with 25 ml. of ether. Potassium hydroxide (5 g.) was added to the aqueous phase which was then extracted with three 25-ml. portions of chloroform. The chloroform extracts were combined, dried, and evaporated to give 475 mg. of the potassium salt of voacangic acid. The product was crystallized from tetrahydrofuran and dried under vacuum, m.p. 204–208°, $\nu_{max}^{CHCl_3}$ 1580 cm.$^{-1}$.

*Benzoic Acid-O-d.* Benzoyl chloride (2 ml.) was added to 0.5 ml. (excess) of deuterium oxide. After 2 days at room temperature, the crystalline benzoic acid-O-*d* was filtered, rinsed with deuterium oxide, and dried under vacuum.

*Phenol-O-d.* Phenol (5 g.) was dissolved in 10 ml. of hot deuterium oxide. The solution was cooled to room temperature and centrifuged. The upper phase was removed and distilled to give crystalline phenol-O-*d* on cooling.

*Potassium Hydroxide-d.* Potassium *t*-butoxide (5 g.) was dissolved in 3.1 ml. of deuterium oxide. The aqueous phase was removed and warmed to 80° under slight vacuum for about 1 hr. to remove any *t*-butyl alcohol, leaving a solution of approximately 50% potassium hydroxide-*d* in deuterium oxide.

*Voacangine-$d_3$ (VIII).* Nitrosomethylurea (0.6 g., dried at room temperature under vacuum) was added to 10 ml. of ether over 2 ml. of 50% potassium hydroxide-*d*. The partially deuterated (67% D) diazomethane was immediately distilled and added to 30 ml. of dry tetrahydrofuran which contained 2 ml. of deuterium oxide and 200 mg. of phenol-O-*d*. (All glassware and pipets were oven dried and cooled in a desiccator prior to use.) After 1 hr. at room temperature in darkness, 200 mg. of voacangic acid potassium salt was added. Benzoic acid-O-*d* (120 mg. in 0.5 ml. of tetrahydrofuran) was added gradually over 2 min. with gentle stirring. After 15 min. of continued stirring, the mixture was reduced to 5 ml. by evaporation under vacuum. Water (50 ml.) was added and the solution was extracted twice with 50 ml. of ether. Bases were isolated by extraction of the ether solution with two 25-ml. portions of 1 *N* hydrochloric acid. The acidic solution was made basic with potassium hydroxide and extracted with ether to give 94 mg. of basic material after drying and evaporation. The ether phase of the acid partition was rinsed with 5% potassium hydroxide, dried, and evaporated to give 95 mg. of neutral compounds. The base fraction was crystallized twice from methanol to give 51 mg. of voacangine-$d_3$ (VIII), m.p. 136–137°. The mother liquors gave an additional 11 mg. of crystalline voacangine-$d_3$ after chromatography on alumina. The neutral fraction was analyzed by mass spectrometry which indicated a mixture of methylbenzoate-$d_3$ and anisole-$d_3$.

*Voacamine-$d_3$ (IV).* Vobasinol (IX, 50 mg.) and 30 mg. of voacangine-$d_3$ (VIII) were refluxed for 9 hr. in 4 ml. of 2% methanolic hydrochloric acid (from concentrated aqueous HCl). Water (5 ml.) was added and most of the methanol was removed under vacuum. After neutralization with sodium carbonate, the product was extracted twice with 10 ml. of chloroform. The combined extracts were rinsed with 5 ml. of water, then dried over sodium sulfate, and evaporated to give 74 mg. of crude product. Crystallization from methanol gave 19 mg. of voacamine-$d_3$ (IV), m.p. 215–220° dec. An additional 8 mg. of crystals was obtained by chromatography of the mother liquor on alumina.

*Acknowledgment.* This investigation was supported in part by Public Health Service Fellowship 5-Fl-GM-21, 624-02 from the National Institute of General Medical Sciences (to D. W. T.) and a research grant from the National Science Foundation (GP-3734).

# Rimocidin. I. Carbon Skeleton, Partial Structure, and Absolute Configuration at C-27[1]

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*Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.* *Received July 12, 1965*

*The carbon skeleton of rimocidin has been established by conversion to the "parent" hydrocarbon, 3-methyluntriacontane, and by the result of oxidative degradation. The aglycone is $C_{32}$ and contains no free carboxyl group. The carbohydrate component has been identified as a glycoside of* D-*mycosamine (3-amino-3,6-dideoxy-D-mannopyranose). The location of the conjugated tetraene system has been determined. The terminus of the macrocyclic lactone is shown to be at C-27 and the absolute configuration at that center is R.*

The isolation of rimocidin, an antifungal antibiotic from *Streptomyces rimosus* cultures, was briefly reported in 1951.[2] Its ultraviolet spectrum ($\lambda_{max}^{ethanol}$ 279, 291, 304, 318 mμ; $E_{1cm}^{1\%}$ 306, 622, 965, 890) was characteristic of a conjugated tetraene.[3] Preliminary structural investigations were conducted at the Chas. Pfizer Laboratories[4] and at Harvard University.[5]

(1) Support was provided in part by the National Institutes of Health through Public Health Research Grant AI-02241. Acknowledgment is made to Chas. Pfizer and Co., Inc. for a generous gift of rimocidin sulfate.

(2) J. W. Davisson, F. W. Tanner, Jr., A. C. Finlay, and I. A. Solomons, *Antibiot. Chemotherapy*, 1, 289 (1951).

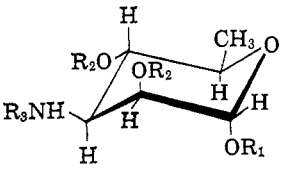
(3) Reported (R. Kunn and C. Grundmann, *Ber.*, 71, 442 (1938)) for the simplest case, 2,4,6,8-decatetraene, is $\lambda_{max}^{hexane}$ 272, 283, 297, and 320 mμ. A much closer analogy is that of pimaricin (O. Ceder, *Acta Chem. Scand.* 18, 77 (1964)), $\lambda_{max}^{ethanol}$ 279, 290, 303, and 318 mμ.

(4) W. M. McLamore and I. A. Solomons, unpublished report.

Potentiometric titrations established the presence of one ester or lactone function and one basic nitrogen atom, and indicated a molecular weight between 700 and 800. Zerevitinov determinations indicated *ca.* ten active hydrogen atoms. Thus it was evident that rimocidin belonged to the diverse and by now extensive class of high molecular weight polyhydroxy many-membered lactones designated as "macrolides."[6,7] The infrared absorption at 1710 cm.$^{-1}$ (potassium bromide or Nujol), thought at first to be at surprisingly low frequency for a saturated lactone, appears to be characteristic for $\alpha,\beta$-saturated macrolides[8] and is not significantly changed on catalytic hydrogenation. Rimocidin hydrogenated in methanol with palladium or platinum as catalyst very rapidly absorbed 4 molar equiv. of hydrogen; in acetic acid with platinum as catalyst hydrogenation proceeded more slowly but did not stop until 6 molar equiv. was absorbed. The products, a crystalline, well-characterized compound (octahydrorimocidin), and an amorphous glass (perhydrorimocidin), respectively, exhibited no ultraviolet absorption (except weak end absorption) and had infrared absorption at 1705 cm.$^{-1}$ (potassium bromide pellet or chloroform solution). Isolation of *n*-dodecanedioic acid as the highest dibasic acid from nitric acid oxidation of octahydrorimocidin proved the presence of ten contiguous methylene groups in the latter. Analyses of rimocidin, octahydrorimocidin, and of their sulfates did not differentiate among a number of possible molecular formulas, $C_{35-41}H_{55-75}O_{11-16}N$, for the free base. Periodate titrations indicated an uptake of 2–2.5 molar equiv. for formulas in this range.

