POLYENE ANTIBIOTICS. VIII
THE STRUCTURE OF RIMOCIDIN[1,2]

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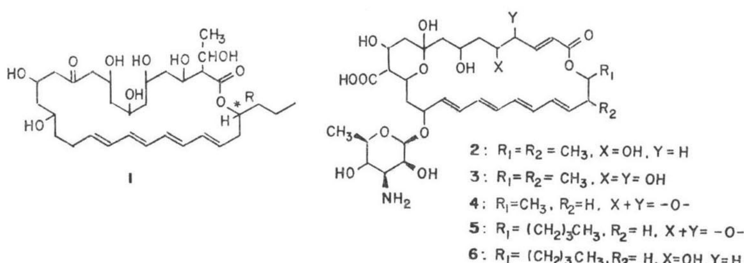
A structure is assigned to the tetraene antibiotic rimocidin, based in part on ^{13}C NMR spectroscopy and field desorption mass spectrometry. The structure assigned revises a structure proposed in 1966 which was anomalous in relation to those of other polyene antibiotics.

Recently, in the course of reviewing the chemistry of polyene antibiotics,[2] we were led to consider the structure of the tetraene antibiotic rimocidin.[3] Partial structure **1** was assigned to rimocidin by COPE, *et al.*,[4] in 1966, but the highly productive structural efforts of that group,[4,5] summarized in part in Fig. 1, were halted by the untimely death of Professor COPE.



The partial structure **1** (for rimocidinolide, to which mycosamine was attached at an unspecified location) assigned to rimocidin seemed to us to be inconsistent with those assigned to other tetraene antibiotics[2,6]—tetrins A[7] and B[8] (**2** and **3**), pimaricin[9] and lucensomycin[10] (**4** and **5**), arenomycin B (**6**).[11] All of those antibiotics (**2**~**6**) have free carboxyl groups and are zwitterionic, while structure **1** lacks a carboxyl group. All of those antibiotics (**2**~**6**) have mycosamine linked to a hydroxyl group adjacent to the tetraene system; while the position of mycosamine was unspecified in **1**, that partial structure lacks the appropriate hydroxyl on C-17 (adjacent to the tetraene). In view of these clear discrepancies we undertook a reinvestigation of the structure of rimocidin.

^{13}C NMR Data. Identification of a Carboxyl and a Second Keto Group

From the outset of our reinvestigation it was clear that our doubts were well founded. The ^{13}C NMR spectrum of rimocidin sulfate (Table 1) contains three absorptions clearly due to carbonyl carbons—at 209.6 ppm (saturated ketone), at 172.6 ppm (lactone), and at 174.8 ppm (carboxyl). [For comparison, the ketone carbon of erythromycin is found at 221.9 ppm and the lactone and carboxyl carbonyl carbons of *N*-acetylamphotericin B are found at 170.5 and 174.3 ppm, respectively.][1] The presence of a carboxyl group in rimocidin was readily confirmed by its conversion (Fig. 2) to a carbomethoxy group (^{13}C NMR, 173.2 ppm; ^{1}H NMR, 3.66 ppm; *cf.* amphotericin B methyl ester, 172.9 ppm and 3.68 ppm)[1] on treatment of rimocidin sulfate with diazomethane. Similarly, derivatives of

Fig. 1. Key degradation products obtained by COPE, *et al.*[4,5] in their studies of rimocidin.

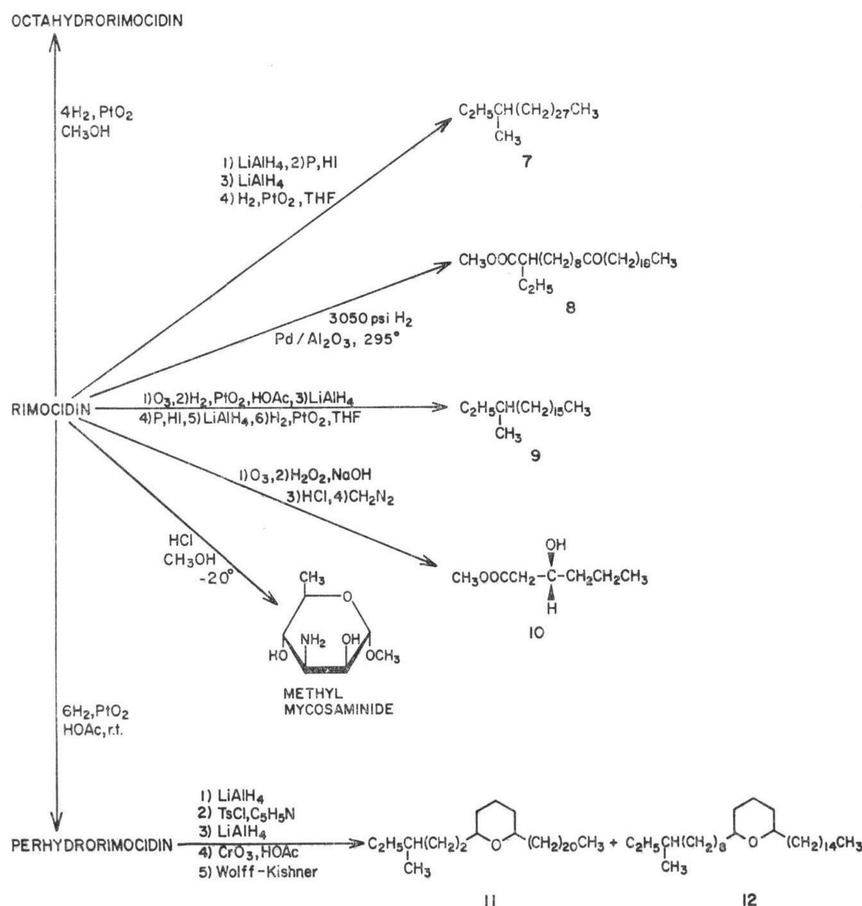
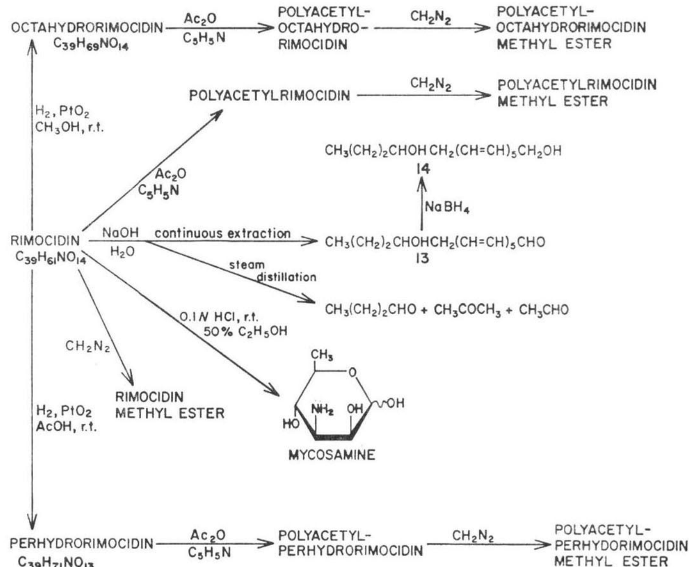


