as it is for that unit in the novobiocins[14]. A route to dipicolinic acid from aspartate has been described[15]. Subsequent oxidations, one stage for rubradirin B and two for rubradirin, could then give rise to the appropriate "hub moieties.".

The oxidation of an amine to a nitro group has been demonstrated with *Penicillium atrovenetum*[16]. A sugar epimeric at C-3 to vancosamine[17] could thus be oxidized to rubranitrose.

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