This summarizes the useful information available at the time our studies were initiated in 1962. It appeared probable that the single basic nitrogen atom of rimocidin resided in an amino sugar; this expectation was realized by the isolation (in *ca.* 85% yield) of a crystalline hydrochloride on treatment of rimocidin with anhydrous methanolic hydrogen chloride at −20° for 3 days. It was identified as methyl 3-amino-3,6-dideoxy-$\alpha$,D-mannopyranoside hydrochloride (methyl $\alpha$,D-mycosaminide hydrochloride, **1a**) of established structure[9] and stereochemistry.[10] The identification was confirmed by a similar hydrolysis followed by acetylation: methyl triacetyl-$\alpha$,D-mycosaminide (**1b**)[9] was isolated in a crude yield of 67%.[11]

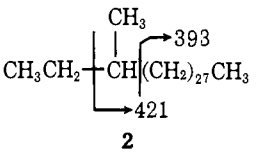
An analysis of the n.m.r. spectra of two derivatives of mycosamine, tetraacetyl-$\alpha$,D-mycosamine (**1c**), and methyl N-acetyl-$\alpha$,D-mycosaminide (**1d**), has been presented by Ceder.[12] The similarities between the spectra of tetraacetate **1c** and triacetate **1b** are noteworthy. The latter shows the C-5 methyl group as a




**1a**, $R_1 = CH_3$; $R_2 = H$; $R_3 = H_2Cl$
**b**, $R_1 = CH_3$; $R_2 = R_3 = Ac$
**c**, $R_1 = R_2 = R_3 = Ac$
**d**, $R_1 = CH_3$; $R_2 = H$; $R_3 = Ac$

doublet ($J$ = 6 c.p.s.) at $\delta$ 1.20. The pattern of the C-5 hydrogen atom, a multiplet centered at $\delta$ 3.93, is very similar in appearance to that described by Ceder for the same proton in tetraacetate **1c** and thus can also be interpreted in terms of further splitting of a quartet ($J$ = 6 c.p.s.), by the (axial) C-4 proton. The three three-proton singlets at $\delta$ 1.92, 2.07, and 2.18 are due to the N-acetyl, the C-4 acetoxy (equatorial), and the C-2 acetoxy (axial) groups, respectively. A three-proton singlet due to the methoxyl group appears at $\delta$ 3.41; the same group in methyl N-acetylmycosaminide (**1d**) appeared at $\delta$ 3.37.[12] The amide proton resonance appears as a doublet ($\delta$ 5.88) with the same coupling constant ($J$ = 8 c.p.s.) as in tetraacetate **1c**. The remaining four protons (C-1, C-2, C-3, C-4) form a complex multiplet at $\delta$ 4.52–5.07.

The carbon skeleton of the aglycone was determined by the phosphorus–hydriodic acid method originally described for fungichromin.[13] We have found this general method amenable to numerous variations and indispensable to the structural investigation of larger macrolides. Perhydrorimocidin was reduced with lithium aluminum hydride to a saturated polyol which on treatment with phosphorus–hydriodic acid followed by lithium aluminum hydride reduction afforded, after filtration through alumina and catalytic hydrogenation, a single hydrocarbon in high yield (34% from perhydrorimocidin). Its retention time on gas chromatography (v.p.c.) was very slightly shorter than that of *n*-dotriacontane and its identity as 3-methyluntriacontane (**2**) followed from inspection of the mass spectrum of a collected sample. The salient features of the spec-




**2**

trum were a weak molecular ion peak at *m/e* 450, moderate peaks at *m/e* 435, 393, and 392, and an extremely intense peak at *m/e* 421 which dominated the spectrum. The last peak was far more intense than any other high-field (*m/e* >120) peak. The presence of an even-numbered peak (*m/e* 392 here) nearly as intense as (and in some other cases of greater intensity than) the expected fragment peak one mass

(5) A. M. Gold, Ph.D. Thesis, Harvard University, 1955. We thank Professor R. B. Woodward for providing us access to the details described therein.

(6) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957).

(7) M. Berry, *Quart. Rev.* (London), 17, 343 (1963).

(8) Reported frequencies of $\alpha,\beta$-saturated macrolides have been in the range 1730–1710 cm.$^{-1}$, *i.e.*, somewhat lower than expected for a saturated ester or six-membered lactone. Two $\alpha,\beta$-unsaturated macrolides, chalcomycin and pimaricin, reportedly absorb at 5.84 $\mu$ (1712 cm.$^{-1}$). Positions of absorption of course can be quite different depending on whether spectra are run in solution or as solids (*cf.* oleandomycin: H. Els, W. D. Celmer, and K. Murai, *J. Am. Chem. Soc.*, **80**, 3777 (1958)).

(9) J. D. Dutcher, D. R. Walters, and O. Wintersteiner, *J. Org. Chem.*, **28**, 995 (1963). An authentic sample was generously supplied by Dr. Dutcher.

(10) M. von Saltza, J. D. Dutcher, J. Reid, and O. Wintersteiner, *ibid.*, 28, 999 (1963).

(11) Identification of the liberated sugar as a specific $\alpha$,D-pyranoside of course does not establish its ring size or anomeric nature in the parent antibiotic.

(12) O. Ceder, G. Eriksson, J. M. Waisvisz, and M. G. van der Hoeven, *Acta Chem. Scand.*, **18**, 98 (1964).

(13) A. C. Cope, R. K. Bly, E. P. Burrows, O. J. Ceder, E. Ciganek, B. T. Gillis, R. F. Porter, and H. E. Johnson, *J. Am. Chem. Soc.*, **84**, 2170 (1962).

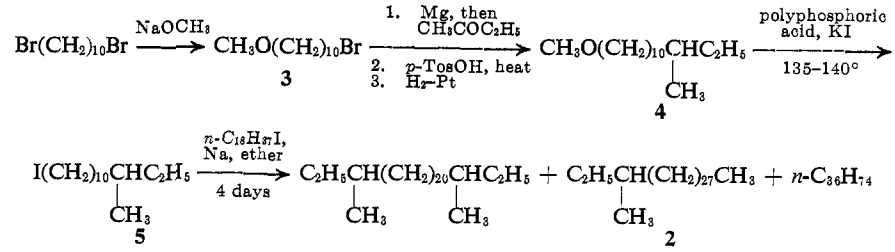


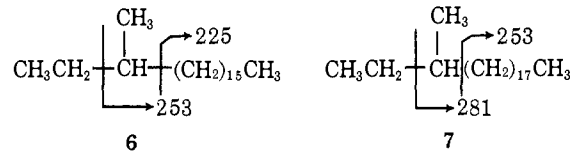

Figure 1. Synthesis of 3-methyluntriacontane (2).

unit higher appears to be general for higher molecular weight branched hydrocarbons.[14]

Albeit no other interpretation of its mass spectrum seemed possible, the identity of hydrocarbon **2** was confirmed by synthesis as outlined in Figure 1. 1-Bromo-10-methoxydecane (**3**) was prepared from 1,10-dibromodecane according to the procedure for the homologous 1,6-bromo ether.[15] Treatment of the Grignard reagent prepared from **3** with excess 2-butanone in ether followed by dehydration and hydrogenation afforded 1-methoxy-11-methyltridecane (**4**). The excellent method of Stone and Shechter[16] provided quantitative cleavage of **4** to the branched iodide **5**. Treatment of an equimolar mixture of iodide **5** and 1-iodooctadecane with sodium in ether under the conditions of the Wurtz reaction gave in high yield a mixture of the expected three hydrocarbons, 21:50:29 in order of increasing v.p.c. retention times. The retention time of the major component was the same as that of 3-methyluntriacontane (**2**) from rimocidin, and the mass spectrum of a collected sample was identical in every detail.