Table 1. Selected ^{13}C NMR signals in spectra of rimocidin sulfate and its derivatives

Assignments	δ, ppm[a,b]			
	Rimocidin sulfate	Rimocidin sulfate methyl ester	Octahydrorimocidin sulfate	Perhydrorimocidin sulfate
-C=O				
ketone	209.6s	208.8s	208.1s	209.6s
carboxyl	174.8s	173.2s	174.3s	176.4s
lactone	172.6s	172.1s	172.4s	173.7s
O-C(-)-O				
hemiketal	97.2s	97.2s	97.1s	
acetal[c]	97.1d	97.1d	96.8d	98.4d
$-OCH_3$		53.2q		
$-CH_3$	17.6q[c]	17.7q[c]	17.8q[c]	17.8q[c]
	13.7q	13.7q	13.7q	13.8q
	11.7q	11.6q	11.6q	11.7q

[a]Ppm from TMS, DMSO-d_6 solutions. [b]Multiplicity in off-resonance spectra: s=singlet, d=doublet, q=quartet. [c]Mycosamine.

Fig. 2. Derivatives and degradation products obtained from rimocidin in the present study.



rimocidin were converted to their methyl esters (*cf.* Fig. 2 and below). The failure of COPE, *et al.*[4] to observe the carboxyl carbon was presumably due to its loss by decarboxylation during reduction to the saturated hydrocarbon **7** (Fig. 1).

In addition to the unrecognized carboxyl, a hemiketal carbon (singlet in off-resonance spectrum) was found at 97.3 ppm, accompanying the mycosamine acetal carbon (doublet in off-resonance spectrum) at 97.1 ppm. Similar absorptions are found for pimaricin at 97.0 and 96.9 ppm, respectively.[1] Thus, rimocidin has two keto groups, rather than the one shown in **1**, and one keto group is in the form of a hemiketal.

Mass Spectrometric Studies. Assignment of Molecular Formula

The molecular formula required for rimocidin by structure **1** for rimocidinolide and the formula of mycosamine is $C_{38}H_{63}NO_{13}$ (mol wt, 741; $C_{32}H_{52}O_{10}+C_6H_{13}NO_4-H_2O$). If a carboxyl is added ($+CO_2$), and a carbinol replaced by a keto group ($-H_2$), a revised molecular formula would be $C_{39}H_{61}NO_{15}$, corresponding to the molecular weight 783. A good bit of effort was expended in the present study in attempts to determine by mass spectrometry the correct molecular formula, which we now assign as $C_{39}H_{61}NO_{14}$ (mol. wt, 767). Elemental analyses of rimocidin sulfate are in agreement with this formula as the hydrate ($C_{39}H_{61}NO_{14}\cdot 1/2H_2SO_4\cdot H_2O$). More importantly, the field desorption (FD) mass spectrum of rimocidin sulfate contained ions at *m/e* 731 ($M-2H_2O$) and *m/e* 714 ($M+H-3H_2O$). However, since the FD spectrum also contained doubly charged ions at *m/e* 815[$(2M+SO_4)/2$], 798 [$(2M+H_2SO_4-2H_2O)/2$], and 780 [$(2M+H_2SO_4-4H_2O)/2$] and exceptionally intense doubly charged ions at *m/e* 690 [$(2M+HSO_4-C_6H_{13}NO_4-2CO_2)/2$] and 681 ($690-H_2O/2$), we attempted to obtain clearer molecular weight results on derivatives.

No molecular ions were observed in the electron impact (EI) or FD mass spectra of polyacetylrimocidin or its methyl ester (Fig. 2), although the former had an ion at *m/e* 897 ($M-2HOAc-CO_2$ for a heptaacetyl derivative) in its FD mass spectrum and the latter had ions at *m/e* 955, 895 and 835 (M−2HOAc, M−3HOAc, M−4HOAc for a heptaacetyl derivative; Table 2) in its EI mass spectrum. Similar mass spectral data were obtained for octahydrorimocidin,[5] and for polyacetyloctahydrorimocidin and its methyl ester (Fig. 2). Octahydrorimocidin gave a difficultly interpretable FD mass spectrum, but the FD mass spectrum of its polyacetyl derivative gave ions at *m/e* 797 ($M-2HOAc-CO_2$) and 737 ($M-3HOAc-CO_2$), while the EI mass spectrum of the third (Table 1) gave the

Table 2. Ions observed in EI mass spectra of polyacetyl derivatives

Assignments	Ions			
	Polyacetyl-rimocidin methyl ester	Polyacetyl-octahydro-rimocidin methyl ester	Polyacetyl-perhydro-rimocidin	Polyacetyl-perhydro-rimocidin methyl ester
M			1055	1069
$M-CO_2$			1011	
M−HOAc			955	1009
$M-HOAc-OCH_3$				978
$M-HOAc-C_2H_2O$			953	967
$M-HOAc-CO_2$			951	
M−2HOAc	955		935	949
$M-2HOAc-C_2H_2O$			893	907
$M-2HOAc-CO_2$			891	
M−3HOAc	895	903	875	
$M-3HOAc-C_2H_2O$			833	847
M−4HOAc	835	843	815	
$M-4HOAc-C_2H_2O$		801	773	
M−5HOAc	775	783		
M−HOAc−288[a]			707	721
$M-HOAc-C_2H_2O-288$[a]			665	679
M−2HOAc−288[a]	666[d]		647	
$M-2HOAc-C_2H_2O-288$[a]			605	619
M−3HOAc−288[a]	606[d]	615	587	
$M-3HOAc-C_2H_2O-288$[a]			545	
M−4HOAc−288[a]	546[d]	555	527	
$C_{12}H_{19}NO_7$[b]	289			
$C_{12}H_{18}NO_6$[c]	272		272	272

[a] 288=mycosaminyloxy. [b] Mycosamine. [c] Mycosaminyl. [d] M−nHOAc−289.

usual series of ions for M−nHOAc and M−nHOAc−triacetylmycosaminyloxy (288 amu), beginning with n=3 (*m/e* 903, *m/e* 615).

The best mass spectral results of all were obtained with perhydrorimocidin[5)] and its derivatives (Fig. 2). The highest ion in the FD mass spectrum of perhydrorimocidin sulfate was at *m/e* 762.5008 ($C_{39}H_{72}NO_{13}$, M+H). The EI mass spectrum of polyacetylperhydrorimocidin (Fig. 2) contained a molecular ion at *m/e* 1055 and its FD mass spectrum also contained a molecular ion, at *m/e* 1056 (M+H). The molecular ion in the EI mass spectrum of polyacetylperhydrorimocidin methyl ester appeared at *m/e* 1069. Fragment ions from these polyacetyl derivatives are shown in Table 2. Polyacetylperhydrorimocidin loses as many as 4 acetic acid units, giving a series of ions at *m/e* 995 (M−HOAc), 935, 875, and 815. The loss of acetylated mycosaminyloxy ($C_6H_9NO_3Ac_3$-O-, 288 amu) gives another series of ions beginning at *m/e* 707 (M−HOAc−288), in addition to triacetylmycosamine (*m/e* 289) and triacetylmycosaminyl (*m/e* 272) ions. The ion at *m/e* 527 (M−4HOAc−288) is especially significant, since it demonstrates conclusively the presence of 7 acetyl groups in polyacetylperhydrorimocidin, *i.e.*, at least 4 hydroxyl groups in the rimocidinolide portion of the molecule. Subtracting $7\times 42=294$ from 1055 gives a molecular weight of 761 for perhydrorimocidin, corresponding to the molecular formula $C_{39}H_{71}NO_{13}$, in agreement with the FD mass spectrum of perhydrorimocidin sulfate. In view of the molecular formula assigned to rimocidin above ($C_{39}H_{61}NO_{14}$) this indicates addition of 5 moles of hydrogen (four of them in the tetraene unit) and loss of one oxygen (by hydro-

genolysis) during the hydrogenation.

The ^{13}C NMR spectrum (Table 1) of perhydrorimocidin sulfate shows characteristic signals at 209.6 (s, ketone), 176.4 (s, carboxyl), 173.7 (s, lactone), and 98.4 ppm (d, mycosamine), but no singlet near 98 ppm in the off-resonance spectrum, indicating absence of the hemiketal group in the perhydroderivatives. Thus, since only one aliphatic ketone carbon is observed (209.6 ppm), the other must have been reduced during hydrogenation, accounting for the absorption of the fifth mole of hydrogen.