The lithium aluminum hydride, phosphorus–hydriodic acid reductive degradation sequence described for perhydrorimocidin was then carried out using three different substrates: (1) octahydrorimocidin, (2) octahydrorimocidin (or perhydrorimocidin) previously treated with sodium metaperiodate, and (3) octahydrorimocidin (or perhydrorimocidin) treated first with methanolic hydrogen chloride and then with periodate. In each case the composition of the hydrocarbon product (obtained in 5–10% over-all yield) was essentially the same. The major product was 3-methyluntriacontane (**2**); small amounts of low molecular weight unbranched hydrocarbons, principally *n*-octadecane and *n*-hexadecane, were also present. No hydrocarbons of retention time between *n*-octadecane and **2** were detected. The significance of these results to the determination of the oxygenation pattern of the aglycone is clear; the possibility of a 1,2-diol or $\alpha$-hydroxy ketone or of an epoxide can be ruled out. Furthermore the glycosidic linkage of mycosamine must not be adjacent to a hydroxyl or keto group.

With a maximum of information gleaned from the "parent" hydrocarbon **2**, we were next able to locate the position of the tetraene system through identification of smaller hydrocarbons resulting from ozonolysis followed by reductive work-up and use of the phosphorus–hydriodic acid method. Initially the result was puzzling. A solution of rimocidin in methanol was ozonized just to completion and hydrogenated immediately over platinum as catalyst. The products were reduced with lithium aluminum hydride and converted to hydrocarbons. The major product was *n*-octadecane; small amounts of lower unbranched hydrocarbons were also present but no trace of a branched hydrocarbon was detected.[17] However, introduction of an extra step in this series of reactions gave an entirely different result. The mixture of products isolated after ozonolysis, hydrogenation in methanol, and removal of the solvent was redissolved in glacial acetic acid and hydrogenated again over platinum as catalyst for 4 days. Lithium aluminum hydride reduction and treatment with phosphorus–hydriodic acid followed just as in the previous series. The hydrocarbon mixture consisted of a major and a minor component, identified as 3-methylnonadecane (**6**) and 3-




methylheneicosane (**7**), respectively. The mass spectra of **6** and **7** were analogous to that of **2** in all respects; each exhibited a weak molecular ion peak, a moderate M − 15 peak, an extremely intense M − 29 peak which dominated the spectrum, and moderate peaks at M − 57 and M − 58. Hydrocarbon **6** was synthesized by the reaction of *n*-hexadecylmagnesium bromide with 2-butanone followed by dehydration and hydrogenation; mass spectra of **6** from both sources were identical.

These results make possible the formulation of the carbon skeleton of rimocidin aglycone as shown in Figure 2. (The reason for numbering in this way will be evident later.) There is no other way to explain the formation of **6** as the major hydrocarbon from ozonolysis. Incomplete ozonolysis of the C-18, C-19 double bond readily explains the presence of the minor product **7**. Isolation of only *n*-octadecane when the ozonolysis products were not hydrogenated in acetic acid prior to conversion to hydrocarbons meant that loss of the C-2 side chain took place at some stage after hydrogenation in methanol. It should be pointed out that a similar loss of the side chain was not observed when octahydrorimocidin (the product of hydrogenation in methanol) was converted to hydrocarbons, although the yield of **2** was much lower than in the

(14) 7,21-Dimethyltritriacontane[13] exhibited significant peaks at *m/e* 196, 168, and 112 as well as at *m/e* 197, 169, and 113 (see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 82, Figure 3-10). Other examples and extensions of this observation will be discussed in subsequent papers.

(15) N. L. Drake, H. D. Anspon, J. D. Draper, S. T. Haywood, J. V. Hook, S. Melamed, R. M. Peck, J. Sterling, Jr., E. W. Walton, and A. Whiton, *J. Am. Chem. Soc.*, **68**, 1536 (1946).

(16) H. Stone and H. Shechter, *J. Org. Chem.*, 15, 491 (1950).

(17) Branched hydrocarbons were detectable by slightly shorter retention times and when there was any doubt, mass spectra of collected samples were taken.

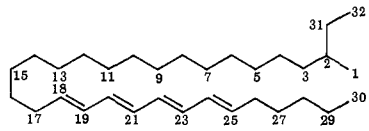
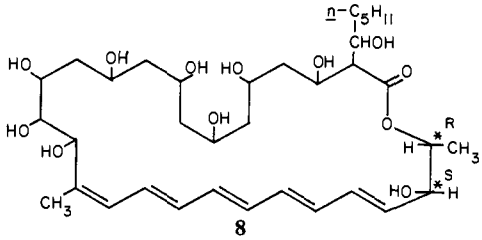


Figure 2. Carbon skeleton of rimocidin aglycone.

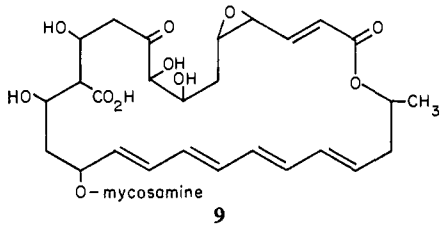
case of perhydrorimocidin (product of hydrogenation in acetic acid). We have no explanation for this apparent anomaly.

Variations of the ozonolysis sequence were then utilized to determine whether an oxygen function was present at C-17. When the ozonolysis products after hydrogenation in methanol were treated with periodate before lithium aluminum hydride reduction and conversion to hydrocarbons, only *n*-octadecane was found. This excluded the presence of a free hydroxyl group at C-17. Rimocidin was next ozonized, hydrogenated in methanol, reduced with lithium aluminum hydride, and treated successively with methanolic hydrogen chloride, periodate, and lithium aluminum hydride followed by conversion to hydrocarbons. *n*-Octadecane and some lower *n*-hydrocarbons were found (see the Experimental Section) but no trace of *n*-heptadecane was detected. Thus the presence of a glycoside at C-17 is excluded as well.

Considering the carbon skeleton of rimocidin (Figure 2) and the established structures of two other related polyenes, fungichromin (**8**)[13] and pimaricin (**9**),[18] it appeared most probable for the lactone carbonyl to be at C-1 or C-32 and the oxygen terminus to be on the $C_6$ fragment, *i.e.*, at C-26–C-30. Ozonolysis of rimocidin followed by steam distillation in the presence of zinc dust provided the first direct evidence for the $C_6$ fragment. 2-Hexenal (**10**) was isolated from the



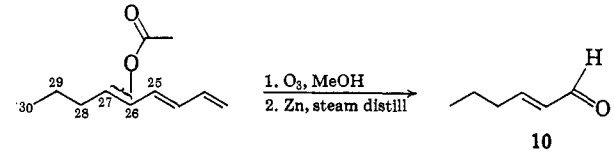
**8**



**9**

steam distillate as the 2,4-dinitrophenylhydrazone. Thus the $C_6$ fragment appeared to possess originally only one oxygen atom, attached most likely to C-27 or possibly to C-26, and the over-all result of ozonolysis would be as depicted in Figure 3. This possibility suggested an experiment which not only established the position of the oxygen atom in question but proved that it was also the terminus of the lactone. Ozonolysis of rimocidin followed by sodium borohydride reduction gave a mixture from which no volatile products could be detected by v.p.c. The mixture was then treated with 5% potassium hydroxide solution and the products

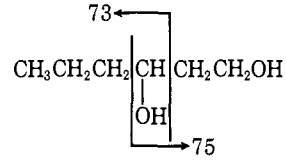
(18) O. Ceder, *Acta Chem. Scand.*, **18**, 126 (1964).