Carbon Skeleton and Oxygenation Pattern from the COPE Studies

Before describing our own degradative studies, we shall review briefly those of the COPE group. We have shown in Fig. 1 what we regard as the most significant degradation products isolated from the COPE studies.[4,5] Exhaustive reduction ("COPE reduction") gave 3-methyluntriacontane (**7**), while ozonolysis followed by COPE reduction gave 3-methylnonadecane (**9**). These hydrocarbons, which have been synthesized,[5] establish the carbon skeleton and locate the tetraene system. The other end of the tetraene system was identified from the sequence ozonolysis-peroxide oxidation-esterification, which gave methyl 3*R*-3-hydroxyhexanoate (**10**). A compound related to **10**, 1,3-hexanediol, was isolated when rimocidin was subjected to the sequence ozonolysis—borohydride reduction—acidification—basic hydrolysis—ether extraction. Since 1,3-hexanediol was not isolated when the basic hydrolysis step was omitted, its 3-hydroxyl (*i.e.*, the C-27 hydroxyl of rimocidin) must have been involved in lactone formation. High pressure hydrogenation followed by esterification with diazomethane ("CEDER reduction")[12] gave methyl 2-ethyl-11-oxotriacontanoate (**8**, which, like **7** and **9**, has been synthesized),[4] locating the lactone carbonyl at C-1 as well as the keto group at C-11. Two other compounds of importance, isolated from a modified COPE reduction carried out on perhydrorimocidin, are the cyclic ethers **11** and **12**, which serve to locate oxygen atoms at C-5 and C-9 (**11**) and at C-11 and C-15 (**12**). On the basis of the degradation products in Fig. 1 the partial structure **a** can be written.

```
              C-5      C-9     C-11    C-15
              O-       O-      O       O-
C-1  |   |    |    |   |    |  ||  |   |    |                                  C-27
CO·C-(C)2-C-(C)3-C - C - C-(C)3-C-(C)2-(CH=CH)4-CH2CH(CH2)2CH3
|    |   |    |    |   |    |      |        |                                 |
|  -C-CH3                                                                      O
|    |                                                                         |
|______________________________________________________________________________|
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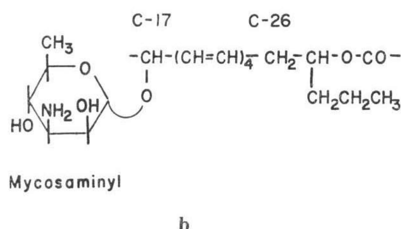
a

Acidic Hydrolysis. Location of Mycosamine

As noted above, the aminosugar mycosamine is located at an allylic position in the other tetraene antibiotics studied. The usual test for this location involves establishing a relatively easier acidic cleavage of the mycosamine glycosidic bond for the native antibiotic than for its hydrogenated derivative *i.e.*, a preferential allylic cleavage. We have now shown that rimocidin sulfate liberates mycosamine at room temperature on treatment with 0.1 N hydrochloric acid in 50 % aqueous ethanol, while under identical conditions perhydrorimocidin sulfate does not yield any mycosamine. As shown for tetrins A[7] and B,[8] these experiments clearly indicate the attachment of the mycosamine unit at a carbon adjacent to the tetraene chromophore. A comparison of the ^{13}C NMR chemical shifts of mycosamine C-1 in rimocidin sulfate and its derivatives (97.6$\pm$0.5 ppm, Table 1) with those reported for C-1 of mycosamine in amphotericin B and its derivatives[1] (96.5$\pm$0.6 ppm), where the anomeric center has been assigned by X-ray crystallography, clearly indicates that the glycosidic linkage is *beta* in rimo-

cidin (as well as in nystatin A_1, tetrins A and B, pimaricin and lucensomycin and their derivatives (96.7±1.4 ppm)).[1]

The partial structure **a** (based on COPE's work) locates the tetraene system from C-18 through C-25; thus, an allylic glycosidic linkage must be at either C-17 or C-26. Location at C-26 is eliminated by the earlier isolation of methyl 3-hydroxyhexanoate (**10**, Fig. 1) following ozonolysis of rimocidin. Thus, the partial structure **b** is assigned.

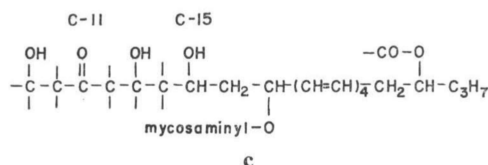


b

Basic Hydrolysis. Isolation of a Pentaenal and Butanal

The location of the tetraene system assigned by COPE, *et al.*[5] was confirmed and amplified in the present study. Rimocidin sulfate on treatment with 2 N aqueous sodium hydroxide during continuous liquid-liquid extraction with ether yielded a yellow crystalline compound which was identified as 13-hydroxy-2,4,6,8,10-hexadecapentaenal (**13**, Fig. 2). The molecular formula of **13** was assigned as $C_{16}H_{22}O_2$ by high resolution mass spectrometry and the oxygens were identified as being in aldehyde and hydroxyl groups by the ^1H NMR ($CDCl_3$) and infrared (KBr) spectra: $-\overset{\text{O}}{\overset{\|}{\text{C}}}\text{H}$, δ 9.49 ppm, d, J=9 Hz, 1675 cm^{-1}; OH, 3445 and 1158 cm^{-1}. The ultraviolet maximum at 370 nm is in accord with that expected for a pentaenal,[7,13] and the compound must be unsubstituted at C-12 from the position of the methylene protons at 2.31 ppm and unsubstituted at C-15 from the splitting of the methyl protons at 0.92 ppm (triplet, J=7 Hz). A peak in the mass spectrum at m/e 73 is in accord with the fragment $CH_3CH_2CH_2\overset{+}{C}HOH \leftrightarrow CH_3CH_2CH_2CH{=}\overset{+}{O}H$, assigning the hydroxyl to C-13 and the structure of the pentaenal as **13**. Reduction with sodium borohydride gave 2,4,6,8,10-hexadecapentaene-1,13-diol (**14**), whose UV spectrum was that of an isolated pentaene.

From the structure of **13** and the location of mycosamine at C-17, a secondary hydroxyl must be located at C-15 of rimocidin. (The isolation of **12** had earlier[4] assigned an oxygen atom at C-15.) In order to trigger the retroaldol reaction which leads to **13**, a keto group at C-13 would be appropriate. However, the isolation of methyl 2-ethyl-11-oxotriacontanoate (**8**), by COPE, *et al.*,[4] as one of the hydrogenolysis products of rimocidin clearly puts a carbonyl group at C-11 and there is no evidence (negative ferric chloride test, no enolic proton in ^1H NMR spectrum) for a β-diketone in rimocidin. Thus, to explain the formation of the pentaenal **13**, rimocidin must have a hydroxyl at C-13 as well as at C-15. Moreover, the lack of a β-diketone indicates that the oxygen at C-9 (found in **11**)[4] must be in a hydroxyl rather than a keto group. Partial structure **c** can then be assigned.



c

In a second basic hydrolysis, rimocidin sulfate was treated with sodium hydroxide and the product was steam distilled into a solution of 2,4-dinitrophenylhydrazine to give the 2,4-dinitro-phenylhydra-

zones of acetaldehyde ($C_8H_8N_4O_4$; mol. wt by EIMS, 224.0543), acetone ($C_9H_{10}N_4O_4$; 238.0703), and butanal ($C_{10}H_{12}N_4O_4$; 252.0861), which were separated by preparative thin-layer chromatography. Acidification of the residue (*ca.* pH 2) and further steam distillation gave only acetaldehyde as a dinitrophenylhydrazone.

Fig. 3. ^{1}H NMR spectrum of rimocidin sulfate (220 MHz, C_5D_5N).

The isolation of butanal from rimocidin was initially puzzling; however, examination of the ^{1}H NMR spectrum of rimocidin sulfate (Fig. 3; see also ^{13}C NMR data in Table 1) revealed that there were three methyl groups: a doublet at 1.46 ppm (J=6 Hz, mycosamine) and two triplets (J=7 Hz) at 0.88 and 1.00 ppm. One of the latter is the terminal methyl group (C-30). The other must be due to the C-2′ methyl group. The substituent at C-2 was pictured as an α-hydroxyethyl group by COPE, *et al.*,[5] but our evidence indicates it must be an ethyl group. Moreover, in order for rimocidin to give butanal on sodium hydroxide treatment, the antibiotic must contain a hydroxyl group at C-3 and there must be either a hydroxyl or a keto group at C-5 (as deduced earlier by COPE *et al.*,[4] from **11**), *i.e.*, partial structure **d** is defined.

Combining the partial formulas **c** and **d** with **a** gives partial formula **e** for rimocidin.

```
d:  C-1 ... C-5
    -O-CO-CH(CH2CH3)-CH(OH)-C-C(O)-      (CH2CH3 = C-2')

e:  C-1, C-3, C-5, C-9, C-11, C-13, C-15
    CO-CH(C2H5)-CH(OH)-C-C(O)-(C)3-C(OH)-C-C(=O)-C-C(OH)-C-C(OH)-CH-CH2-CH(O-mycosaminyl)-(CH=CH)4-CH2-CH(O)-CH2C2H5
    (ring closed between C-1 carbonyl and the terminal O)
```

d **e**

The formation of acetone in the basic cleavage could, and probably does, come from C-10, C-11, and C-12 by retroaldol cleavage involving the hydroxyls at C-9 and C-13. Acetaldehyde presumably comes from C-13 and C-14 following two retroaldol cleavages.

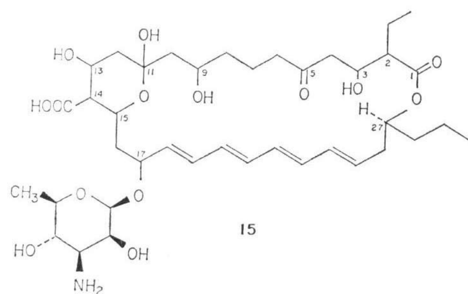
Structure of Rimocidin

The partial structure **e** contains 12 oxygen atoms which, with the two oxygen atoms of the carboxyl group, constitute the total of 14 oxygen atoms required by the molecular formula $C_{39}H_{61}NO_{14}$. The known functional groups—4 olefinic double bonds, 3 carbonyl double bonds (carboxyl, lactone, ketone), and three rings (hemiketal, mycosamine, and lactone)—require a total of 10 elements of unsaturation, in agreement with the molecular formula. Except for the carboxyl group, the only additional element of unsaturation to add to **e** is the second ketone. In order to trigger the retroaldol cleavage which leads to butanal this function must be at C-5 (since there is no oxygen at C-7).

Placement of the carboxyl is allowed by the observation that acetaldehyde is generated by steam distillation of the acidified retroaldol reaction mixture. Acetaldehyde must have come from decarboxylation of malonaldehydic acid ($HOOCCH_2CHO$) in the solution, and the latter compound must have come from C-13 and C-14, the source of acetaldehyde in rimocidin. Thus, the carboxyl is on C-14 and the structure of rimocidin is assigned as **15**.* Placement of the carboxyl on C-14 agrees with the corresponding location in tetrins A and B (**2** and **3**), pimaricin (**4**), lucensomycin (**5**) and arenomycin B (**6**) and, in keeping with that location, the ^{13}C NMR absorption of the carboxyl is like those of **2**~**5** (174.3±0.2 ppm).[1]

We have drawn rimocidin in **15** with the 11-keto group in the hemiketal form, since the corresponding keto groups (the only keto groups) in other tetraene antibiotics (**2**~**5**) are in the hemiketal form and their hemiketal carbon's ^{13}C NMR absorption is at the same position (97.1±0.2 ppm).[1]

The structure of octahydrorimocidin is obvious but that of perhydrorimocidin is somewhat less clear. It seems likely that it is the 11-keto group which has been reduced since the ^{13}C NMR spectrum of perhydrorimocidin contains a carbonyl carbon's absorption at 209.6 ppm, identical to that of the 5-keto carbon of rimocidin (Table 1). Oxidation of perhydrorimocidin with nitric acid earlier gave 2-ethylglutaric acid,[5] indicating that the 3-hydroxyl group had been hydrogenolyzed. Thus, the most likely structure for perhydrorimocidin is 3-deoxy-11-dihydrorimocidin.



Experimental

General. Melting points, determined on a Kofler hot stage apparatus, are uncorrected. Infrared spectra were determined on Beckman, Model IR12, and Perkin-Elmer, Model 137, infrared spectrophotometers. Ultraviolet spectra were taken on Beckman, Models DB and ACTA MVI, and Perkin-Elmer, Model 202, ultraviolet-visible spectrophotometers. Optical rotations were measured on a Zeiss polarimeter. ^1H NMR spectra were determined by Mr. M. L. MILLER or Mr. S. K. SILBER on Varian HR-220, equipped with Nicolet Instrument Corporation TT220 FOURIER transform accessory, and HA-100 spectrometers. ^{13}C NMR spectra were recorded by Mr. S.K. SILBER and Dr. S. E. ULRICH on a Varian XL-100 spectrometer operated in the FOURIER transform mode with Digilab computer. ^1H and ^{13}C NMR chemical shifts are reported as ppm relative to tetramethylsilane as internal standard.

* Subsequent to our preliminary report,[2] which we discussed with one of Professor BOROWSKI's coworkers in July 1975, BOROWSKI, *et al.*, have, in their own preliminary report [FALKOWSKI, L.; J. GOLIK, J. ZIELINSKI & E. BOROWSKI: The structure of rimocidin. J. Antibiotics 29: 197~198, 1976], employed quite different derivatives and arguments to assign the same structure (**15**) to rimocidin.

Low resolution mass spectra were determined by Mr. J. WRONA with a Varian MAT spectrometer, Model CH5 DF, employing the direct probe technique. High resolution and field desorption mass spectrometric measurements were made by Mr. J. C. COOK, Jr., on a Varian MAT 731 mass spectrometer. Microanalyses were determined by Mr. J. NEMETH and his associates. Gas chromatography was carried out with a Varian gas chromatograph, Series 1700, using 6 ft × 2 mm (id) helical glass columns. Analytical and preparative thin-layer chromatography was carried out on Analtech precoated (250 μ or 2000 μ) silica gel G plates. The spots were visualized either by iodine vapor, UV light, ninhydrin spray, 2,4-dinitrophenylhydrazine - 2 N hydrochloric acid spray (DNPH spray) or sulfuric acid spray.