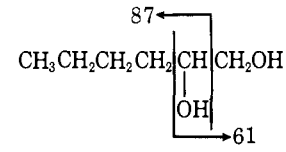

**10**

Figure 3. $C_6$ fragment from rimocidin.

were again subjected to v.p.c. analysis. A single major component (*ca.* 60% yield based on a hexanediol from a precursor of mol. wt. 750) was collected and identified as 1,3-hexanediol (**11**); its mass spectrum was identical with that of authentic material and very different



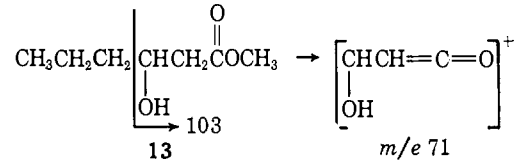
**11**



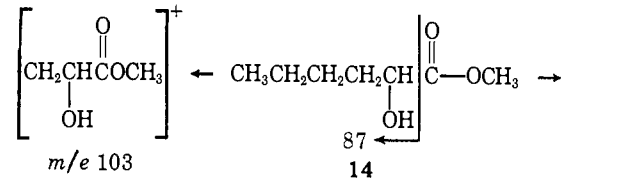
**12**

from that of 1,2-hexanediol (**12**). The salient features of these spectra were in accord with expectation and are summarized with the respective structures **11** and **12**. The spectrum of **11** showed important peaks at *m/e* 71 and 72 as well as at *m/e* 73 and 75, in order of decreasing intensity 75 > 71 > 72 > 73. The peak at *m/e* 72 corresponds to the frequently observed loss of ethylene and water from the molecular ion in alcohols, for which a six-membered transition state has been postulated.[19] The structure of **12** obviously does not permit this process. The base peak of the spectrum of **12** is at *m/e* 69 and corresponds to loss of water from the ion of *m/e* 87.

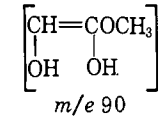
1,3-Hexanediol from rimocidin was optically active, $[\alpha]D$ −11°, but the absolute configuration had not previously been determined. Consequently rimocidin was again ozonized and an oxidative work-up (alkaline hydrogen peroxide) was used. Esterification and analysis of the mixture of products by v.p.c. revealed a component with the same retention time as methyl 3-hydroxyhexanoate (**13**) (in 10–15% yield). Mass spectra of the two samples were identical and very different from that of methyl 2-hydroxyhexanoate (**14**). Principal peaks in the spectrum of **13** are at *m/e* 71,



**13** *m/e* 71



*m/e* 103 **14**



*m/e* 90

(19) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, p. 33.

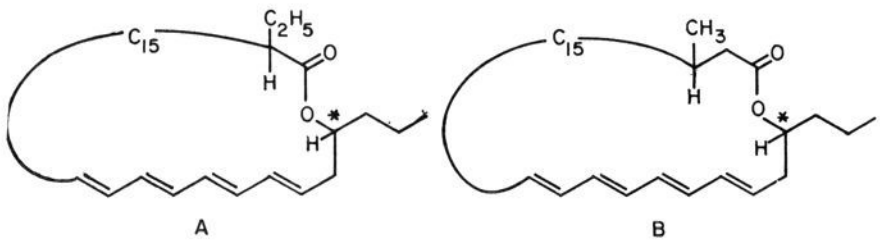



Figure 4. Partial structures for rimocidin aglycone.

74, and 103, the last being very nearly as intense as the base peak at *m/e* 43. Characteristic of **14** are the base peak at *m/e* 69 (87 − $H_2O$), a second most intense peak at *m/e* 87, and moderate peaks at *m/e* 103 and 90. The features of these spectra are in accord with Ryhage and Stenhagen's generalizations for methyl esters of 2- and 3-hydroxy acids.[20]

Methyl 3-hydroxyhexanoate from rimocidin was optically active, $[\alpha]_D$ −18 ± 1°. The configuration of (−)-methyl 3-hydroxyhexanoate has been determined as *R*[21] and the maximum reported rotation (measured under approximately the same conditions as our sample) was $[\alpha]_D$ −26 ± 2°. The configuration at C-27, the terminus of the lactone ring, is consequently *R*.

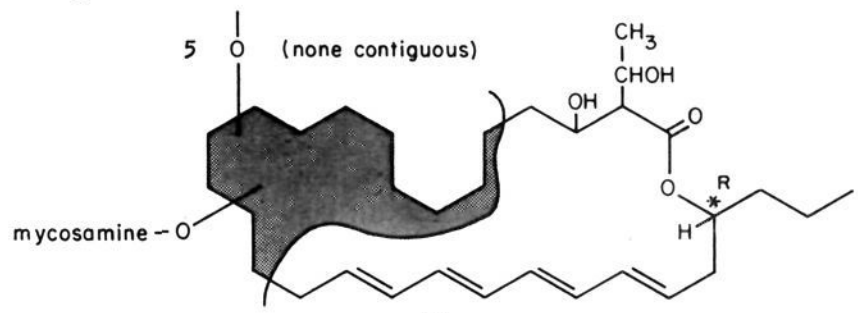
Ignoring for the time being oxygen functions other than the lactone, the two partial structures possible for rimocidin aglycone are shown in Figure 4. To determine which one was correct, rimocidin was ozonized and the products were hydrogenated first in methanol and then in glacial acetic acid. Oxidation of the resulting mixture with fuming nitric acid, esterification of the surviving acids, and distillation of the volatile esters yielded (in addition to the dimethyl esters of succinic, glutaric, adipic, and pimelic acids) two branched dimethyl esters as major and minor products. They were identified as dimethyl α-ethylglutarate and dimethyl α-ethylsuccinate, respectively, by comparison of infrared and mass spectra with those of authentic samples. No higher homologs of these esters were detected. Dimethyl β-methylglutarate was separable from the esters of the two other isomeric $C_6$ acids on the v.p.c. columns used and was not found among the oxidation products. Thus the correct partial structure is "A." Furthermore, these results definitely exclude the presence of a free carboxyl group in rimocidin.

When the ozonolysis–hydrogenation–oxidation sequence was carried out omitting the hydrogenation in acetic acid, no branched acids were found. This fact necessitated the hydrogenolysis of at least one hydroxyl group β to the lactone carbonyl during hydrogenation in acetic acid. Now we recall that on hydrogenation in acetic acid rimocidin absorbed 2 molar equiv. of hydrogen in excess of the 4 required for saturation of the tetraene. It has been demonstrated that rimocidin cannot possess an epoxide or tertiary hydroxyl group; consequently uptake of the second mole of hydrogen must be due either to reduction of a keto group or hydrogenolysis of a second β-hydroxyl group. Work to be described in a subsequent paper on the oxygenation pattern of rimocidin eliminated the first possibility.

(20) R. Ryhage and E. Stenhagen, "Mass Spectrometry of Organic Ions," F. W. McLafferty, Ed., Academic Press Inc., New York, N. Y., 1963, pp. 435, 436.

(21) K. Serck-Hansen, *Arkiv Kemi*, **10**, 135 (1956). For a discussion of the use and advantages of the *R,S* system see E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 88–95.

At the present time the degradative work here described in combination with analytical data on rimocidin, octahydrorimocidin, and their sulfates (see the Experimental Section) supports partial structure **15** for rimocidin. The shaded area shows where the oxygenation pattern remains to be established.




**15**

## Experimental Section[22]

*Rimocidin.*[4] To a solution of rimocidin sulfate[2] (9.96 g.) in 700 ml. of 80% aqueous methanol was added 1 equiv. of barium chloride dihydrate (1.285 g.) in 9 ml. of water. The barium sulfate was removed by filtration and washed with 80% methanol. One equivalent of sodium hydroxide (0.418 g.) in methanol was added to the filtrate and the methanol was evaporated. The crystalline rimocidin was collected, washed with water, and dried under reduced pressure over calcium chloride, yield 7.7 g. The analytical sample was dried under reduced pressure at 56° to constant weight.