Rimocidin Sulfate. The sample used in the present investigation* showed a single spot in thin-layer chromatography using 1-butanol-ethanol-water; 1 : 1 : 1 (Rf 0.70) or the upper layer of 1-butanol-ethanol-water; 4 : 1 : 5 (Rf 0.37), giving a violet spot with ninhydrin spray, an orange yellow spot with DNPH spray, a black spot with sulfuric acid spray. The sample started darkening at 145 °C, softened at 155 °C, and melted above 250 °C with decomposition (lit.[1] mp 151°C dec.); $[\alpha]_D^{25}$ +72.1° (*c* 0.74, MeOH), [lit.[1] $[\alpha]_D^{25}$+75.2 ° (*c* 1, MeOH)]. The ultraviolet spectrum had $\lambda\lambda_{max}$ (ε) 317 (62,722), 303 (69,606), 290 (45,894), and 278 (25,242) nm. Its IR spectrum (KBr) had bands at 3440, 1070 (OH), 1715 (C=O), and 1635 (C=C) cm^{-1}. Its ^1H NMR spectrum contained characteristic peaks (DMSO-d_6, 220 MHz) at 0.84 (t, J=7 Hz), 0.88 (t, J=7 Hz), and 1.21 (d, J=6 Hz); and (C_5D_5N, 220 MHz) at 0.88 (t, J=7 Hz), 1.00 (t, J=7 Hz), 1.46 (d, J=6 Hz). The ^1H NMR spectrum in C_5D_5N is shown in Fig. 3. Its ^{13}C NMR spectrum (DMSO-d_6) contains characteristic peaks at 209.6 (ketone >C=O), 174.8 (carboxyl C=O), 172.6 (lactone C=O), 97.2 (hemiketal carbon), 97.1 (acetal carbon of mycosamine), 17.6 (–CH_3 of mycosamine), 13.7 (CH_3), and 11.7 (CH_3). The electron impact mass spectrum did not give ions above *m/e* 580, while the field desorption mass spectrum showed the highest ion at *m/e* 815 (see text).

Anal.	Calcd for $C_{39}H_{61}NO_{14}\cdot 1/2\ H_2SO_4$:	C, 57.35; H, 7.48; N, 1.72; S, 1.96.
	Calcd for $C_{39}H_{61}NO_{14}\cdot 1/2H_2SO_4\cdot H_2O$:	C, 56.12; H, 7.67; N, 1.68; S, 1.92.
	Found:	C, 56.12; H, 7.78; N, 1.90; S, 2.22.

Rimocidin Sulfate Methyl Ester. A solution of diazomethane in tetrahydrofuran was added to a solution of rimocidin sulfate (220 mg) in methanol (10 ml) and the yellow solution was left at room temperature for 8 hours. Excess diazomethane was decomposed by a few drops of acetic acid, the solution was filtered, solvent was removed and the residue was precipitated from ether to afford 149 mg of the product; ^1H NMR (DMSO-d_6, 220 MHz) 0.85 (t, J=7 Hz), 0.89 (t, J=7 Hz), 1.25 (d, J=6 Hz), 3.66 (s, –$COOCH_3$); ^{13}C NMR data in Table 1.

Polyacetylrimocidin. A solution of 200 mg of rimocidin sulfate, 0.5 ml of pyridine, and 2 ml of acetic anhydride was stirred for 24 hours at 22 °C, excess acetic anhydride was decomposed with cold deionized water, solvent was removed at room temperature under vacuum. The foamy residue was taken up in chloroform-methanol (1 : 1), and the solution was treated with Norite, filtered, and concentrated to afford 276 mg of a foamy residue; mp 110~114 °C (d); $\lambda\lambda_{max}$ 318, 303, 290, 279 (sh), and 206 nm; IR ($CHCl_3$) 1740, 1238, 670 (acetate), 3440, 1680 (amide), 1635, 1515 cm^{-1} (C=C); ^1H NMR (C_5D_5N, 220 MHz) 0.88 (t, J=7 Hz), 0.91 (t, J=7 Hz), 1.30 (d, J=6 Hz), 2.02 (br. s), 2.09 (s), 8.31 (br. –N<u>H</u>–, lost with D_2O shake), 12.18 (–COO<u>H</u>, lost with D_2O).

Polyacetylrimocidin methyl ester was prepared by treating 65.7 mg of polyacetylrimocidin in 10 ml of tetrahydrofuran with a solution of diazomethane in tetrahydrofuran (*ca.* 10 ml). Excess diazomethane was decomposed with a few drops of acetic acid, solvent was removed and the residue was dried to afford 69 mg of the product, which was passed through a column of silica gel (19 × 1.5 cm), eluting with chloroform and chloroform-methanol (90 : 10). Removal of solvent afforded 32 mg of a yellow brown foamy residue, mp 119~125 °C (d), showing a single spot on tlc [chloroform - methanol (90 : 10), ninhydrin negative, DNPH spray positive]. The product had $\lambda\lambda_{max}$ 318, 303, 290, 274 (sh) nm; IR ($CHCl_3$) 1740, 1240, 667 (acetate), 3440, 1677 (amide), 1550, 1518 (C=C); ^1H NMR (C_5D_5N, 220 MHz) 3.68 ppm (carbomethoxy).

* Obtained from Dr. W. D. CELMER, Pfizer Laboratories.

Perhydrorimocidin sulfate was prepared according to the method of COPE, *et al.*[2] Rimocidin sulfate was hydrogenated over pre-reduced ADAMS platinum catalyst (200 mg) in glacial acetic acid (50 ml) at atmospheric pressure and room temperature. Hydrogenation was stopped after 48 hours and the catalyst was filtered and washed with fresh acetic acid. Removal of solvent afforded an amorphous powder (259.3 mg), mp 140~143 °C, which showed two very close spots on tlc using BEW 415 (Rf 0.41 and 0.45) and a single spot using BEW 111 (Rf 0.73). It gave a light yellow color with DNPH spray, a violet color with ninhydrin spray and a black color with sulfuric acid spray. The product showed very weak end absorption ($\varepsilon_{206}=721$). Its IR spectrum (KBr) showed bands at 3440, 2940, 2860, 1725, 1635, and 1075 cm^{-1}. The ^{1}H NMR spectrum (DMSO-d_6, 220 MHz) did not show any olefinic protons from 4.90 to 10.00 ppm but showed signals at 0.86 (br. s, t after addition of D_2O), 1.27 (br. s), 1.47 (br. s), and 5.0 ppm (br. –OH, lost with D_2O); for ^{13}C NMR data see Table 1. Its FD mass spectrum (17 ma) showed ions at *m/e* 762 (M+H), 745, 729, 703, and 685 and doubly charged ions from *m/e* 330 to 425.

Anal. Calcd for $C_{39}H_{71}NO_{13}\cdot 0.5H_2SO_4$: C, 57.78; H, 8.89; N, 1.73; S, 1.98.
Found: C, 58.68; H, 9.12; N, 2.01; S, 2.31.
Calcd for $C_{39}H_{72}NO_{13}$ (M+H): 762.5004.
Found: 762.5008 (HRFDMS).

Polyacetylperhydrorimocidin. A mixture of perhydrorimocidin sulfate (165 mg), pyridine (0.5 ml) and acetic anhydride (2 ml) was stirred at 22 °C for 24 hours. Excess acetic anhydride was decomposed at 0 °C with water, solvent was removed under high vacuum and the residue was dissolved in chloroform. The chloroform solution was washed with water and brine and dried over sodium sulfate. Removal of solvent afforded a residue which was passed through a column of silica gel (20 cm × 1.5 cm), eluting with chloroform and chloroform - methanol to afford 176 mg of the derivative, which showed a single spot on tlc and the characteristic mass spectral fragmentation reported in Table 2.

Anal. Calcd for $C_{53}H_{85}NO_{20}$: mol wt, 1055. Found: mol wt, 1055 (MS).

Polyacetylperhydrorimocidin Methyl Ester. A solution of diazomethane in tetrahydrofuran was added to a solution of 40 mg of polyacetylperhydrorimocidin in 5 ml of methanol until the yellow color persisted. The mixture was left at room temperature for 4 hours, excess diazomethane was decomposed with a few drops of acetic acid, solvent was removed under vacuum, and the residue was passed through a column of silica gel (19 cm × 1.5 cm). The product was eluted with chloroform - methanol, and solvent was removed to afford a viscous residue (32.8 mg). The product showed a single spot on tlc (chloroform - methanol, 90 : 10) which was yellow when sprayed with DNPH, black when sprayed with sulfuric acid, and was ninhydrin-negative. The UV spectrum showed only end absorption below 208 nm, the IR spectrum ($CHCl_3$) had characteristic peaks at 1735, 1235, 665 (acetate), 3435, 1675 cm^{-1} (amide) and no peaks for hydroxyl groups, the ^{1}H NMR spectrum ($CDCl_3$, 220 MHz) showed signals at 0.90 (t, 6, J=7 Hz; CH_2–CH_3), 1.27 [br. s, $(-CH_2)_x-$], 1.88, 2.03, 2.04, 2.05, 2.14 (all s, 21, $-OCOCH_3$ and $-NH-COCH_3$), and 3.65 ppm (s, 3, $-COOCH_3$). The mass spectral fragmentation is reported in Table 2.

Anal. Calcd for $C_{54}H_{87}NO_{20}$: mol wt, 1069. Found: mol wt, 1069 (MS).

Polyacetyloctahydrorimocidin Methyl Ester. Rimocidin (274.8 mg, prepared according to COPE's procedure[5]) from rimocidin sulfate) was hydrogenated over pre-reduced ADAMS platinum catalyst (100 mg) in 60 ml of methanol at room temperature and atmospheric pressure. After hydrogenation had ceased (4 hours), the catalyst was filtered and washed with fresh methanol, solvent was removed and the residue was precipitated from ether. The precipitate was centrifuged, washed with fresh ether, and dried to give 237.7 mg of octahydrorimocidin, mp 125~130 °C (d), single spot on tlc in BAW 415 (Rf 0.37), BEW 415 (Rf 0.43), and BEW 711 (Rf 0.27), positive to ninhydrin (violet) and DNPH (deep yellow) sprays. Its IR (KBr) spectrum had bands at 3430, 1065 (OH) and 1715 cm^{-1} (C=O). The UV spectrum had ε_{280} (2,019) and ε_{208} (1,579). The ^{1}H NMR spectrum (C_5D_5N, 100 MHz) showed peaks at 0.91 (t, J=7 Hz), 1.04 (t, J=7 Hz), 1.32 (s), and 1.54 ppm (d, J=6 Hz), but no olefinic protons from 5.00 to 10.00 ppm; ^{13}C NMR data are in Table 1.

Octahydrorimocidin (50.4 mg) was stirred with pyridine (0.5 ml) and acetic anhydride (2 ml) under anhydrous conditions at 22 °C for 22 hours. Excess acetic anhydride was decomposed with 2 ml of water at 0 °C and solvent was removed under vacuum. The residue was dissolved in chloroform (40 ml), washed with water (2×25 ml) and brine (25 ml), and dried over sodium sulfate. Solvent removal gave a foamy residue (67.7 mg) which was converted into the methyl ester as described in the next paragraph.

Polyacetyloctahydrorimocidin (50 mg) was dissolved in 5 ml of methanol and an excess of diazomethane in ether was added. The reaction mixture was left at room temperature for 1 hour, excess diazomethane was decomposed by a few drops of acetic acid and solvent was removed. The residue was passed through a column of silica gel (20 cm×1.5 cm) and the product was eluted with chloroform - methanol (90 : 10). Solvent removal gave a viscous residue (41.4 mg) which showed a single spot on tlc, yellow with DNPH spray, black with sulfuric acid. Its IR spectrum ($CHCl_3$) showed bands at 3440 (very weak), 3020, 2935, 2860, 1737, 1675, 1517, 1438, 1375, 1240, 1070, 1050, and 667 cm^{-1}. The ^1H HMR spectrum ($CDCl_3$, 220 MHz) showed signals at 3.65 ($-COOCH_3$), 1.88, 2.02, 2.04, 2.06, 2.14 ($-OCOCH_3$, $-NHCOCH_3$), 1.28 [$-(CH_2)_x-$], and 0.9 ppm (CH_3).