*Anal.* Calcd. for $C_{38}H_{63-65}NO_{13}$: C, 61.5; H, 8.43; N, 1.89. Calcd. for $C_{38}H_{63-65}NO_{13}\cdot H_2O$: C, 60.3; H, 8.25; N, 1.85. Found: C, 60.43; H, 7.93; N, 1.92, 1.94.[23]

*Octahydrorimocidin.*[4] Rimocidin sulfate trihydrate[2,4] (2.027 g., equivalent to 1.891 g. anhydrous) dissolved in 100 ml. of 95% methanol was hydrogenated over prereduced platinum oxide (0.277 g.) at atmospheric pressure. Hydrogen uptake ceased after 2.7 hr. and was 99% of theoretical for 4 equiv. based on mol. wt. 800. A white precipitate which had formed during the hydrogenation was dissolved by the addition of 1.0 ml. of 1.09 *N* hydrochloric acid and the catalyst was removed by filtration. To the filtrate was added 2 ml. of 1.09 *N* sodium hydroxide and the mixture was concentrated under reduced pressure. Water (20 ml.) was added and the crystalline octahydrorimocidin was collected by filtration and dried under reduced pressure, yield 1.312 g., m.p. 210–213° dec. when placed in a bath at 200°. The ultraviolet spectrum in neutral or acidic aqueous alcoholic solutions displayed only weak

(22) Melting points, taken on a Kofler micro hot stage, are corrected. Unless noted otherwise, microanalyses were performed by the Scandinavian Microanalytical Laboratory, Herlev, Denmark. Mass spectra were determined using a CEC 21-103C or a CEC 21-130 instrument, and more recently a Hitachi Perkin-Elmer RMU-6D instrument. Nuclear magnetic resonance (n.m.r.) spectra were recorded on a Varian Associates A-60 instrument. Optical rotations were measured wth a Zeiss photoelectric precision polarimeter and the values at the sodium D-line (589.2 mμ) were calculated from the measured values at 546.1 and 578 mμ. Except for the earliest experiments an F and M Model 720 gas chromatograph was employed for analyses and collections. All-purpose columns were 2 ft. × 0.25 in. 10 or 20% silicone rubber (SE-30) on 60–80 mesh Chromosorb W or Diatoport S (silanized Chromosorb W) and 8 ft. × 0.25 in. 20% silicone rubber on the same supports. An 8 ft. × 0.5 in. 15% silicone rubber column (on 60–80 mesh Chromosorb W) was used for larger-scale collections. More exotic liquid phases were used occasionally when the need arose and are described at appropriate places in the text. Solvents were removed under reduced pressure using a rotary evaporator, and magnesium sulfate was used as a drying agent unless noted otherwise,

(23) Performed at the Chas. Pfizer Laboratories.

end absorption. Octahydrorimocidin from a similar preparation[24] was recrystallized for analysis by the addition of standard hydrochloric acid to a 95% ethanol suspension and addition of 1 equiv. of standard sodium hydroxide to the resulting solution with heating on a steam bath, followed by cooling overnight.

*Anal.* Calcd. for $C_{38}H_{71-73}NO_{13}$: C, 60.9; H, 9.43; N, 1.87. Calcd. for $C_{38}H_{71-73}NO_{13}\cdot H_2O$: C, 59.6; H, 9.22; N, 1.83. Found: C, 59.76; H, 9.24; N, 1.83.[25]

*Octahydrorimocidin Sulfate. A. For Microanalysis.*[26] To a suspension of octahydrorimocidin (97) mg.) in 1 ml. of methanol were added 1 *N* sulfuric acid (3 drops), acetone (2 ml.), and water (1 ml., just enough to make a homogeneous solution when the mixture was warmed). The warm solution was centrifuged and the supernatant liquid was allowed to stand at 0°. The sulfate (71 mg.) was collected by filtration, recrystallized by a similar procedure, and dried under reduced pressure.

*Anal.* Calcd. for $C_{38}H_{71-73}NO_{13}\cdot 0.5H_2SO_4$: C, 57.2; H, 8.96; N, 1.75. Found: C, 57.64; H, 8.61; N, 1.81.[25]

*B. By Hydrogenation of Rimocidin Sulfate.* In a typical preparation, rimocidin sulfate (2.01 g.) was hydrogenated over prereduced platinum oxide (0.25 g.) in methanol (100–125 ml.) at atmospheric pressure. After 35 min. the hydrogen uptake was 218 ml. and after 2 hr. it had ceased at 227 ml.; this represented 93% of 4 molar equiv. for mol. wt. 800. Attempts to separate the white precipitate formed during hydrogenation from the catalyst after initial filtration by treatment of the filter cake with dilute hydrochloric acid followed by neutralization of the acidic solution yielded little organic material (<100 mg.). Consequently this step was not repeated, and the precipitate and the catalyst were discarded together. The original filtrate on evaporation to constant weight yielded 1.51 g. of crude octahydrorimocidin sulfate which was used without purification.

*Perhydrorimocidin.* In a typical preparation, rimocidin sulfate (2.675 g.) was hydrogenated over prereduced platinum oxide (0.30 g.) in glacial acetic acid (100 ml.) at atmospheric pressure. Hydrogen uptake was slower than in methanol as solvent, but continued slowly for *ca.* 2 days. After 4 hr., uptake was 335 ml.; after 20 hr., 460 ml.; after 42 hr., 515 ml. The hydrogenation was stopped after 45 hr. at a total uptake of 520 ml., representing 106% of 6 molar equiv. for mol. wt. 800. The catalyst was removed by filtration, the solvent was evaporated, and the residue was dried at 0.1 mm. overnight. It was an amorphous powder (2.22 g.) and was used without purification.

*Isolation of Methyl α,D-Mycosaminide Hydrochloride* (**1a**) *from Rimocidin.* Rimocidin sulfate (1.65 g.) was dissolved in 100 ml. of absolute methanol saturated with hydrogen chloride and allowed to stand for 72 hr. at −20°. Removal of hydrogen chloride and methanol left a light brown solid which was extracted with two 10-ml. portions of water. The aqueous extract was washed until colorless with chloroform and with 1-butanol (five 5-ml. portions of each), and then was stirred for 3 hr. with barium chloride dihydrate (0.25 g.). After removal of water the residue was treated with 25 ml. of absolute ethanol and the barium salts were removed by filtration. Evaporation of the ethanol yielded 0.356 g. of crude methyl α,D-mycosaminide hydrochloride, m.p. 177–180°. Two recrystallizations from hexane–ethanol (2:1) raised the melting point to 196–198° dec., undepressed when mixed with an authentic sample.[9] The infrared spectra of the two samples were identical.

*Isolation of Methyl Triacetyl-α,D-mycosaminide* (**1b**) *from Rimocidin.* Rimocidin sulfate (1.75 g.) was treated with anhydrous hydrogen chloride in methanol as described above. After being washed with chloroform and with 1-butanol the colorless aqueous solution was evaporated to a dry residue (0.42 g.) which was dissolved in pyridine (5 ml.) and acetic anhydride (6 ml.) and allowed to stand for 13 hr. at room temperature. Water (6 ml.) was added and the mixture was extracted with five 3-ml. portions of chloroform. The combined extracts were washed successively with equal volumes of 1 *N* hydrochloric acid, water, saturated sodium bicarbonate, and water, and dried, and the chloroform was removed. The residue (0.42 g., m.p. 138–140°) was recrystallized four times from pentane–ether and yielded pure methyl triacetyl-α,D-mycosaminide, m.p. 140–142°, undepressed on admixture with an authentic sample,[9] m.p. 140–141°. The infrared spectra and the specific rotations ($[\alpha]^{25}D$ +30 ± 2° and $[\alpha]^{23}D$ +33 ± 2°, respectively, in ethanol at *c* 1.0) of the two samples were identical. Triacetate **1b** displayed n.m.r. absorption (in deuteriochloroform with tetramethylsilane as the internal standard) at δ 1.20 (3 H, d, $J$ = 6 c.p.s.), 1.92 (3 H, s), 2.07 (3 H, s), 2.18 (3 H, s), 3.41 (3 H, s), 3.93 (1 H, five lines of $ABX_3$ octet), 4.52–5.07 (4 H, m), and 5.88 (1 H, d, $J$ = 8 c.p.s.).

*Conversion of Rimocidin to 3-Methyluntriacontane. A. Without Sodium Periodate Treatment.* Perhydrorimocidin (2.13 g.) was stirred for 24 hr. with 2.74 g. of lithium aluminum hydride in 150 ml. of refluxing tetrahydrofuran (THF). Work-up was accomplished by successive addition of water (2.74 ml.), 15% sodium hydroxide solution (2.74 ml.), and water (8.3 ml.).[27] The mixture was filtered and the filtrate was evaporated to dryness yielding 1.87 g. of polyol. An additional 0.13 g. was obtained after extraction of the filter cake with THF in a Soxhlet extractor.[28] The polyol was heated under reflux for 20 hr. with hydriodic acid (70 ml.) and red phosphorus (2.0 g.), and the cooled mixture was then diluted with water (135 ml.) and extracted with ether. The ether solution was filtered and washed with water, 2% sodium thiosulfate solution, and water, and dried. Evaporation of the ether gave an iodine-containing oil (2.94 g.) which was dissolved in THF (100 ml.) and stirred under reflux for 12.5 hr. with lithium aluminum hydride (2.05 g.). The mixture was worked up by the *n*, *n*, 3*n* method and the pentane-soluble product[29] was filtered through a column of

(24) Reference 5, pp. 99, 100.

(25) Reference 5, p. 96. Performed by W. Manser of the Eidg. Tech. Hochschule, Zürich.

(26) Reference 5, p. 101.

(27) Hereafter referred to as "the *n*, *n*, 3*n* method."

(28) When octahydrorimocidin was subjected to this treatment the relative amounts of polyol from the filtrate and from Soxhlet extraction were *ca.* 1:1 and the total recovery was substantially lower (*ca.* 60%).

(29) Lithium aluminum hydride reductions of these iodo compounds always gave some unidentified materials which were insoluble in hydrocarbon solvents and consequently were ignored.

Woelm alumina (15.5 g.) in pentane. The resulting solid hydrocarbon (0.48 g.) was hydrogenated over 45 mg. of platinum oxide in 20 ml. of THF.[30] Filtration to separate the catalyst and evaporation of the filtrate yielded 3-methyluntriacontane (**2**) (0.43 g., 34% yield from perhydrorimocidin), *ca.* 99% pure by v.p.c. The mass spectrum (CEC) of a collected sample was superimposable on that of the synthetic material (see below).

*B. With Sodium Periodate Treatment.* Perhydrorimocidin (or octahydrorimocidin) treated with sodium metaperiodate in a chloroform–water mixture by the procedure used to cleave the 1,2,3-triol system in fungichromin[13] followed by conversion to hydrocarbons as described above gave largely **2** and no other major product. The same result was obtained when perhydrorimocidin (or octahydrorimocidin) was allowed to stand overnight with anhydrous hydrogen chloride in methanol at 0° before periodate treatment followed by conversion to hydrocarbons.

*Synthesis of 3-Methyluntriacontane* (**2**). *A. 1-Bromo-10-methoxydecane* (**3**). A solution of 100 g. of 1,10-dibromodecane (Aldrich Chemical Co.) in 120 ml. of ether was stirred under gentle reflux while a solution prepared from 7.7 g. of sodium and 160 ml. of methanol (distilled from magnesium methoxide) was added slowly during 1 hr. The mixture was then stirred under reflux for 16 hr. The sodium bromide was filtered and rinsed with ether (150 ml.), and the filtrate was washed with 300-ml. portions of water and sodium chloride solution, and dried. Two successive distillations through a semimicro column yielded 1-bromo-10-methoxydecane (**3**) in two fractions: 16.1 g., b.p. 117–118° (3 mm.), and 20.0 g., b.p. 118° (3 mm.). Analysis by v.p.c. showed that the first fraction contained *ca.* 10% 1,10-dimethoxydecane and that the second contained *ca.* 5% 1,10-dibromodecane. A sample of **3** collected by v.p.c. was analyzed.

*Anal.* Calcd. for $C_{11}H_{23}BrO$: C, 52.59; H, 9.23. Found: C, 53.05; H, 9.29.

*B. 1-Methoxy-11-methyltridecane* (**4**). To a solution of the Grignard reagent prepared from 14.95 g. of bromo ether **3** (fraction 2, above) and 1.59 g. of magnesium turnings in 60 ml. of ether was added dropwise a solution of excess 2-butanone (6 g.) in ether (30 ml.). The mixture was stirred for 16 hr. at room temperature and then cooled while water was added dropwise. Sufficient 10% sulfuric acid was added to dissolve the hydroxides, the layers were separated, the aqueous layer was extracted with ether, and the combined organic layers were washed successively with sodium chloride solution, sodium bicarbonate solution, and sodium chloride solution, dried, and evaporated. The semisolid residue was dissolved in benzene (150 ml.), and *p*-toluenesulfonic acid monohydrate (6 g.) was added to the solution, which was then heated under reflux for 2 hr. After cooling, the mixture was shaken successively with water, 5% sodium carbonate solution, and water (twice). The benzene solution was dried, the solvent was evaporated, and the residue was dissolved in pentane and filtered through a column of Merck acid-washed alumina (60 g.) packed in pentane. Evaporation of the solvent left a residue of 13.0 g. (96% of theory for $C_{15}H_{30}O$) which was dissolved in ethyl acetate (50 ml.) and hydrogenated over 1.3 g. of platinum oxide prereduced in ethyl acetate (25 ml.). Uptake of hydrogen (1380 ml., 97% of theory for one double bond) ceased after 2 hr. Gas chromatographic analysis of the product obtained after removal of the catalyst and concentration of the filtrate revealed a single major component. A collected sample exhibited infrared and mass spectra (CEC) consistent with the expected 1-methoxy-11-methyltridecane (**4**) and was analyzed.

*Anal.* Calcd. for $C_{15}H_{32}O$: C, 78.87; H, 14.12. Found: C, 79.16; H, 14.18.

The crude hydrogenation product was combined with that from a similar preparation starting with 5.0 g. of **3** and was distilled through a semimicro column yielding **4** in two fractions: 2.92 g., b.p. 108–111° (1.2 mm.), >99.8% pure by v.p.c., and 3.49 g., b.p. 112° (1.2 mm.), *ca.* 98% pure. The other fractions consisted of mixtures of **4** with a lower boiling and a higher boiling component.

*C. 1-Iodo-11-methyltridecane* (**5**). A solution of 0.80 g. of phosphorus pentoxide in 2 ml. of 85% phosphoric acid was prepared and to it was added with stirring 3.32 g. of potassium iodide followed by 0.90 g. of the ether **4** (>99.8% pure). The mixture was stirred and heated at 135–140° (bath temperature) for 5.25 hr., then cooled, and to it were added water (20 ml.) and ether (20 ml.). The ether layer was separated, washed successively with water, sodium thiosulfate solution, and sodium chloride solution, and dried. Removal of the solvent left 1.27 g. (99%) of the colorless iodide **5**, *ca.* 98% pure by v.p.c. It was used without purification for the Wurtz reaction described below. A sample collected by v.p.c. was analyzed.