Mycosamine from Acidic Hydrolysis of Rimocidin Sulfate. Rimocidin sulfate (50.9 mg) was stirred at room temperature with 0.1 N hydrochloric acid in 50 % aqueous ethanol (25 ml) for 24 hours. Ethanol was removed under vacuum at room temperature and the residue was freeze-dried to afford 34.5 mg of product. Thin-layer chromatographic analyses indicated a positive ninhydrin spot corresponding to mycosamine [Rf 0.21 (BAW 415), Rf 0.41 (BEW 415), Rf 0.72 (methanol : 30 % aq. ammonia - water (4 : 1 : 5)] and no spot corresponding to rimocidin sulfate. The identity of mycosamine was further confirmed by preparing trimethylsilyl derivatives of the above hydrolyzate and of an authentic sample of mycosamine and injecting the derivatives on a glass column 6′×2 mm (id) containing 3 % OV-17 on 100~120 GCQ. With a temperature programming of 8 °/min from 80 °C to 300 °C and a flow of 18 ml/min the TMS derivatives of authentic mycosamine and the hydrolysis product had the same retention time, 10 minutes.

Acidic Hydrolysis of Perhydrorimocidin Sulfate.

Perhydrorimocidin sulfate (35.1 mg) was stirred with 15 ml of 0.1 N hydrochloric acid in 50% aqueous ethanol at room temperature for 26 hours; it was recovered unchanged (35 mg) after employing the usual workup as discussed above.

Basic Hydrolysis of Rimocidin Sulfate.

A. Isolation and Characterization of 13-Hydroxy-2,4,6,8,10-hexadecapentaenal.

A mixture of rimocidin sulfate (500 mg) and 2 N aqueous sodium hydroxide (150 ml) was extracted continuously with ether for 45 hours at room temperature. The light yellow ether extract was washed with water (2×100 ml) and brine (100 ml), dried over sodium sulfate, and concentrated. The residue was crystallized from ether-hexane to afford 33.0 mg of yellow crystalline compound: mp 113~115°C(d); single spot on tlc (C_6H_6-Et_2O-EtOAc, 30: 30: 40, Rf 0.81); $[\alpha]_D^{25}$ −47.0° (*c* 0.9, $CHCl_3$); λ_{max} (MeOH) 370 nm; IR bands (KBr) at 3445, 1158 (OH), 1675 (conjugated C=O), 1620, 1575, 1012 (C=C); ^1H NMR ($CDCl_3$, 220 and 100 MHz) signals at 9.49 (d, 1, J=9 Hz, =CH-C$\underline{H}$O), 6.14 (q, 1, J=15 Hz and 9 Hz, =C$\underline{H}$-CHO), 5.85 (m, 1, =C$\underline{H}$-CH_2-), 2.31 (m, 2, =CH-$\underline{CH_2}$-), 0.93 (t, 3, 7 Hz); mass spectrum (electron impact), *m/e* 247 (17), 246 (75, M^+), 175 (14), 174 (100), 145 (12), 117 (10), 106 (16), 73 (16), 55 (12).

Anal. Calcd for $C_{16}H_{22}O_2$: mol wt, 246.1619. Found: mol wt, 246.1615 (HRMS).

Sodium borohydride (30 mg) was added to a solution of 13-hydroxy-2,4,6,8,10-hexadecapentaenal (10 mg) in 15 ml of methanol at 0°C and the mixture was stirred at that temperature for 30 minutes and at room temperature for another 30 minutes. Water (10 ml) was then added, methanol was removed under vacuum at room temperature and the residue was extracted with ether (4×25 ml). The combined ether extract was washed with water (3×25 ml) and brine (25 ml) and dried over sodium sulfate. Removal of solvent afforded 2,4,6,8,10-hexadecapentaene-1,13-diol as a light yellow residue, which was precipitated from a mixture of ether - pentane (1:1), filtered, and dried (6 mg): mp 116~120°C(d); λ_{max} (MeOH) 343, 325, 311, 298 nm.

Anal. Calcd for $C_{16}H_{24}O_2$: mol wt, 248. Found: mol wt, 248 (mass spectrum).

B. Isolation of Acetone, Acetaldehyde and Butanal.

A mixture of rimocidin sulfate (500 mg) and aqueous sodium hydroxide (500 mg in 50 ml of water) was subjected to steam distillation into a solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. The distillate (2,500 ml) was left overnight at *ca.* 5°C and the crystals were filtered and dried; 17.0 mg, mp 120~125°C. The derivative showed two major spots on tlc which were separated by preparative tlc using benzene as solvent. The faster moving yellow-orange spot was identified as butanal 2,4-dinitrophenylhydrazone by its mass spectrum and ^1H NMR spectrum, which were superimposable on the spectra of an authentic sample.

Anal. Calcd for $C_{10}H_{12}N_4O_4$: mol wt, 252.0858. Found: mol wt, 252.0861 (HRMS).

The slower moving yellow spot was crystallized from ether-hexane; mp 145~155°C and was identified as a mixture of acetone and acetaldehyde 2,4-dinitrophenylhydrazones by mass spectral fragmentations and a high resolution mass spectrum of the molecular ions.

Anal. Calcd for $C_9H_{10}N_4O_4$: mol wt, 238.0702. Found: 238.0703 (HRMS).
Calcd for $C_8H_8N_4O_4$: mol wt, 224.0545. Found: 224.0543 (HRMS).

After steam distillation from base the residue was adjusted with hydrochloric acid to pH 2 and again steam distilled into acidic dinitrophenylhydrazine. The derivative formed was filtered and purified by preparative tlc using benzene-ethyl acetate (95:5) as solvent. The single major band was crystallized from ether-hexane; 5.7 mg, mp 145~149°C and was identified as acetaldehyde 2,4-dintrophenylhydrazone by mixture melting point and comparison of its mass spectrum with an authentic sample.

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