*Anal.* Calcd. for $C_{14}H_{29}I$: C, 51.85; H, 9.01. Found: C, 53.60; H, 9.30.[31]

*D. 3-Methyluntriacontane.* To a stirred suspension of 0.80 g. of finely cut sodium in ether (75 ml.) was added a solution of 1-iodo-11-methyltridecane (1.26 g.) and 1-iodooctadecane (1.56 g.) in 35 ml. of ether. A blue color began to develop after 4–5 hr.; the mixture was stirred at room temperature with protection from moisture for 4 days. Methanol was added dropwise to destroy the remaining sodium; then hexane (50 ml.) and water (75 ml.) were added and the mixture was shaken thoroughly. The organic layer was separated, dried, and concentrated to *ca.* 4 g. of a semisolid. Gas chromatographic analysis revealed three major components in relative amounts of 21:50:29 in order of increasing retention times. The retention time of the middle component was identical with that of 3-methyluntriacontane from rimocidin. The mass spectrum (CEC) of a collected sample showed a weak molecular ion peak at *m/e* 450 and an extremely intense peak at *m/e* 421 which dominated the spectrum; the other differences between this spectrum and that of an unbranched hydrocarbon were moderately intense peaks at *m/e* 435, 393, and 392.

*Anal.* Calcd. for $C_{32}H_{66}$: C, 85.24; H, 14.76. Found: C, 85.00; H, 14.66.

*Ozonolyses of Rimocidin. A. Conversion to 3-Methylnonadecane.* A solution of 2 g. of rimocidin

(30) Solvent mixtures of *ca.* 2:1 hexane–ethyl acetate were later found preferable for the hydrogenation.

(31) This represented the best of three trials; a satisfactory analysis could not be obtained. Carbon and hydrogen values found varied but were consistently higher than theoretical.

sulfate in 70 ml. of methanol at −70° was ozonized just to completion (first yellow color in potassium iodide exit trap). The solution was hydrogenated over 0.2 g. of platinum oxide for 3 hr. and filtered. The solvent was removed from the filtrate and the residue was dissolved in glacial acetic acid (100 ml.) and hydrogenated for 4 days over 0.2 g. of platinum oxide. The catalyst was separated and the filtrate was evaporated to dryness. Treatment of the residue successively with lithium aluminum hydride, phosphorus–hydriodic acid, and lithium aluminum hydride, followed by filtration through alumina and hydrogenation as described for the conversion of rimocidin to 3-methyluntriacontane yielded a hydrocarbon mixture which was shown by v.p.c. to consist of a major and a minor component with retention times slightly less than those of *n*-eicosane and *n*-docosane, respectively. The major component was identified as 3-methylnonadecane (**6**) by comparison of its mass spectrum with that of an authentic sample (see below) collected from the same column. The mass spectrum (CEC) of the minor component (a weak molecular ion peak at *m/e* 310 and one major highfield peak at *m/e* 281) established its identity as 3-methylheneicosane (**7**).

*B. Conversion to n-Octadecane.* When hydrogenation in acetic acid was omitted from the series of reactions described above, the major hydrocarbon product was *n*-octadecane; no trace of 3-methylnonadecane was found. The same result was obtained when a sodium periodate oxidation step (in chloroform–water[13]) was inserted between the hydrogenation in methanol and the first lithium aluminum hydride reduction; there was no trace of *n*-heptadecane.

*C. Conversion to n-Octadecane, n-Hexadecane, and Lower Hydrocarbons.* Rimocidin sulfate (2 g.) was ozonized and hydrogenated in methanol as described above. The products were reduced with 2 g. of lithium aluminum hydride in 75 ml. of tetrahydrofuran, and the resulting mixture of polyols (1.5 g., including material from a Soxhlet extraction of the filter cake) was allowed to stand overnight with anhydrous hydrogen chloride in methanol at 0°. The solution was concentrated and the residue was dissolved in chloroform and washed with water. The chloroform solution was stirred with aqueous sodium periodate solution and the procedure for conversion of the products to hydrocarbons was followed. The relative yields of hydrocarbons, as estimated by comparison of peak areas with those from solutions of known concentrations of authentic hydrocarbons, were as follows: *n*-octadecane, 2.5%; *n*-heptadecane, 0.0%; *n*-hexadecane, 5.5%; *n*-dodecane, 6%; *n*-decane, 14%; *n*-nonane, 45%; *n*-octane, 25%.

*Synthesis of 3-Methylnonadecane* (**6**). To a solution of the Grignard reagent prepared from 6.0 g. of 1-bromohexadecane and 0.4 g. of magnesium turnings in 30 ml. of ether was added a solution of 2-butanone (2 g.) in ether (10 ml.). The mixture was refluxed for 2 hr. The work-up procedure and the dehydration following it were carried out as described for the preparation of 1-methoxy-11-methyltridecane. *p*-Toluenesulfonic acid (2 g.) in 50 ml. of benzene was used for the dehydration; the product was filtered through a column of Woelm alumina (30 g.) in pentane. The solvent was evaporated and the residue was hydrogenated in a mixture of ethyl acetate (40 ml.) and acetic acid (30 ml.) using platinum as catalyst. Analysis of the hydrogenated products (4 g.) by v.p.c. revealed a major component (82%) with the same retention time as **6** from rimocidin, and mass spectra (CEC) of the collected samples were identical.

*Isolation of 2-Hexenal from Rimocidin.* Rimocidin sulfate (1.0 g.) in 20 ml. of methanol at −70° was ozonized just to completion and the solvent was removed below room temperature. Water (10 ml.) and zinc dust (2 g.) were added to the residue and the mixture was steam distilled until 3 ml. of distillate was collected. The distillate was treated with 8 ml. of 3.3% 2,4-dinitrophenylhydrazine in diglyme[32] and allowed to stand overnight. The precipitate was collected, dissolved in benzene, and passed through a magnesium sulfate column to remove excess 2,4-dinitrophenylhydrazine. Thin layer chromatography of the resulting 2,4-dinitrophenylhydrazones (210 mg.) on Kieselgel G Merck (hexane–ethyl propionate 7:3) showed at least three spots. Chromatography on Merck acid-washed alumina (12 g.) using 5% methyl acetate in hexane afforded 40 mg. (12% yield) of 2-hexenal 2,4-dinitrophenylhydrazone in the earliest fractions. It was recrystallized from ethanol as dark red needles, m.p. 148°, undepressed on admixture with authentic material.[33] The infrared and ultraviolet ($\lambda_{max}^{ethanol}$ 375 mμ) spectra and $R_f$ values on Kieselgel G of the two samples were identical.

*Isolation of (−)-1,3-Hexanediol from Rimocidin.* Rimocidin sulfate (2.0 g.) in 50 ml. of methanol at −70° was ozonized just to completion. To the cold solution, stirred and kept in an ice bath, was added 1.5 g. of sodium borohydride in portions during 15 min. The mixture was stirred for 2.25 additional hr. while the ice melted and an additional portion of sodium borohydride (0.5 g.) was added. The mixture was concentrated below room temperature to 30 ml. and then was stirred and cooled in an ice bath while water (50 ml.) and 3 *N* hydrochloric acid were added rapidly to pH 7. Addition of hydrochloric acid was then continued dropwise to pH 4, and the resulting mixture was extracted with two 100-ml. portions of ether. The ether extract was dried and concentrated; it contained a large quantity of boric acid and no trace of volatile material by v.p.c. analysis. Potassium hydroxide (5 g. in 5 ml. of water) was added to the aqueous solution and the mixture was heated under reflux for 1 hr., then cooled, and extracted with two 100-ml. portions of ether. Gas chromatographic analysis of the residue (220 mg.) after removal of the solvents from the dried ether extract revealed a single major component. It was collected and identified as 1,3-hexanediol by comparison of its mass spectrum (Hitachi) with that of an authentic sample[34]; infrared spectra of the two samples also were superimposable. The optical rotation of a larger sample (25.2 mg.) was determined in

(32) H. J. Shine, *J. Org. Chem.*, **24**, 252 (1959).

(33) 2-Hexenal was prepared (30% yield) from *n*-propylmagnesium bromide and 3-(N-methylanilino)-2-propenal as described by C. Jutz, *Ber.*, **91**, 1867 (1958). The 2,4-dinitrophenylhydrazone melted at 148°, the semicarbazone at 173–174°.

(34) 1,3-Hexanediol was prepared by reduction of ethyl 3-oxohexanoate (supplied by K & K Laboratories) with lithium aluminum hydride in ether. 1,2-Hexanediol was prepared by treatment of 1-hexene with performic acid followed by alkaline hydrolysis. The two diols were not separable on the silicone rubber columns used.

absolute ethanol (1.0 ml.): $\alpha_{546}$ − 0.325°; $\alpha_{578}$ − 0.290°; $\alpha_{589}^{calcd}$ −0.280°; $[\alpha]_{589}^{calcd}$ −11.1 ± 0.5°. Homogeneity of this sample was demonstrated by reinjection on the analytical silicone rubber column.

Continuous ether extraction of the basic aqueous solution from the saponification gave 66 mg. of material which by v.p.c. analysis was largely 1,3-hexanediol with a small amount (*ca.* 15%) of an unidentified component of shorter retention time, probably ethylene glycol. The exact yield of 1,3-hexanediol from rimocidin was not determined but was estimated to exceed 60%.

*Isolation of (R)-(−)-Methyl 3-Hydroxyhexanoate from Rimocidin.* Rimocidin sulfate (2.0 g.) in 50 ml. of methanol was ozonized as described above and to the cold, stirred solution was added immediately 10% sodium hydroxide solution (20 ml.), followed by 20 ml. of 30% hydrogen peroxide slowly in portions. The stirred mixture was allowed to come to room temperature, warmed gently on a steam bath, and finally heated under reflux for 2 hr. The cooled solution gave a negative test for peroxides (potassium iodide–starch paper). It was brought to pH 8 by addition of 6 *N* hydrochloric acid, concentrated to *ca.* 70 ml. and then acidified to pH 5. Water (10 ml.) was added and the mixture was heavily salted and extracted with two 75-ml. portions of ether. The solvent was removed from the dried extracts and the residue was esterified with diazomethane. Analysis by v.p.c. revealed a complex mixture with a major peak at the same retention time as authentic methyl 3-hydroxyhexanoate (**13**).[35] The mass spectrum (Hitachi) of this major component was identical with that of authentic **13** and very different from that of the isomer **14**. A larger sample (23.7 mg.) was collected and the optical rotation was measured in chloroform (1.0 ml.): $\alpha_{546}$ −0.490°; $\alpha_{578}$ −0.440°; $\alpha_{589}^{calcd}$ −0.425°; $[\alpha]_{589}^{calcd}$ −18.0 ± 1°. Homogeneity of the sample was demonstrated by reinjection on the analytical silicone rubber column.

The yield of **13** from this experiment was estimated as 10–15%, and was not increased by continuous ether extraction of the salted acidic aqueous solution.

*Nitric Acid Oxidation of Ozonized Rimocidin.* Rimocidin sulfate (2.0 g.) was ozonized and hydrogenated first in methanol and then in glacial acetic acid as described previously. The solid product was placed in a porcelain evaporating dish and oxidized on a steam bath with portions of 90% fuming nitric acid for 2 days. Extraction of the black, gummy residue with ether gave a yellow liquid (0.47 g.) which was esterified with diazomethane and distilled at 0.15 mm. in a short-path still (bath temperature increased slowly from 30 to 100°). Analysis of the distillate (0.20 g.) by v.p.c. revealed seven major components: a single peak with the same retention time as dimethyl succinate followed by three doublets with retention times roughly corresponding to the dimethyl esters of glutaric, adipic, and pimelic acids, respectively. The three doublets were collected (30–36 mg. of each) and the first two were separated into four single compounds by reinjection separately on a 4 ft. × 0.25 in. XF-1150 column (15% of the liquid phase, a silicone nitrile, on Chromosorb W). These were identified as dimethyl α-ethylsuccinate, dimethyl glutarate, dimethyl α-ethylglutarate, and dimethyl adipate by comparison of their mass spectra (CEC) with those of authentic samples. The third doublet was identified as dimethyl pimelate plus an impurity which was only partially separable on silicone rubber and not resolved at all on XF-1150, Versamide 900, or LAC-728. The retention time of the unknown[36] and the infrared (1795, 1745 cm.$^{-1}$) and mass spectra of a partially separated sample proved that it was not dimethyl α-ethyladipate, but identification was not possible. Dimethyl β-methylglutarate[37] was separable from the esters of the two other isomeric $C_6$ acids on silicone rubber and was not detected in the mixture from nitric acid oxidation.

*Synthesis of Dimethyl α-Ethylglutarate.* To a solution of freshly cut potassium (0.5 g.) in dry *t*-butyl alcohol (12 ml.) was added 8.0 g. of ethyl α-ethylcyanoacetate.[38] The mixture was cooled in an ice bath and stirred while a solution of excess redistilled acrylonitrile (4.5 g.) in *t*-butyl alcohol (6 ml.) was added dropwise. The ice bath was removed and stirring was continued for 12.5 hr. Hydrochloric acid (1 *N*) was added until the mixture was strongly acidic, then ether and water were added, and the mixture was filtered. The ether layer was washed with three portions of sodium chloride solution and dried; the solvent was removed. A portion (5.0 g.) of the thick oily residue (11.0 g.) was heated under reflux with concentrated hydrochloric acid (20 ml.) for 7 hr. The mixture was cooled, diluted with water (40 ml.), and extracted successively with methylene chloride (50 ml.), ether (50 ml.), and chloroform (30 ml.). The combined extracts were washed with water and dried, and the solvents were removed. The oily residue (1.48 g., 36% yield) crystallized on standing and was recrystallized once from ether, m.p. 53–60° (lit.[39] m.p. 58–60°). A portion (0.3 g.) was esterified with diazomethane and a sample of the ester was collected from the XF-1150 column for analysis by mass spectrometry.

(35) Authentic **13** was prepared by sodium borohydride reduction of ethyl 3-oxohexanoate in 96% aqueous methanol followed by treatment of the acidic product with diazomethane. 2-Hydroxyhexanoic acid is available commercially (K & K Laboratories) and was similarly esterified. The silicone rubber columns used did not separate the isomeric hydroxyesters.

(36) On silicone rubber the order of increasing retention times of the esters was glutarate (1), α-ethylsuccinate (2), adipate (3), α-ethylglutarate (4), unknown (5), pimelate (6). On XF-1150 the order was $2 < 1 < 4 < 3 < 5 \equiv 6$.

(37) β-Methylglutaric and α-ethylsuccinic acids were available from K & K Laboratories.

(38) E. R. Alexander and A. C. Cope, *J. Am. Chem. Soc.*, **66**, 886 (1944).

(39) M. F. Ansell and D. H. Hey, *J. Chem. Soc.*, 1683 (1950).